2021

Short-Term Fasting and Autonomic Control

Joshua Gonzalez
Michigan Technological University, jegonzal@mtu.edu

SHORT-TERM FASTING AND AUTONOMIC CONTROL

By

Joshua Eric Gonzalez

A DISSERTATION

Submitted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

In Integrative Physiology

MICHIGAN TECHNOLOGICAL UNIVERSITY

2021

This dissertation has been approved in partial fulfillment of the requirements for the Degree of DOCTOR OF PHILOSOPHY in Integrative Physiology.

Department of Kinesiology and Integrative Physiology

Dissertation Advisor: *William H. Cooke*

Committee Member: *Jason R. Carter*

Committee Member: *John J. Durocher*

Committee Member: *Kui Zhang*

Department Chair: *Megan C. Frost*

Table of Contents

List of Tables, Figures, & Equations

Acknowledgements

The pursuit of this PhD has been an enduring wonderful adventure that would not have been possible without the giants on whose shoulders I stand.

My Grandparents Roman and Olga Martinez who instilled in me an insatiable curiosity and love for learning. Their passion lives on in me.

My Gonzalez Grandparents who came to the United States bringing with them the immigrant spirit of limitless possibility.

My mother, Sandra Gonzalez, who's unwavering love gave me the confidence to risk failing spectacularly while striving to achieve my dreams. Everything I am is because of you.

My father, Rene Gonzalez, who worked tireless hours to give me a life where I would be able to use my mind instead of muscle to make a living.

My siblings, Venessa, Veronica, Emily, Mark, and Michael. Y'all are my greatest supporters and have showered me with love all my life. Y'all are my luckiest break and greatest gift.

My polymathic advisor William Cooke whose mentorship, expertise, and encouragement have been paramount in my development as a scientist. Dr. Cooke is equally as brilliant and creative as he is kind and patient. I am overwhelming grateful for his guidance and investment in my growth over the years. Dr. Cooke helped me discover the potential within myself and inspired me to learn more, dream more, and be more.

My dissertation committee whose invaluable advice and plentiful experience made this dissertation possible. John Durocher who exemplifies and perfectly balances intellectual prowess, grit, and levity. Dr. Durocher has been a constant source of encouragement throughout my graduate training. Jason

Carter whose contagious charisma, curiosity and deep knowledge motivated me to challenge myself and expand my thinking. Kui Zhang whose kindness and statistical knowledge have been a great asset to this dissertation.

I would like to thank my fellow graduate students and dearest friends Jeremy Bigalke, Ian Greenlund, Hannah Cunningham, Issac Wedig, and Jessica Bruning. Throughout my graduate training they each in turn have played the role of sounding board, help hotline, test subject, ping-pong partner, confidant, consoler, brainstormer, advisor, troubleshooter, and hype man/women.

Special thanks to the gems of the KIP Department Steve Elmer, Terry Anderson, and Meagan Frost. Dr. Elmer never failed to deliver a memorable experience and is a fantastic teaching mentor. Terry was the glue that kept my scattered brain together and was always available to provide vital support. Dr. Frost has always been quick to offer encouragement and share a laugh.

Critical research assistance for this project was generously provided by Steven Stelly, Stephanie Jewell, Corey Miller, and Autumn Weiderman.

I would like to thank my funding sources the Portage Health Foundation and the Michigan Space Grant Consortium. Lastly, I would like to thank all my hungry participants.

List of Abbreviations

ABPM: Ambulatory blood pressure monitor

ACH: Acetylcholine

ANS: Autonomic nervous system

AU: Arbitrary units

BMI: Body mass index

CALERIE: Comprehensive assessment of long-term effects of reducing intake of energy trial

CHD: Coronary heart disease

CSI: Cumulative stress index

cvBRS: Cardiovagal baroreflex sensitivity

DAP: Diastolic arterial pressure

DNP: Duration of negative pressure

ECG: Electrocardiogram

FBF: Forearm blood flow

FFM: Fat-free mass

FFT: Fast-Fourier transform

FVR: Forearm vascular resistance

HDL: High-density lipoproteins

HF: High frequency

HR: Heart Rate

HRV: Heart rate variability

hs-CRP: High sensitivity C-reactive protein

LBNP: Lower body negative pressure

LDL: Low-density lipoprotein

LF: Low frequency

MAP: Mean arterial pressure

MSNA: Muscle sympathetic nerve activity

NE: Norepinephrine

PNS: Parasympathetic nervous system

RSA: Respiratory sinus arrythmia

SA: Sinoatrial Node

SAP: Systolic arterial pressure

SNS: Sympathetic nervous system

SP: Spontaneous

TG: Triglycerides

TPR: Total peripheral resistance

VM: Valsalva maneuver

VOP: Venous occlusion plethysmography

WASO: Wake after sleep onset

Abstract

Obesity is a chronic metabolic disorder associated with increased risk of cardiovascular disease. Evidence suggests that chronic intermittent fasting improves cardiometabolic health and reduces arterial blood pressure. However, the mechanisms underlying the reductions in blood pressure and improved cardiovascular health observed from chronic fasting studies remain unclear. The autonomic nervous system has a central role in the regulation of blood pressure and is essential for cardiovascular homeostasis. We conducted a study to investigate how acute fasting influences autonomic control of blood pressure at rest and during stress. Twenty-five young, healthy, normal weight, normotensive participants were tested twice, once in the fed state (3 hours postprandial) and again in the fasted state (24 hours postprandial). **Aim 1** of the study was to determine the influence of an acute fast on hemodynamics, peripheral neural activity, and cardiovascular control at rest. To fulfill this aim we measured 24-hour ambulatory blood pressure for both conditions leading up to an autonomic function test. During the autonomic function test, we controlled breathing at 0.25 Hz and measured blood pressure, heart rate, muscle sympathetic nerve activity, and forearm blood flow for 10 minutes. Fasting reduced overall ambulatory blood pressure and heart rate compared to the fed condition. From the autonomic test we measured enhanced vagal modulation of the heart through 1) increased R-R interval and heart rate variability measured via spectral analysis; 2) Increased spontaneous (rest) and dynamic (Valsalva Maneuver) cardiovagal baroreflex sensitivity indicating enhanced reflexive vagal activation. Fasting did not alter peripheral sympathetic activity or blood pressure during the autonomic test. However, forearm vascular resistance and stroke volume were increased during the fasting condition. **Aim 2** investigated if fasting influenced cardiovascular and neural reactivity to a mental stressor (5 min mental arithmetic). Fasting did not augment neural or cardiovascular reactivity to a

mental stress challenge. **Aim 3** investigated if fasting reduced orthostatic tolerance to intense lower body negative pressure (LBNP). LBNP was applied in a stepwise manner until participants became presyncopal. Fasting reduced the duration of negative pressure participants could tolerate before presyncope occurred. The reduced tolerance to central hypovolemia seems to have been caused by an impaired ability to increase peripheral resistance as measured from the forearm. This dissertation provides novel insight into how systemic energy balance influences autonomic regulation of blood pressure. Specifically, that fasting reduces 24-hour ambulatory blood pressure, increases vagal modulation of the heart, and enhances cardiovagal baroreflex sensitivity.

1 INTRODUCTION

1.1 The Problem of Plenty

In the United States, overeating, inactivity, and obesity have emerged as serious public health challenges that reduce life expectancy (Peeters et al., 2003). The problem has only gotten worse as we progress through the 21st century. Caloric intake in the United states has increased 22% in women and 7% in men between 1971 and 2000 (Wright, Kennedy-Stephenson, Wang, McDowell, & Johnson, 2004). The prevalence of obesity in the U.S. population increased from ~31% to ~42% from 2000 to 2018 (Hales, Carroll, Fryar, & Ogden, 2020). Accessible countermeasures are needed to abate the rise in obesity and improve life expectancy and health. It is well established that physical activity enhances cardiometabolic health and increases life expectancy in humans in a dose dependent manner (Mandsager et al., 2018; Moore et al., 2012; Reimers, Knapp, & Reimers, 2012). However, only ~23% of American adults over 18 years old engage in the recommended amount of physical activity (Blackwell & Clarke, 2018). Recently, caloric restriction and intermittent fasting have been proposed as countermeasures to improve cardiometabolic health and potentially increase life expectancy.

1.2 An Evolutionary Perspective

In developed societies such as the United States hyperpalatable foods are abundant and accessible to a majority of the population. Undoubtedly, this abundance of food has allowed for sustained population growth throughout the 21st century. However, regular intake of calorically dense food represents a significant divergence from homo sapiens evolutionary history. Early human societies that subsisted on hunting and gathering evolved in environments where they intermittently experienced periods of little to no caloric intake (Crittenden & Schnorr, 2017). In these societies caloric intake was

opportunistic, and periods of fasting were not uncommon. In order to survive these environments our ancestors had to constantly make the metabolic shift from glucose utilization and fat storage (lipogenesis) during times of plenty, to fat breakdown (lipolysis) and ketone utilization for energy when food was scarce. The switch to lipolysis and the mobilization of fatty acids for energy production only occurs once glycogen stores in the liver are depleted. Depending on energy expenditure and liver glycogen stores, the switch to lipolysis occurs approximately 12 to 36 hours after caloric cessation and is characterized by the production of ketones (β-hydroxybutyrate) from triglycerides (Merimee, Misbin, & Pulkkinen, 1978). In the fasted condition, ketone bodies provide a major source of energy. However, in the United States it is common for individuals to consume 3 meals a day plus snacks. This constant energy intake keeps ketone levels low, as there is a near continuous infusion of glucose for energy. People who adhere to the 3 meals a day eating pattern never make the metabolic switch to the production and utilization of ketones for energy unless they engage in strenuous exercise. We as a species are physiologically well suited for periods of caloric restriction and complete caloric cessation. Sections 1.3 and 1.4 will briefly cover the emerging evidence supporting the potential health benefits of chronic caloric restriction and intermittent fasting.

1.3 Caloric Restriction, Cardiometabolic Health, and Lifespan

Caloric restriction has been reported to significantly increase the lifespan and delay the onset of age-related disease. In lower order eukaryotes, such as yeast and worms, caloric restriction extends their lifespan by 3 fold (Fontana, Partridge, & Longo, 2010). Caloric restriction is also reported to extend the lifespan of higher order eukaryotes like flies (by 2 fold) and mice (by 30-50%) (Fontana et al., 2010). There may also be benefits

to the lifespan of primates who consume a calorie restricted diet. In 1989 a longitudinal study commenced in rhesus monkeys investigating caloric restriction and lifespan. By 2013, 63% (24/38) of the control animals died of age-related causes, compared to only 26% (10/38) of the animals in the caloric restricted group (Colman et al., 2014). The rhesus monkey study is still active. The most recent report in 2017 reported that six calorie restricted monkeys had lived beyond the age of 40 years old, which was previously thought to be maximal lifespan for the species (Mattison et al., 2017). The degree of caloric restriction that confers maximal benefits to the lifespan is unknown. However, the evidence suggests that lower caloric intake improves lifespan in animals.

To date, there is no direct evidence to support that caloric restriction increases the lifespan of humans. The most prominent caloric restriction study conducted in humans is the Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy trial (CALERIE). The CALERIE trial was conducted in 218 healthy non-obese men and women and prescribed a diet that had 25% less calories than needed to maintain energy balance over a 2-year period. While the diet prescription was 25% below energy needs, actual adherence in the caloric restriction group averaged out to 12% over the 2-year period. As expected, participants in the caloric restriction group lost about 10.4% of their body weight, 71% of which was fat (Kraus et al., 2019). The study showed improvements in cardiometabolic health greater than those conferred by the concordant weight loss. The caloric restriction group consistently had lower low-density lipoprotein (LDL) and serum triglycerides (TG) concentrations, which was accompanied by elevated high-density lipoprotein (HDL) concentrations. The caloric restriction group also reported lower systolic, diastolic, and mean arterial pressure. Insulin sensitivity was improved and fasting glucose was lower in the caloric restriction group. Lastly, high sensitivity C-reactive protein (hs-CRP), a measure of low-grade

chronic inflammation was significantly reduced at the 2 year time point (Kraus et al., 2019). Caloric restriction of just 12% on average in this population improved multiple indicators of cardiometabolic health and reduced risk factors for cardiovascular disease. Additional support for the hypothesis that caloric restriction has cardiovascular benefits comes from observational studies. 18 individuals who reported self-imposed caloric restriction for 3-15 years exhibited similar cardiovascular benefits as the participants in the CALERIE trail. The observational study reported that in the caloric restricted group blood pressure, carotid artery intima media thickness, LDL, TG were lower and HDL were higher than the age-matched comparison group (Fontana, Meyer, Klein, & Holloszy, 2004). The CALERIE trial and the observational study provide evidence that caloric restriction may be beneficial for cardiovascular health. However, the metabolic switch from preferential glucose utilization to ketone bodies for energy does not occur during caloric restriction as energy intake does not cease. Without exercise, fasting is required for the metabolic switch to occur and for ketone production to commence. This may be an important metabolic distinction between caloric restriction and fasting because the ketone β-hydroxybutyrate has been reported to inhibit sympathetic nerve activity in cell cultures and rat models (Kimura et al., 2011). Intermittent fasting requires the complete cessation of energy intake for a specific time period resulting in the production of ketone bodies. Section 1.4 will discuss the beneficial association between chronic intermittent fasting and cardiovascular health.

1.4 Chronic Intermittent Fasting and Cardiovascular Health

Intermittent fasting is a rapidly growing dieting concept popular with individuals seeking to lose weight. The most popular intermittent fasting regimens are alternate day fasting, 5:2 intermittent fasting (fasting 2 days out of the week), and time restricted feeding (Anton et al., 2018). 5:2 and

alternate day fasting commonly involve fasting for an entire 24 hours. Studies have demonstrated that intermittent fasting can promote weight loss and increase fat oxidation in obese and non-obese populations (Heilbronn, Smith, Martin, Anton, & Ravussin, 2005; Varady et al., 2013) Additionally, intermittent fasting has been utilized by weight lifters to successfully reduce body fat mass while maintaining muscle mass (Moro et al., 2016). Separate from weight loss fasting may convey other health benefits. Two observational studies have reported that populations who periodically fast as infrequently as once a month have reduced prevalence of diabetes and coronary artery disease (Horne et al., 2012). When adjusted for age, sex, BMI, hypertension, hyperlipidemia, smoking, and family history, the meta-analysis suggested that odds of diabetes were 43% lower and coronary artery disease 42% lower in the infrequent fasters (Horne et al., 2008; Horne et al., 2012) suggesting that even an acute periodic fast may have beneficial cardiovascular and metabolic outcomes. The cardiovascular benefits of fasting have primarily been investigated in chronic fasting models.

In rats, intermittent fasting increases heart rate variability and reduces blood pressure and heart rate (Mager et al., 2006). Reductions in blood pressure measured in the rats was attributed to enhanced parasympathetic activation at the heart. The ability for fasting to reduce blood pressure has also been observed in humans. A recent review reported that chronic intermittent fasting is beneficial for lowering blood pressure in obese and non-obese individuals (Malinowski et al., 2019). One month of alternate day fasting effectively lowers blood pressure and heart rate, suggesting that chronic fasting may enhance parasympathetic activity (Stekovic et al., 2019). A drop in blood pressure has also been measured in men observing Ramadan fasting (Samad et al., 2015). However, the influence of fasting on the autonomic nervous system and the mechanism responsible for the observed reduction in blood pressure remains unclear. The autonomic

nervous system is intimately linked to cardiovascular health. Remarkably, few studies have investigated how an acute fast influences autonomic balance in humans (section 2.5) or how fasting affects autonomic cardiovascular control of blood pressure. **Therefore, the purpose of this research study is to identify how an acute 24-hour fast influences autonomic cardiovascular and neurovascular control of arterial pressure at rest and during stress.**

1.5 Summary

We are far removed from the eating and activity patterns of our early ancestors. Nevertheless, humans are metabolically well equipped to compensate for periods of complete cessation in energy intake. Humans are capable of making the metabolic switch from glucose for energy production to ketones through the breakdown of fat. It is well established that reducing caloric intake through restriction or fasting will result in weight loss. Current evidence suggests that fasting could have cardiovascular benefits separate from weight loss. However, knowledge on how the autonomic nervous system influences cardiovascular control of blood pressure during periods of fasting is limited. This dissertation will contribute new knowledge to the field of autonomic physiology by investigating how acute fasting influences cardiovascular and neurovascular control of blood pressure at rest and during stress.

2 LITERATURE REVIEW

2.1 Obesity

2.1.1 State of the Disease

Obesity in adults is most commonly defined as having a body mass index greater than or equal to 30 kg/m^2. According to a 2020 report from the Centers for Disease Control and Prevention in 2017-2018, 42.4%(~138 million people) of the population in the United States is considered obese (Hales et al., 2020). These data show an approximate 12% increase in the overall prevalence of obesity since the year 2000. Obesity is a chronic metabolic disorder associated with increased risk of cardiovascular disease, stroke, type 2 diabetes, and overall mortality (Poirier et al., 2006). In 2008 alone, medical costs associated with obesity were estimated to be 147 billion dollars (Finkelstein, Trogdon, Cohen, & Dietz, 2009). Obesity affects some groups disproportionally. Non-Hispanic Black adults and Hispanic adults have the highest prevalence of obesity at 49.6% and 42.2% (Ogden et al., 2017). The association between obesity, income, and educational level is complex and varies by sex, race, and ethnicity. Obesity prevalence is lower among college graduates for White men and women, Black women, and Hispanic women but not for Black and Hispanic men. In general, women with a higher income are less likely to be obese. However, there is no difference in obesity prevalence between the lowest and highest income groups in men (Ogden et al., 2017). Obesity is often considered to be a result of either excessive energy intake and/or of insufficient energy expenditure. This is known as energy imbalance and will be further explored in the next section.

2.1.1.1 Energy Balance Theory

The first law of thermodynamics states that internal energy of a system equals the net heat transfer into the system minus the net work done by the system. This can be explained by the following equation

Equation 1: 1st Law of Thermodynamics

$$\Delta \boldsymbol{U} = \boldsymbol{Q} - \boldsymbol{W}$$

ΔU= change in internal energy of the system

Q = is the sum of all heat transfer into and out of the system

W = the net work done on or by the system

Applied to human metabolism, **Q** is heat transfer out of the body and lost to the environment (meaning **Q** is always negative and relatively constant). **W** is the sum of work done by the body (energy expenditure) and also work done on the body in the form of food (energy intake). There are three uses for energy in human metabolism; heat transfer, doing work, or storage in the form of fat. The first law of thermodynamics assures that body weight cannot change if over time energy intake and energy expenditure are equal. Energy balance has played a central role in the study of human obesity. Weight gain must be caused by a positive energy balance just as weight loss must be caused by a negative energy balance. The equations below summarize this concept.

Equation 2: Energy Balance

Energy Intake > Energy Expenditure = Weight Gain (positive energy balance; +U)

Energy Intake < Energy Expenditure = Weight loss (negative energy balance; -U)

Multiple studies have attempted to ascertain if excessive energy intake or insufficient energy expenditure is the primary driver for human obesity. Those who claim excessive energy intake as the cause of obesity have reported that highly palatable energy dense foods lead to accidental excess energy consumption (Prentice & Jebb, 2003). Others who claim insufficient energy expenditure as the cause of obesity have reported that over the last 50 years daily energy expenditure has declined by more than 100 calories (Church et al., 2011). It is likely that both excessive energy intake and insufficient energy expenditure contribute to the increase in the prevalence of obesity in the United States. Bariatric surgery is currently the most effective treatment for obesity and all procedures effectively decrease energy intake or energy absorption to result in weight loss. (Waseem, Mogensen, Lautz, & Robinson, 2007). However, bariatric surgery is an invasive and expensive intervention. Other lifestyle weight loss strategies such as diet and exercise should always be the first options for promoting weight loss.

The first law of thermodynamics cannot be violated in regard to the onset, maintenance, or reversal of obesity. However, obesity is an extremely complex disease sensitive to genomic, metabolic, and environmental influences. The autonomic nervous system has been proposed to have an integrated regulatory role in maintaining constant energy storage and expenditure. Studies in both animals and humans have reported that the autonomic nervous system responds to changes in systemic energy balance. The following sections will describe the autonomic nervous system and how it responds to acute energy intake and energy deprivation.

2.2 Autonomic Nervous System

2.2.1 The Autonomic Nervous System

2.2.1.1 A Brief History and Organization of the Autonomic Nervous System

The first identification and description of the Peripheral Vegetative Nervous System (i.e. Autonomic Nervous System; ANS) was first made by Galen in 150 A.D by dissecting pigs. However, Galen's physiological ideas were heavily influenced by humoral theory and he thus believed that the nerves he identified were used to transfer animal spirits to muscle and organs in what he described as sympathetic action (Ackerknecht, 1974). The term Autonomic Nervous System (ANS) would not be coined until 1898 by John Newport Langley. Langley is recognized for his work concerning the anatomical organization of the ANS. Langley is also credited with distinguishing the sympathetic and parasympathetic branches of the ANS. The basis for the separate distinction of the sympathetic and parasympathetic branches of the ANS was the recognition that multiple organ systems are under the opposing influences of two sets of nerves. The importance of the balance between the sympathetic and parasympathetic branches of the autonomic nervous system would become evident in the work of Claude Bernard. Bernard's work identified numerous autonomic effector targets, such as the influence of the vagus nerve on heart rate, the glycemic function of the liver, and the link between blood flow and temperature regulation. Bernard also famously coined the phrase *milieu interior* or internal environment stating that "The constancy of the internal environment is the condition for free and independent life."(Wehrwein, Orer, & Barman, 2011) The concept that maintenance of a constant internal environment was revolutionary at the time and is a fundamental tenet in the discipline of human physiology. Walter B. Canon expanded upon Bernard's work and highlighted the central role of the ANS in maintaining a constant internal environment. Cannon coined the term

homeostasis to described factors that maintain internal stability, stating “If a state remains steady it does so because any tendency towards change is automatically met by increased effectiveness of the factor which resist the change.” Cannon went on to describe that thirst, hypoglycemic reaction, and thermogenic functions all become more intense as the disturbance to homeostasis is more pronounced (Walter B Cannon, 1929). These corrective factors are necessary for the maintenance of constant internal state and governed by the branches of the ANS.

2.2.1.2 The Sympathetic Branch

The sympathetic nervous system (SNS) is a division of the autonomic nervous system whose preganglionic neurons are located between the first thoracic and first few lumbar spinal segments (C8-L2). Sympathetic preganglionic neurons are relatively short compared to the receiving postganglionic neurons that proceed to the effector organ. Sympathetic preganglionic neurons release acetylcholine (ACH) to their receiving nerve terminals that then transmit the nerve action potential to release norepinephrine (NE) at the target effector organ. Sympathetic postganglionic neurons mainly terminate on smooth muscle, but also project into the thoracic cavity to innervate the heart, bronchi, and visceral targets such as the gastrointestinal tract, urinary bladder, and kidneys. Most blood vessels are exclusively innervated by the sympathetic postganglionic neurons making the sympathetic nervous system the primary regulator of peripheral vascular resistance and the governor of blood flow throughout the body.

The unique characteristic of the SNS to control blood flow and thus direct necessary resources to organs and muscle makes it the primary system called upon when responding to danger and stress. This responsibility of the sympathetic nervous system to respond to danger earned it the designation the “fight or flight response.” The SNS prepares the body to fight

or flee by 1) increasing heart rate and contractility thus cardiac output by direct stimulation; 2) increasing blood flow to active muscles while concurrently decreasing blood flow to metabolically inactive organs not needed for motor function such as the gastrointestinal tract and kidneys; 3) bronchodilation in the lungs to facilitate greater ventilation; 4) enhancing utilization of glycogen stores to increase blood glucose; and 5) mobilizing epinephrine from the adrenal glands into the blood stream to enhance glycogenolysis and reduce muscle fatigue (Walter Bradford Cannon, 1922). The sum of these mechanisms allows for the best possible conditions for maximal physical effort to escape or neutralize the threat.

Two classes of adrenergic receptors are primarily responsible for mediating the sympathetic cascade of the fight or flight response, alpha (α_1, α_2) and beta (β_1, β_2). The agonists for alpha and beta receptors are epinephrine, released mostly from adrenal medulla and norepinephrine released predominantly from sympathetic nerves. Briefly, α_1 receptors are expressed on vascular smooth muscle and facilitate vasoconstriction through the binding of epinephrine or norepinephrine. The α_1 receptor has high affinity to both epinephrine and norepinephrine. In low concentrations epinephrine paradoxically causes vasodilation until a threshold saturation is reached and vasoconstriction is facilitated. The α_2 receptor are primarily localized on presynaptic nerve terminals and act to inhibit norepinephrine release to attenuate excitatory transmission. The β_1 receptor are present in the heart and have a greater affinity for epinephrine and increase heart rate and contractility when activated. Lastly, β_2 receptors are present in the smooth muscle of the respiratory tract and have a high affinity for epinephrine that induces relaxation or dilation (Wehrwein et al., 2011; William Tank & Lee Wong, 2011).

The circulating levels of epinephrine in conjunction with circulating and directly released norepinephrine increase during stress and physical activity (Zouhal, Jacob, Delamarche, & Gratas-Delamarche, 2008). Epinephrine and norepinephrine are the primary agonists of the sympathetic nervous system responsible for resting vascular tone and mediating rapid mobilization of resources for physical activity. However, many autonomic effector organs are influenced by the parasympathetic nervous system. The sympathetic and parasympathetic nervous system are anatomically and functionally distinct and control autonomic effector organs synergistically, antagonistically, and independently.

2.2.1.3 The Parasympathetic Branch

The parasympathetic nervous system (PNS) is a division of the autonomic nervous system whose preganglionic neurons project from cranial nerves III, VII, IX, X and the sacral region of the spinal cord (S2-S4). A divergent characteristic of the PNS is that all preganglionic and postganglionic neurons of the parasympathetic nervous system release acetylcholine (ACh). Unlike the sympathetic nervous system, the preganglionic neurons of the parasympathetic nervous system are quite long and synapse with their respective postganglionic neurons close to or embedded in their effector organs. Parasympathetic fibers innervate lacrimal, oral, and nasal glands as well as the gastrointestinal tract, rectum, kidneys and bladder. However, The majority of parasympathetic fibers (75%) are located in cranial nerve X or the vagus nerve (McCorry, 2007). The vagus nerve is central to autonomic physiology because of its powerful direct influence on the sinoatrial node and atrioventricular nodes of the heart.

ACh released from the vagus nerve binds to muscarinic receptors (M_2) on the sinoatrial node of the heart and effectively slow heart rate (Brodde, Bruck, Leineweber, & Seyfarth, 2001). The sinoatrial node is the predominant

pacemaker of the heart and is under the constant influence of the vagus nerve. Without the influence of the vagus nerve the sinoatrial node would fire at its intrinsic rate of >100 beats per minute (Shaffer, McCraty, & Zerr, 2014). However, the heart is innervated by both sympathetic and parasympathetic nerves. If both divisions of the ANS innervate the heart, why is heart rate under the dominant control of the PNS? Simply put, parasympathetic nerves exert their effects faster, with a vagal impulse reaching the SA node in less than a second allowing for beat-to-beat modification. Cardiac sympathetic nerves can take upward of 5 seconds to exert their influence (Nunan, Sandercock, & Brodie, 2010). Therefore, a single vagal impulse triggers an immediate response within the cardiac cycle and its affect is eliminated after one or two cardiac cycles, due to rapid action of acetylcholinesterase which breaks down ACh (Shaffer et al., 2014; Wehrwein et al., 2011). This is a foundational concept in heart rate variability and with be expounded upon in section 2.2.2.1.

ACh acts on two primary receptor types, nicotinic (N_N) and muscarinic (M_1, M_2, M_3) receptors. Transmission of signals in autonomic ganglia are primarily facilitated by nicotinic (N_N) receptors which should not to be confused with the nicotinic receptors (N_M) that facilitate skeletal muscle contraction. The only muscarinic receptors found in the autonomic synapses of the PNS are M_1, M_2, and M_3. M_1 receptors are found in autonomic ganglia while M_2 and M_3 are concentrated on PNS target organs. M_2 receptors are highly concentrated on the heart and are responsible for enabling vagal influence over heart rate (SA node), contractility (atrial muscle), and conduction velocity (AV node & His-Purkinje system) (Brodde et al., 2001; Wehrwein et al., 2011). M_3 receptors are associated with exocrine glands influenced by the PNS such as pancreas β cells responsible for insulin secretion.

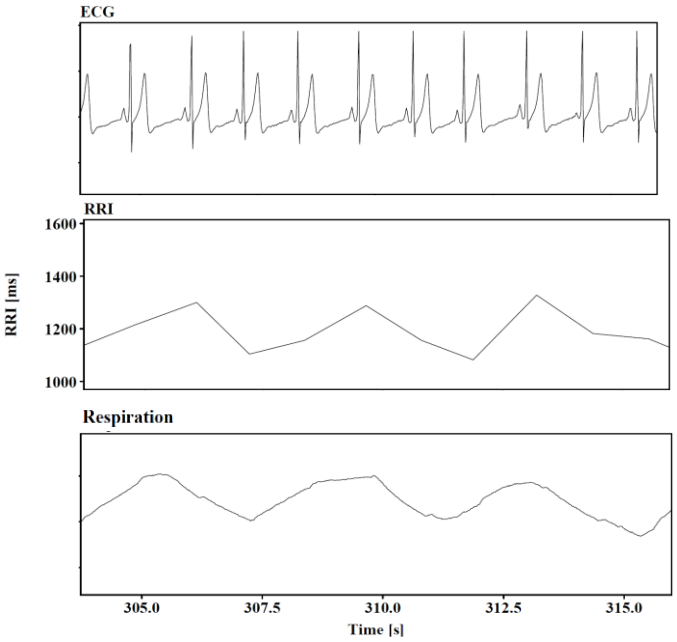
The summation of the influences of the PNS is why it is commonly referred to as the “rest and digest system.” The SNS and PNS work in tandem to maintain homeostasis via widespread innervation of glands, smooth muscles, and the heart. Frequently, the PNS and SNA are thought of conceptually in stark dichotomy. However, the PNS and SNS work in harmony as part of an integrated regulatory system and neither works in isolation. Section 2.2.2 will discuss how balance between these two systems is measured and interpreted.

2.2.2 Techniques Used to Access Autonomic Balance in Humans

2.2.2.1 Heart Rate Variability

The heart is not a metronome. Each cardiac cycle is deliberately and meticulously controlled by the autonomic nervous system to ensure adequate perfusion of organs critical to sustain life. Heart rate variability (HRV) is the term used to describe changes in the time interval between consecutive cardiac cycles at the R-wave of the electrocardiogram (ECG) or contraction of

Figure 1: ECG and R-R Interval Example



the ventricles this can be seen in figure 1. All HRV assessments are calculated from the time interval from R wave to adjacent R wave, known as the R-R interval. The time interval between R waves is influenced by multiple factors including autonomic neural activity, blood pressure, and respirations (W. H. Cooke et al., 1998; Hirsch & Bishop, 1981). The influence of respiration on R-R interval can be seen in figure 1. The most used methods for assessing HRV are time-domain analyses and frequency domain analyses (power spectral density).

2.2.2.1.1 Time Domain Analysis of Heart Rate Variability

To calculate time-domain HRV in a continuous ECG, each R wave is detected and the time between each R wave is measured in a given time period. The time between each R wave of the QRS complex is commonly referred to as the normal-to-normal (NN) interval in time domain analysis. The most commonly used measured derived from the NN interval differences include standard deviation of the NN intervals (SDNN, unit: ms), square root of the mean squared differences of the NN intervals (RMSSD, unit: ms), and the percentage of successive NN intervals that differ by more than 50 ms (PNN50, unit: %) (Electrophysiology, 1996). All these assessments measure respiratory frequency variations between cardiac cycles. It is necessary that when comparing these measurements of heart rate variability, the duration of recordings is the same.

Interpreting time domain analysis of heart rate variability is relatively straight forward. In general, and increase in SDNN, RMSSD, and PNN50% indicate greater vagal activity at the level of the heart when comparing short-term recordings. Time domain analysis of HRV is also valuable for cardiac and mortality risk stratification. Over a 24-hour period SDNN is good cardiac risk stratification tool in the acute phase of myocardial infarction (Casolo et al., 1992). Short term ECG recordings indicate that low heart rate variability

(SDNN) is associated with risk of death in middle-aged and elderly men (Dekker et al., 1997). Additionally, one study found that a pNN50 <3% is strongly associated with occurrence of a future coronary event in patients admitted to a coronary care unit (Manfrini, Pizzi, Trerè, Fontana, & Bugiardini, 2003). After clinical stabilization 56% of patients with a pNN50 <3% had a subsequent coronary event (Manfrini et al., 2003). These studies indicate that reductions in time-domain measurements of heart rate variability are strong predictors of deleterious health outcomes.

2.2.2.1.2 Frequency Domain Analysis of Heart Rate Variability

Frequency domain analysis of HRV requires the same initial steps of time domain analysis of HRV. R waves of a continuous ECG are identified, and the R-R time intervals are measured and plotted over time. A fast Fourier transform (FFT) is performed on the R-R intervals data at a selected time point. The FFT creates a power spectrum that provides information on how variance (power) distributes over frequency. This process yields two physiologically relevant frequency bands, the low frequency band (LF; 0.04 – 0.15 Hz) and the high frequency band (HF; 0.15 – 0.40 Hz). The HF band reflects parasympathetic activity at the level of the heart (Hayano et al., 1991).The physiological relevance of the LF band, traditionally associated with sympathetic activity, has been questioned. The ability of the LF band to reflect sympathetic activity is dubious because there is significant vagal contribution to power density in the LF band (Eckberg, 1997). Evidence for vagal contribution to the LF power comes largely from autonomic blockade studies. High dosage atropine administration blocks sinoatrial responses to acetylcholine released from the vagus nerve (Epstein et al., 1990). When atropine is administered HF power is abolished and LF power is severely reduced (Pomeranz et al., 1985). Thus, the reductions of LF power via atropine administration reveals the significant vagal contribution to LF power density. Moreover, the low frequency component of heart rate variability does

not correlate with cardiac norepinephrine spillover, the gold standard index of cardiac sympathetic outflow (Moak, 2007). In summary, cardiac vagal activity is the primary contributor to the HF band and heavily influences power density at the LF band. The LF component of heart rate variability does not reflect cardiac sympathetic activity. The significant contribution of vagal activity to the HF band makes HF power density a powerful reflection of vagal modulation (parasympathetic activity) at the heart.

Spectral analysis of heart rate variability is a useful tool in evaluating autonomic function. Similar to time domain measurements of HRV frequency domain analysis can be used for disease risk stratification. Multiple cardiovascular diseases exhibit a decrease in HF power and/or an increase in LF power, such as myocardial infarction (Lombardi et al., 1996) and hypertension (Piccirillo, Munizzi, Fimognari, & Marigliano, 1996). In sepsis patients a reduction in LF band power below 18 ms^2 is associated with development of multiple organ dysfunction syndrome and a 64% mortality rate (Pontet et al., 2003). Similarly, in chronic heart failure patients' diminishment of LF band power below 11 ms^2 is an independent predictor of sudden death (Rovere et al., 2003). Both sepsis and chronic heart failure are associated with extreme sympathetic overactivity so why does the diminishing of LF band power act as a predictor for deleterious health outcomes? Reduced LF power during diseases associated with sympathoexcitation may be due to an impaired baroreflex, reduced responsiveness of the SA node, or vagal withdrawal (Rovere et al., 2003). Nevertheless, while the usefulness of the LF band for evaluating autonomic balance is dubious it is useful as a risk stratification tool.

While power density is typically expressed in absolute values of power (ms^2) LF and HF can also be quantified in normalized units. Normalization is done by dividing the LF or HF power by the total power of the spectrum and

multiplying by 100 (Malliani, Pagani, Lombardi, & Cerutti, 1991). Total spectral power varies greatly between individuals. Reporting normalized units emphasizes the balance between the LF and HF power bands relative to total power for better interindividual comparison. Lastly, it should be noted that cardiovascular rhythms and respirations are intimately linked through respiratory sinus arrythmia (RSA). Variations in heart rate are synchronized with respirations, by which the intervals between R waves on an ECG are shortened during inspiration and prolonged during expiration. Since RSA's initial description and recording it has been further explored and understood to be the modulation of the cardiac vagal efferent activity through respiratory central drive (Shykoff, Naqvi, Menon, & Slutsky, 1991). The influence of breathing on autonomic neural outflow make breathing a potential confounding factor in human autonomic testing (W.H. Cooke, 1998). Controlled (or paced) breathing ≥0.2 Hertz has been advised as a means to increase the reproducibility and stability of heart rate variability measurements during short-term autonomic testing (T. E. Brown, Beightol, Koh, & Eckberg, 1993; W. H. Cooke et al., 1998; Pitzalis et al., 1996).

2.2.2.2 Blood Pressure

Blood pressure is the force exerted by the blood against any unit area of the arterial vessel wall. When blood pressure is recorded two numbers are typically reported, systolic pressure (mmHg) which is arterial pressure when the ventricles are contracting, and diastolic pressure (mmHg) which is arterial pressure when the ventricles are refilling. Blood pressure should be recorded at the level of the heart, therefore non-invasive measurements are recorded at the upper arm.

Blood pressure is a fundamental variable of the cardiovascular system, and short-term regulation is driven by the autonomic nervous system. Maintaining blood pressure chronically within normotensive ranges (Systolic:

<120 mmHg; Diastolic: <80 mmHg) is one of the homeostatic responsibilities of both the ANS and the kidney. Adequate perfusion pressure is necessary to maintain consciousness and avoid syncope and potential injury from falling (Hainsworth, 2004). Conversely, chronically elevated blood pressure is a primary contributor to cardiovascular disease and potentially organ damage. Therefore, highly integrative responses are necessary for the control of blood pressure. Autonomic control of blood pressure with be discussed in detail in section 2.2.3. This section will cover non-invasive beat-to-beat and ambulatory blood pressure recordings.

2.2.2.2.1 Noninvasive Beat-to-beat Arterial Pressure

Noninvasive continuous measurement of arterial pressure from the finger was first introduced in the 1980's (Imholz, Wieling, van Montfrans, & Wesseling, 1998). Since its introduction noninvasive finger arterial pressure has been extensively validated as a reliable method to track changes in blood pressure (Imholz et al., 1998; Truijen, van Lieshout, Wesselink, & Westerhof, 2012). When calibrated to brachial blood pressure noninvasive finger arterial pressure are comparable to invasively measured intraarterial brachial pressures (Guelen et al., 2008).

Continuous measurement of arterial pressure allows for accurate evaluation of the pressor response to stressful stimuli such as mental stress, cold pressor test, and isometric exercise. Additionally, beat-to-beat blood pressure measurements are essential for measuring hemodynamic responses to the Valsalva maneuver and orthostatic stressors such as lower body negative pressure (van Wijnen et al., 2017). When paired with ECG, continuous arterial pressure can be used to assess spontaneous and dynamic cardiovagal baroreflex sensitivity (described in section 2.2.3.1.). Using the Modelflow method, beat-to-beat arterial pressure can be used to

estimate stroke volume and cardiac output (Langewouters, Wesseling, & Goedhard, 1984).

In conclusion, continuous blood pressure is a valuable and reliable measurement for the assessment of pressor response, orthostatic intolerance, and baroreflex sensitivity. However, this technique has limitations. Continuous blood pressure recordings are often short-term and done in a controlled laboratory setting with participants mostly stationary. Due to these limitations continuous blood pressure recordings fail to capture the circadian rhythm or blood pressure during normal living. The deployment of ambulatory blood pressure devices has allowed for a more holistic look of blood pressure over a 24 period.

2.2.2.2.2 Ambulatory Blood Pressure

Ambulatory blood pressure (ABPM) allows for the recording of multiple blood pressure readings several times an hour across a 24-hour period. Recording blood pressure over a 24-hour period gives ABPM the unique ability to group blood pressure measurements into time windows of wake and sleep recordings and render a 24-hour mean. ABPM can also be used to evaluate the morning surge and nighttime dipping patterns of blood pressure. Given these advantages, ABPM has become a valuable tool for the diagnosis and management of hypertension and the prediction of cardiovascular disease (Krakoff, 2013).

ABPM is the best measurement methodology for assessing nighttime blood pressure dipping when paired with sleep diaries or actigraphy measurement (Dolan et al., 2005). Measurement of nocturnal blood pressure dipping is an increasingly important prognostic parameter for cardiovascular morbidity and mortality. Ambulatory recordings also allow for the ruling out of white-coat hypertension as blood pressure recordings are taken outside the laboratory during the subject's normal life. White-coat hypertension is a

phenomenon wherein individuals show elevated blood pressure in clinical, or laboratory settings. White-coat hypertension can occur in approximately 15 to 30 percent of subjects (Franklin, Thijs, Hansen, O'Brien, & Staessen, 2013). Despite the advantages of ABPM, it also has limitations. A successful ABPM recording requires at least 20 valid wake measurements and 7 valid sleep measurements which can be difficult to obtain in some participants (O'Brien, Parati, & Stergiou, 2013). ABPM monitoring can cause potential discomfort during nighttime periods that disrupt sleep. Due to the potential for sleep disruption, it is important to pair ABPM measurements with an objective sleep measurement such as actigraphy, to accurately assess sleep time for nighttime dipping calculation. Additionally, ABPM monitoring does not account for body position at the time of measurement (seated, supine, etc.)

In conclusion, ABPM allows for the measurement of 24-hour blood pressure and assessment of circadian driven blood pressure activity such as nocturnal dipping and morning surge. ABPM allows for a more holistic assessment of blood pressure compared to short-term beat-to-beat recordings and allows for the ruling out of white-coat hypertension. In the future ABPM may allow for the timing of hypertension medications and lifestyle interventions to the endogenous circadian rhythm of blood pressure.

2.2.2.3 Microneurography

Microneurography is a technique used to directly record multifiber postganglionic nerve impulses in human subjects. The microelectrode used for the technique is made from tungsten and is inserted through the skin into the accessible peripheral nerve. Microneurography is commonly performed in peripheral nerves such as the peroneal neve of the leg and/or the ulnar, median, or radial nerves of the arm. The technique was first developed by Vallbo and Hagbarth at Academic Hospital in Uppsala, Sweden in 1966 (Vallbo, Hagbarth, & Wallin, 2004). Vallbo and Hagbarth effectively developed

the technique to measure both efferent and afferent muscle and skin neural activity. Muscle sympathetic nerve activity can be quantified by bursts per minute (burst frequency) and/or by burst per 100 cardiac cycles (burst incidence). Both burst frequency and burst incidence give insight into neural control of the vasculature. Section 2.2.2.3.1 will expound upon the measurement of efferent muscle sympathetic nerve activity.

2.2.2.3.1 Description, Measurements, and Reliability

Efferent postganglionic nerve activity that innervates muscle vascular beds is a stable and reproducible measurement at baseline within individuals (Fonkoue & Carter, 2015; Grassi et al., 1997; G Sundlof & Wallin, 1977). Muscle sympathetic nerve activity (MSNA) is characterized by strong intra-individual reproducibility and large inter-individual variability (Fagius & Wallin, 1993). The cause for the interindividual variability of MSNA is unknown, but a genetic component may influence MSNA as identical twins have similar basal MSNA (B G Wallin, Kunimoto, & Sellgren, 1993). At rest, MSNA is highly correlated with both cardiac and renal norepinephrine spillover (B. Wallin, Thompson, Jennings, & Esler, 1996; B.G. Wallin et al., 1992) The relationship between MSNA and norepinephrine spillover does not stand during measurements taken during stressful stimuli, such as mental stress or isometric handgrip (B.G. Wallin et al., 1992).

The relationship between transient rises in blood pressure and quiescence in MSNA was first reported by Wallin (G. Sundlof & Wallin, 1978). Wallin also observed that stimulation of the carotid sinus produced a clear inhibition of MSNA (B. G. Wallin, Sundlöf, & Delius, 1975). Later, the arterial baroreceptors dominance over MSNA would be proven in an elegant study by Allyn Marks group. Upon the infusion of phenylephrine, arterial pressure sharply increases inducing a baroreflex mediated reduction in MSNA (Sanders, Ferguson, & Mark, 1988). Baroreflex inhibition of MSNA has been

observed when humans consume other drugs that exhibit a pressor response such as nicotine (Gonzalez & Cooke, 2021; Grassi et al., 1994). The observation of baroreflex mediated inhibition of MSNA is the foundation for the development of sympathetic baroreflex analyses.

MSNA is a major contributor in the creation and control of peripheral vascular resistance in humans. Nonetheless, there is a paradoxical lack of correlation between MSNA and arterial pressure (Skarphedinsson, Elam, Jungersten, & Wallin, 1997; G. Sundlof & Wallin, 1978), meaning young individuals whose blood pressure fall in the normotensive range can have widely varying basal MSNA. Not until middle age (~40 years) does a relationship between resting blood pressure and MSNA develop (Michael J. Joyner, Charkoudian, & Wallin, 2010; Narkiewicz et al., 2005). There does exist a negative relationship between MSNA and cardiac output in men, with higher MSNA being associated with lower cardiac output (Michael J. Joyner et al., 2010). Furthermore, in normotensive young men there is a positive relationship between total peripheral resistance and MSNA; there is no relationship in women (Emma C Hart et al., 2009). Lastly, greater waist circumference and fat mass are strongly correlated with an increase in MSNA in men (Jones, Davy, Alexander, & Seals, 1997).

Another persistent characteristic of MSNA is that it tends to increase with age. These age-related changes in MSNA also differ between the sexes. MSNA in men and women are similar at age 20. MSNA and women will then decrease until about age 30. From age 30 on MSNA in both women and men increase, with women having significantly lower activity until age 50 (Keir et al., 2020). After age 50, average activity converges again between men and women. Additionally, in both men and women younger than age of 40 no relationship exists between baseline mean arterial pressure and MSNA. However, after the age of 40 a positive relationship emerges in both sexes

between baseline MSNA and mean arterial pressure (Narkiewicz et al., 2005). In women, resting MSNA is also influenced by menstrual phase. Resting MSNA has been reported to be higher during the mid-luteal phase of the menstrual cycle compared to the earlier follicular phase (Minson, Halliwill, Young, & Joyner, 2000). However, this finding was not replicated by Carter & Lawrence who found similar resting MSNA during the mid-luteal and early follicular phases of the menstrual cycle (Carter & Lawrence, 2007). A later study explained the inconstancies by investigating to what degree sex steroid surges contributed to resting MSNA (Carter, Fu, Minson, & Joyner, 2013). Specifically, it was reported that the magnitude of estradiol increase during the mid-luteal phase was significantly correlated with decreases in resting MSNA (Carter, Fu, Minson, & Joyner, 2013). Hormonal contraceptives are used by ~80% of women in the United States during their lifetime. Studies have reported that women on oral contraceptives have higher resting blood pressure but no change in resting MSNA when compared to normal menstruating women (Harvey et al., 2015). Women on contraceptives also do not have significant differences in resting MSNA during the low hormone phase (placebo period) and high hormone phase (17-20 days after start of estrogen-progestin pills) (Minson et al., 2000). However, it is important to note that blood pressure, sympathetic baroreflex sensitivity, and cardiovagal baroreflex sensitivity are all greater during the low hormone phase of contraceptive use (Minson et al., 2000). Indicating that oral contraceptive use can modify the mechanisms by which blood pressure is regulated in women.

2.2.3 Autonomic Control of Blood Pressure and Blood Flow

The autonomic nervous system plays a central role in the maintenance of mean arterial pressure and cardiovascular homeostasis. An intact and responsive ANS is paramount in the maintenance of perfusion pressure during postural changes, food consumption, exercise, and hemorrhage.

Concurrently, the ANS must be able to respond to sharp increases in blood pressure to prevent serious cardiovascular complications such as stroke, myocardial infarction, organ hyperperfusion injury and blood vessel rupture. The ANS relies on feedback from the mechanoreceptors of the baroreflex to regulate arterial pressure. The ANS uses feedback from the baroreflex to regulate mean arterial pressure by altering either cardiac output and/or peripheral resistance. Cardiac output is influenced by both heart rate and stroke volume. The creation of vessel resistance is best described by Poiseuille's law (Equation 5). The radius of the vessel, which is influenced by the sympathetic nervous system, has the greatest contribution to the creation of peripheral resistance. The relationship between cardiac output and vessel resistance describes mean arterial pressure.

Poiseuille's law can also be used to understand how pressure and resistance influence blood flow. The equation clearly shows the influence of vessel radius on flow rate, explaining how the ANS influences both local blood flow and mean arterial pressure through its control of the heart and vessel radius in the periphery. The next section will focus on the negative feedback loop of the baroreflex and how it is assessed. Additionally, the next section will also contain a brief description of venous occlusion plethysmography and how the technique estimates blood flow.

Equation 3: Determinants of Mean Arterial Pressure

$$MAP = Cardiac\ Output\ X\ Total\ Periphearl\ Resistance$$

Equation 4: Cardiac Output

$$Cardiac\ Output = Stroke\ Volume\ X\ Heart\ Rate$$

Equation 5: Determinants to Resistance to Flow (Poiseuille's Equation)

$$Vessel\ Resitance = \frac{8(viscosity\ of\ fluid)(length\ of\ vessel)}{\pi(radius\ of\ vessel)^4}$$

Equation 6: Determinants of Flow Rate (Poiseuille's Equation)

$$Flow\ Rate = \frac{\pi(Pressure\ Differences)(Radius\ of\ Vessel)^4}{8(Viscosity\ of\ Fluid)(Length\ of\ Vessel)}$$

2.2.3.1 Baroreflex Sensitivity

The primary mechanoreceptors of the baroreflex negative feedback loop are located in the aortic arch and the carotid sinuses. The baroreflex is the fastest mechanism to regulate acute changes in blood pressure through both fast vagal action at the heart and slower sympathetic action in peripheral vessels (de Boer, Karemaker, & Strackee, 1985). In other words, if blood pressure is perturbed, sympathetically mediated changes in peripheral resistance and sympatho-vagal modulation of heart rate and contractility (stroke volume) work together to adjust cardiac output and maintain blood pressure homeostasis. The ability of the baroreflex to appropriately sense acute changes in blood pressure and respond appropriately by activating or inhibiting the branches of the autonomic nervous system is called baroreflex sensitivity. Impairment of the baroreflex sensitivity is associated with multiple disease conditions such as myocardial infarction (Farrell et al., 1992), heart failure (Mortara et al., 1997), and even type II diabetes (Kück et al., 2020). Additionally, impaired baroreflex sensitivity is an independent prognostic value in predicting cardiac mortality (Rovere et al., 2001). Aerobic exercise has been reported to improve baroreflex sensitivity in healthy individuals and improve survival after a myocardial infarction (La Rovere, Bersano, Gnemmi, Specchia, & Schwartz, 2002; Monahan et al., 2000). Aerobic exercise has also been reported to reverse the age associated decline in baroreflex sensitivity (Monahan et al., 2000). Section 2.2.3.2 will discuss the hearts beat-to-beat control of arterial pressure known as cardiovagal baroreflex sensitivity.

2.2.3.2 Cardiovagal Baroreflex Sensitivity

Cardiovagal baroreflex sensitivity (cvBRS) is described as the slope of the relationship between the R-R interval and systolic blood pressure during acute changes in arterial blood pressure. cvBRS contributes to the beat-to-beat control of arterial blood pressure and is an indicator of cardiac autonomic regulation (Parlow, Viale, Annat, Hughson, & Quintin, 1995). In particular, the sequence method of cvBRS is a good indicator of fast vagal regulation of the heart because the parameters for the technique are usually shorter than six beats (Silva, Dias, da Silva, Salgado, & Fazan, 2019). That cvBRS is primarly regulated by vagal modulation has been proven through atropine infusion which eliminates the baroreflex slope (Parlow et al., 1995). Spontaneous baroreflex function can be assessed at rest and during forced dynamic changes in blood pressure. At rest, cvBRS can be assessed as blood pressure and heart rate naturally oscillate in response to respiratory rate and Mayer waves, which have a ~10 second (0.1 Hz) rhythm (deBoer, Karemaker, & Strackee, 1987). Assessment of the baroreflex can also be done by perturbing blood pressure. This will be discussed in section 2.3.1 with the Valsalva maneuver.

2.2.3.3 Venous Occlusion Plethysmography and Blood Flow

Venous occlusion plethysmography (VOP) is a minimally invasive, relatively simple, and effective technique for assessing vascular function of the limbs. As the name implies it requires occlusion of the veins by inflating a cuff located proximal to the measurement site above venous pressure, but below diastolic arterial pressure. Therefore, Inflation pressure of the cuff prevents venous blood from leaving the limb but does not inhibit arterial blood from entering. When taking measurements in the forearm, it is standard practice to exclude hand circulation by inflating a secondary cuff above arterial pressure throughout the measurement. This results in a linear increase in limb volume over time. Changes in limb volume are then

measured by a plethysmograph (Whitney, 1953). Measurements are made successively, with upper cuff inflated for a 7 second recording, and then deflates for 7 seconds to allow for venous emptying. At rest, approximately 70% of forearm blood flow is through skeletal muscle and the rest is through skin blood vessels (Cooper, Edholm, & Mottram, 1955). VOP provides a estimate of blood flow rate to the part of forearm enclosed by the two cuffs.

Blood flow rate depends on arterial pressure and vessel resistance (Equation 6). Due to this relationship vascular resistance in the limb can be *estimated* by taking mean arterial pressure and dividing by blood flow rate.

Equation 7: Forearm Vascular Resistance Equation

$$Forearm\ Vascular\ Resistance = \frac{Mean\ Arterial\ Pressure}{Forarm\ Blood\ Flow}$$

This technique does have serious limitations. The forearm vascular resistance equation assumes laminar flow of a Newtonian fluid, driven at a constant pressure, through a fixed resistance (Benjamin et al., 1995). However, Blood is not a Newtonian fluid, that can have turbulent flow, driven by a pulsatile pressure through a distensible vessel. Therefore, calculated resistance should be interpreted with caution.

2.2.3.4 Sex Differences in Blood Pressure Regulation

There are multiple clinically relevant sex-related differences in blood pressure regulation between men and women. First, young men have a higher prevalence of hypertension and in general have higher blood pressure than women (Burt et al., 1995; Wiinberg et al., 1995). This trend continues until age 70 and then the prevalence of hypertension in women surpasses men. Second, young women are at higher risk of orthostatic intolerance (Ali et al., 2000). These sex related differences in blood pressure regulation are influenced by the sex steroids testosterone, estrogen and progesterone.

In premenopausal eumenorrheic women the menstrual cycle is characterized by 4 phases; menstruation, follicular phase, ovulation, and the luteal phase. In general, studies that investigate sex difference in blood pressure regulation divide the menstrual cycle into 2 phases, the early follicular and mid-luteal phase. The early follicular (2-5 days after menstruation) is commonly used among autonomic studies because it is characterized by low estradiol and low progesterone levels. The mid-luteal (8 to 10 days after luteinizing hormone surge) serves as a good comparison time point in the menstrual cycle because it is characterized by high estradiol and high progesterone. Occasionally studies will include the late follicular phase (10-14 days after menstruation) to isolate the influence of progesterone because late follicular is characterized by low estradiol and high progesterone. These phases are important in understanding sex differences in blood pressure regulation because they can alter baroreflex sensitivity. Sympathetic baroreflex sensitivity is greater in the mid-luteal phase but cardiovagal baroreflex is not affected (Minson et al., 2000). There is no reported difference between vascular transduction of muscle sympathetic activity between the two phases (Minson et al., 2000). Resting MSNA is elevated in the mid-luteal phase, but this does not translate to increases in blood pressure (Minson et al., 2000). Later, studies demonstrated that the increase in MSNA observed in the mid-luteal phases is related to the ratio and concentration of the sex steroid estradiol and progesterone. Specifically, increases in estradiol are associated with sympathoinhibition and progesterone with sympathoexcitation (Carter et al., 2013). Another important distinction between young men and women is that men have a positive relationship between peripheral resistance and MSNA and women do not (Emma C Hart et al., 2009). The lack of a relationship between peripheral resistance and MSNA appears to be due to a reduced vasoconstrictive response to norepinephrine in young women. However, when β-adrenergic

receptors (vasodilate in response to norepinephrine) are blocked and norepinephrine is infused, women exhibit vasoconstriction similar to men (Emma C. Hart et al., 2011). Suggesting that in young women α-adrenergic vasoconstriction may be countered by β-adrenergic vasodilation in the periphery (Emma C. Hart et al., 2011). β-adrenergic vasodilation in the periphery would account for the lack of relationship between MSNA and peripheral resistance, and could contribute to the increase in orthostatic intolerance in young women. Lastly, approximately 80% of women in the United States will use hormonal contraceptives during their lifetime. Hormonal contraceptives are reported to increase mean arterial pressure, but the mechanism is unclear as MSNA does not change (Harvey et al., 2015) Further Investigation into how sex influences blood pressure regulation is an important and necessary field of study.

2.3 Autonomic Function Tests

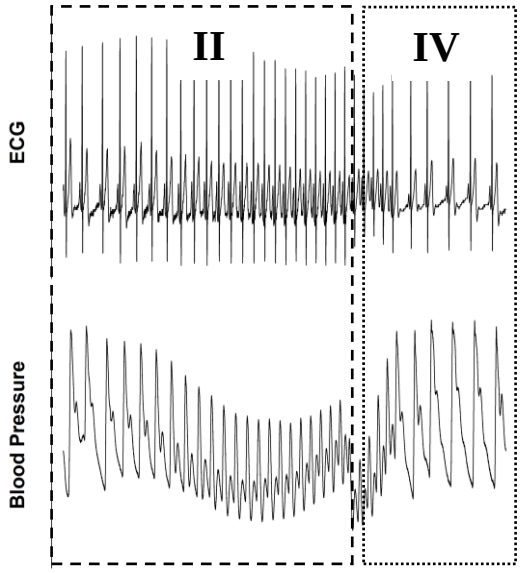
As mentioned previously the autonomic nervous system has a central role in the maintenance of homeostasis. The purpose of an autonomic functions test is to perturb homeostasis through a physical and/or psychological stress and measure the response. Commonly, cardiovascular and neurovascular measures are used to quantify the autonomic response to the test.

2.3.1 The Valsalva Maneuver

The cardiovascular and neurovascular response to the Valsalva maneuver provides reliable and reproducible information regarding the integrity of the baroreflex loop. The Valsalva maneuver requires a human subject to forcefully exhale air through a mouthpiece while blocking airflow through their nose for 15 seconds at a pressure of 40 mmHg. The Valsalva maneuver increases intrathoracic pressure and thus impedes venous return to the heart. This results in a fall in blood pressure that begins to rise as heart

rate and peripheral resistance increase (Phase II). When the intrathoracic pressure is released, venous return is restored, and cardiac output is significantly increased. The increased cardiac output is forced into elevated peripheral resistance causing blood pressure to rapidly rise (Phase IV). The rise in blood pressure is blunted by a reduction in heart rate allowing for blood pressure to stabilize. The Valsalva maneuver is a reliable and reproducible tool to assess the arterial baroreflex sensitivity to hypotensive (Phase II) and hypertensive (Phase IV) stimuli (displayed in figure 2) (Palamarchuk, Ives, Hachinski, & Kimpinski, 2014).

Figure 2: Representative Valsalva Maneuver



2.3.2 Mental Stress

Throughout human evolution the sympathetic nervous system played a critical role in helping our ancestors survive physical threats. In the modern world, physical threats are usually limited. However, the evolutionarily conserved sympathetic nervous system does not discriminate between real threats and perceived threats. Psychological stress can be induced through a

speech task (Trier social stress test), attention task (Stroop test), and mental arithmetic (rapid serial subtraction) (Carter & Goldstein, 2015). Mental arithmetic induces sympathetically mediated increases in heart rate, blood pressure, skin sympathetic nerve activity, and also causes variable muscle sympathetic activation in humans (Carter, Kupiers, & Ray, 2005; Muller, Sauder, & Ray, 2013). The cardiovascular and neurovascular responses to mental stress are reproducible across laboratory sessions separated by at least 1 month (Fonkoue & Carter, 2015). Individuals who exhibit an exaggerated hemodynamic response to mental stress may be at heightened risk for the development of hypertension (Chida & Steptoe, 2010). Modern societies have mostly eliminated the need to activate the sympathetic nervous system to survive physical threats. However, managing acute hemodynamic reactivity to mental stress remains an important target in the prevention and treatment of cardiovascular disease.

2.3.3 Lower Body Negative Pressure

Homo Sapiens being upright bipeds with their brains located above their heart have a unique gravitational problem. A majority of our blood volume (70%) is located below the level of the heart (Rowell, 1993). Gravity and the location of the heart create the problem of venous return in upright humans. Fortunately, we have many solutions to the problem of being a terrestrial biped. A change in posture stimulates the otolithic organs initiating the vestibulosympathetic reflex. The vestibulosympathetic reflex can evoke sympathetic nerve firing to increase vascular resistance before blood pressure changes are detected by the baroreflex (Yates, Bolton, & Macefield, 2014). The respiratory pump creates negative intrathoracic pressure that assists in venous return. Meanwhile, large leg muscles contract and move blood from the veins in the legs back towards the heart, known as the muscle pump (Rowell, 1993). The muscle pump in the legs is effective due to one-way valves in the veins. The one-way valves in the veins prevent the backflow

of blood after muscle contraction displaces volume towards the heart. Additionally, rapid and precise control of the heart and adjustments in arterial vascular resistance are constantly fine-tuning perfusion pressure.

Lower body negative pressure (LBNP) induces a shift in blood volume from the central circulation of the upper body to the peripheral veins of the lower body. When performed in the supine position LBNP does not stimulate the otolith organs and renders the muscle pump ineffective as no significant leg contractions occur. At high pressures, LBNP results in central hypovolemia which unloads the arterial and cardiopulmonary baroreflexes. The result of LBNP induced hypovolemia is dramatic cardiovascular and neurovascular compensation to maintain arterial pressure and cerebral perfusion (W. H. Cooke, K. L. Ryan, & V. A. Convertino, 2004). Without the assistance of the muscle pump for venous return the maintenance of perfusion pressure falls to the sympathetic nervous system. Sympathetic activation maintains perfusion pressure by facilitating the creation of peripheral resistance and enhancing cardiac output. The central hypovolemia induced by LBNP is immediately reversed upon release of pressure. This feature of LBNP makes it a valuable non-invasive method for investigating sympathetic compensation to hypovolemia and orthostatic stress. LBNP is a particularly useful tool for investigating physiological responses to simulated hemorrhage (W. H. Cooke et al., 2004).

2.4 The Influence of Energy Intake on Autonomic Balance

The sympathetic nervous system has a fundamental role in energy expenditure, weight stabilization, and heat production in mammals. In rats,

short-term overfeeding increases norepinephrine turnover at the heart, indicating activation of the sympathetic nervous system (Landsberg & Young, 1978). Dogs also exhibit significant increases in norepinephrine after 1 week of being overfed a high fat diet (Rocchini, Moorehead, DeRemer, & Bondie, 1989). In humans, norepinephrine turnover in plasma increases significantly with elevated energy intake (O'Dea, Esler, Leonard, Stockigt, & Nestel, 1982). These studies demonstrate a clear evolutionarily conserved pattern in mammals, that acute overfeeding induces sympathetic activation. Landberg postulated that overeating stimulates the sympathetic nervous system in order to initiate thermogenesis and stabilize body weight (Landsberg, 1986) Unfortunately, the increase in sympathetic activity influences the vasculature and thus increases blood pressure. These studies laid the foundation for postulating that human obesity is characterized by sympathetic activation and increases the risk for hypertension. Increased sympathetic activity has been confirmed by measuring significantly elevated renal norepinephrine spillover and muscle sympathetic nerve activity in obese individuals (Grassi et al., 1995; Vaz et al., 1997). However, even without overeating energy intake induces sympathetic activation. Sections 2.4.1 and 2.5.2 will explore how energy intake influences autonomic balance.

2.4.1 Cardiovascular Responses to Food Ingestion

The consumption of food begins a cascade of physiological processes essential for the maintenance of life. Almost every major biological system responds to the ingestion of food. Macronutrient composition (% protein, % fats, % carbohydrate) of a meal is a contributing factor to the physiological responses to food consumption, with carbohydrates having the most influence. This section will highlight literature in which carbohydrates consumed during a meal were adequate so that glucose production was not required liver. Due to the fact that if a meal is deficient in carbohydrates, insulin is still released at a greater rate than basal, causing the liver to initiate

gluconeogenesis to maintain blood glucose concentrations (Cahill Jr, 1978). Essentially, when meals are consumed with low-carbohydrates, the liver produces glucose as if no meal had been consumed.

Following a meal there is an increase in energy expenditure, commonly referred to as the thermogenic effect of food. (K. Acheson, Jéquier, & Wahren, 1983; K. J. Acheson, Ravussin, Wahren, & Jéquier, 1984). This increase in energy expenditure is accompanied by an increase in sympathetic nervous system activity. Increased sympathetic nervous system activity has been demonstrated by postprandial rises in both systemic and local (adipose tissue) norepinephrine spillover (Patel, Eisenhofer, Coppack, & Miles, 1999), and increases in muscle sympathetic nerve activity (Berne, Fagius, & Niklasson, 1989; C. N. Young, Deo, Chaudhary, Thyfault, & Fadel, 2010). This increase in MSNA can last up to 2 hours post ingestion (C. N. Young et al., 2010). Meal ingestion will also cause a rise in insulin, which facilitates an increase in arterial baroreflex gain of MSNA (C. N. Young et al., 2010). Spontaneous cardiac baroreflex sensitivity is not affected by meal ingestion (C. N. Young et al., 2010). After a meal, splanchnic blood flow and cardiac output will increase (Høost et al., 1996). The increase in cardiac output compensates for the postprandial fall in total peripheral resistance resulting in no, or very little decrease in blood pressure (Sidery, Macdonald, Cowley, & Fullwood, 1991). In general, after a meal local vasodilation occurs in splanchnic areas, adipose tissue, and the heart but not in other areas such as the limbs, skeletal muscle, and the kidney (van Baak, 2008). The sympathetic response to food ingestion is essential for the maintenance of arterial pressure, as cardiac output is shunted to visceral areas. An example of the importance of the sympathetic response to food ingestion can be seen in individuals with autonomic dysfunction and in the elderly. After a meal, elderly individuals and those with autonomic dysfunction can experience post-prandial hypotension, resulting in a profound decrease in both systolic and

diastolic blood pressure (Jansen & Lipsitz, 1995; Robertson, Wade, & Robertson, 1981). Post-prandial hypotension can result in dizziness, syncope, and falls (Aronow & Ahn, 1997), highlighting the importance of sympathetically mediated cardiovascular responses to food ingestion.

2.4.2 Hormonal Responses to Food Ingestion

Postprandial hormonal responses are important for the initiation of thermogenesis, satiety, and maintenance of blood glucose concentrations. BMI and sex can influence some key baseline circulating hormones and their response to meal consumption. Obese subjects have greater increases in insulin and glucose when compared to normal weight subjects (Carroll, Kaiser, Franks, Deere, & Caffrey, 2007). Circulating levels of ghrelin are also lower in obese individuals. Obese individuals experience little to no change in ghrelin levels upon meal consumption, while lean subjects exhibit significant declines in circulating ghrelin after a meal (English, Ghatei, Malik, Bloom, & Wilding, 2002). Stress hormones such as cortisol (Stimson et al., 2014) and norepinephrine increase after a meal (Astrup, Simonsen, Bulow, Madsen, & Christensen, 1989). The importance of norepinephrine for initiating postprandial increases in energy expenditure and glucose uptake was demonstrated in a study utilizing β-adrenoreceptor blockade. After the blockade, postprandial increases in energy expenditure and glucose uptake were blunted (Astrup et al., 1989). Circulating epinephrine levels exhibited a significant decrease immediately after a meal (Astrup et al., 1989; Penev, Spiegel, Marcinkowski, & Van Cauter, 2005). There are sex specific differences in the hormonal responses to food ingestion. Men have greater baseline and postprandial glucagon and a slightly greater decline in leptin after a meal compared to women (Carroll et al., 2007). In men, meal consumption also temporarily reduces serum testosterone (Habito & Ball, 2001). The influence of meal consumption on estradiol in women has not been studied. However, estradiol replacement therapy improves postprandial

lipid metabolism and endogenous estrogens attenuate postprandial lipemia (Westerveld, 1998). The cardioprotective benefits of estrogen could partially be due to estrogen's beneficial influence on lipid metabolism.

2.5 The Influence of Fasting on Autonomic Balance

In the 1970's Lewis Landsberg and John Young set out to investigate how acute fasting influenced the sympathetic nervous system in rats. Their initial hypothesis was that fasting would increase sympathetic activation as fasting initiates mobilization of fat stores and was thought to be "stressful," overall. However, in an elegant study they found that after 48 hours of food deprivation there was a marked suppression of sympathetic activity (James B Young & Landsberg, 1977). They measured sympathetic activation at the heart using the norepinephrine turnover technique. They found that fasting significantly reduced norepinephrine turnover at the heart indicating reduced sympathetic activity. This study was repeated in 1982 and they again found reduced norepinephrine turnover at the heart that was associated with decreased blood pressure in hypertensive rats (Einhorn, Young, & Landberg, 1982). Initially these findings were counterintuitive to the group because if sympathetic activity is suppressed how do fat stores get mobilized? In another series of studies Lewis and Landberg discovered that in the fasted state sympathetic activity at the heart is disassociated from sympathetic activity at the adrenal medulla. They reported that in fasted rats the adrenal medulla increases secretion of epinephrine and norepinephrine while cardiac sympathetic activity remains suppressed (J. B. Young, Rosa, & Landsberg, 1984). Landsberg and Lewis's work laid the foundation for our understanding of how fasting influences the autonomic nervous system. Nevertheless, translating this work to humans is difficult because a rat's metabolic rate is much higher. Rats heart rate is on average 4 fold faster than humans and their metabolic rate is ~6 fold faster than humans (Agoston, 2017). The

following sections will discuss how short-term fasting affects the autonomic nervous system and hormonal responses to energy deprivation.

2.5.1 Cardiovascular Responses to Short-term fasting

Short term fasting is defined as a period of complete caloric cessation lasting from 12 to 72 hours. When compared to food consumption, an overnight fast (~12 hours) enhances parasympathetic modulation of the heart as suggested by a study utilizing spectral analysis of heart rate variability. The overnight fasting study measured no change in heart rate, decreased LF/HF ratio and increased HF power in the fasting group (Kuwahara, Okita, Kouda, & Nakamura, 2011). A follow-up study by the same group repeated the overnight fasting study but in women during the follicular and luteal menstrual phases. The primary findings were that heart rate decreased during fasting in both phases, HF power increased during fasting during both phases and salivary cortisol was decreased during the fasting luteal phase (Ohara et al., 2015). However, a similar overnight fast vs. fed comparison done in an older sample population (~59±9 yrs; mean±SD) was unable to replicate the changes in HF power and even reported an increase in diastolic pressure (Rodrigues et al., 2019).

Few studies have utilized a 24-hour fasting time point in humans. Herbert et al., conducted a study in 20 healthy young women who fasted for 24 hours in a metabolic ward. Herbert's study in 20 women reported that fasting for 24-hours increased heart rate and cardiac output and decreased high-frequency normalized units of spectral power (Herbert et al., 2012). Mazurak et al., studied 16 healthy women and was unable to replicate the changes in heart rate or heart rate variability after 24-hours of fasting. Interestingly, Mazurak reported a numerical but not statistically significant increase in RRI and heart rate variability (SDNN, RMSSD, HFnu) at 24 hours fasted. However, after 48 hours of fasting Mazurak reported a decrease in SDNN, a time domain

indicator of heart rate variability. Marurak et al., included a tilt test and reported that after 24 & 48 hours of fasting participants exhibited greater vagal withdrawal when exposed to tilt. Vagal withdrawal was indicated by decreased RRI, SDNN, and LogHF power (Mazurak et al., 2013). Greater vagal withdrawal and increased heart rate responses to head-up tilt were also exhibited after a 72-hour fast (S. J. Brown, Bryant, Mündel, & Stannard, 2012). It should be noted that the studies conducted by Mazurak and Herbert were done exclusively in women and they did not control for menstrual cycle or for breathing during heart rate variability measurements.

A cornerstone study in understanding the influences of acute fasting in humans was conducted by Webber and Macdonald. Webber and Macdonald investigated the influence of fasting for 12, 36, and 72 hours on cardiovascular and hormonal changes in 29 (17F) individuals. The 36-hour and 72-hour timepoints were compared to the 12-hour fasting timepoint. The authors reported that fasting did not change blood pressure but increased heart rate at both the 36- and 72-hour timepoint. Forearm blood flow was significantly increased at the 36- and 72-hour time point and plasma epinephrine and norepinephrine increased at the 72 hours fasted (J. Webber & Macdonald, 1994). Webber and Macdonald repeated their fasting study and they again found that heart rate and forearm blood flow increased at 36 and 72 hours fasted compared to the 12 hour fasted timepoint (J Webber et al., 1995). It was suggested that forearm vasodilation found could potentially be caused by the significant increase in ketones (J Webber et al., 1995). However, there is an important methodological caveat to the studies conducted by Macdonald and Webber. Both studies compare the 36-hour and 72-hour fasting timepoint to an overnight fast of ~12-hours. At the beginning of this section, it was highlighted that a 12-hour fast may enhance parasympathetic activity at the heart (Kuwahara et al., 2011; Ohara et al., 2015). The studies by Macdonald and Webber report an increase in

sympathetic activity, measured via heart rate and blood catcholamines, as fasting reaches 36 and 72 hours. However, because they are comparing their time points to a 12 hour fast, how fasting influences autonomic activity at 12-24 hours post caloric cessation remains equivocal.

To date, only one study has directly measured sympathetic neural outflow in fasted participants. The study was conducted in 11 obese middle-aged women, before and after a 48 hour fast. Only 6 nerves were recorded, and they reported a slight but statistically significant increase in muscle sympathetic nerve activity (42.0±5.5 vs. 44.5±5.8 bursts/min) (Andersson, Wallin, Hedner, Ahlberg, & Andersson, 1988). Counterintuitively, the authors also reported a reduction in systolic and diastolic blood pressure in response to the 48 hour fast (Andersson et al., 1988).

In conclusion, short-term fasting influences autonomic balance and the cardiovascular system. While the influence of fasting on autonomic activity is equivocal at the early hours post caloric cessation (~12-24 hours) as fasting time extends a clear shift towards increased sympathetic activity occurs. This dissertation seeks to elucidate the influence of a 24 hour fast on autonomic activity.

2.5.2 Hormonal Responses to Short-term Fasting

Short-term fasting alters blood biomarkers and circulating hormone concentrations. A 24-hour water only fast that focused on blood biomarker expression reported that participants increased human growth hormone and circulating cholesterol levels in the fasted state (Horne et al., 2013). Additionally, participants in the fasted state exhibited increased red blood cell count and hemoglobin without loss in plasma volume (Horne et al., 2013). Leptin serum levels fall by ~50% after a 24 hour fast (Boden, Chen, Mozzoli, & Ryan, 1996). After 12-hours of fasting ghrelin levels increase ~31% (Ariyasu et al., 2001). However, upon the cessation of food intake ghrelin

secretions take on a diurnal pattern, increasing at habitual meal times (Natalucci, Riedl, Gleiss, Zidek, & Frisch, 2005). Ghrelin also tends to be higher in females and has a strong inverse relationship to cortisol in the fasted state (Espelund et al., 2005). Serum insulin continuously decreases during the first 30 hours of fasting; meanwhile plasma glucagon continuously increases during 72 hours of fasting (Højlund et al., 2001). Both plasma epinephrine and norepinephrine concentrations fluctuate in a circadian rhythm during fasting. Studies have reported significant increases in plasma epinephrine and norepinephrine during fasting at the 24-, 48-, and 72-hour time points (Højlund et al., 2001; J. Webber & Macdonald, 1994). Plasma glucose steadily decreases until it plateaus after approximately 2 days of fasting (Haymond, Karl, Clarke, Pagliara, & Santiago, 1982; Højlund et al., 2001) Concurrently, ketones (β-hydroxybutyrate) progressively increase as fasting time continues (Haymond et al., 1982; J. Webber & Macdonald, 1994). Notably, women have higher concentrations of ketones during fasting when compared to men (Merimee et al., 1978).

3 EXPERIMENTAL APPROACH

3.1 Research Methods Common Across Aims 1-3

3.1.1 Subject Inclusion and Exclusion Criteria

Male and female participants between the ages of 18 and 40 years were invited to participate. Participants received a verbal briefing from the Principal Investigator or Co-Investigator, and a written description of all procedures and risks associated with the experiment was provided. Voluntary written informed consent to participate in the study was obtained. Because of the potential effect on vascular volume and autonomic control, participants were asked to refrain from exercise and stimulants such as caffeine, cold medications that might alter autonomic function (e.g., those containing diphenhydramine), and alcohol 24 hours prior to autonomic testing. Because of the potential influence of ovarian hormones on autonomic function (Carter et al., 2013; Minson et al., 2000), all female participants were tested in the early follicular phase of their menstrual cycles (days 3 to 8). Participants visited the laboratory for a familiarization session before the first day of experimentation, during familiarization all procedures and equipment were explained to them and they had the opportunity to ask questions of the investigators. Participants read and signed an informed consent document that was approved by the Committee for the Protection of Human Subjects in Research at Michigan Technological University.

All Participants filled out an information sheet prior to being officially enrolled in the study (Appendix A.1). This information sheet was used to generate an alphanumerical code (e.g. AA001) that was assigned to that participant for the duration of the study. Thus, all electronic data files are unidentifiable in the unlikely event of an electronic security breach. Paper files containing the participant's information are treated with strict confidentiality.

Participant Inclusion:

- Individuals between the ages of 18-40 years old
- All women tested in early follicular phase
- Individuals able to not eat for 24 hours

Participant Exclusion:

- Individuals who have been diagnosed with diabetes
- Individuals who have a history of blood clots
- Individuals with a history of hyperthyroidism
- Women currently taking oral contraceptives, are pregnant or trying to become pregnant
- Individuals with respiratory illnesses (e.g. asthma, chronic obstructive pulmonary disease, reactive airways disease, etc.)
- Hypertensive (Systolic ≥130 mmHg and/or diastolic ≥80 mmHg)
- Individuals with a history of tobacco or vaporized nicotine use

3.1.2 Measurements

- **Actigraphy**: Actigraphy was used to determine sleep/wake patterns and to confirm compliance to requirements. Subjects will continuously wear a wrist actiwatch (Respironics, Murrysville, PA) to monitor 24-hour activity levels (1 min epoch) on the days of monitoring. This is a safe, noninvasive procedure that poses no risk to the subjects.

- **Ambulatory Blood Pressure Monitor:** The ABPM is a lightweight battery powered device that measures blood pressure using an arm cuff 3 times per hour during the day time and 2 times per hour during the programmed sleep time.

- **Electrocardiogram**: 3-lead ECG for identification of R-waves for time- and frequency domain analysis of heart rate variability.

- **Finger Photoplethysmography**: A finger cuff housing an infrared sensor was placed on the middle finger and equilibrated to brachial arterial pressure with a servo motor (Finometer, Finapres Medical Systems, Arnhem, Netherlands) to record beat-by-beat arterial pressures from the finger.

- **Muscle Sympathetic Nerve Activity:** Muscle sympathetic nerve activity (MSNA) was measured directly with a Nerve Traffic Analyzer (Model 662C-2, University of Iowa Bioengineering, Iowa City, IA). To accomplish this, multifiber efferent sympathetic nerve traffic from peroneal nerve muscle fascicles at the popliteal fossa was recorded with tungsten microelectrodes (Frederick Haer and Co., Bowdoin, ME). The course of the nerve was mapped by stimulating the nerve through the skin with a pencil shaped electrode (10 - 50 v; 0.1 ms duration). Once the nerve was located, two sterile wire electrodes (diameter approximately 0.2 mm) were introduced through the skin at a depth of approximately 0.5 – 1 cm; one electrode served as the ground, and the other as the recording electrode inserted directly into the nerve. Both electrodes were connected to a differential preamplifier and then to an amplifier (total gain of 90,000), where the nerve signal was band-pass filtered (700-2000 Hz) and integrated (time constant 0.1 s) to obtain mean voltage neurograms. The recording electrode was manipulated into the region of the nerve until a characteristic “bursting” sound was detected. At this point minute adjustments were made to the electrode position until adequate sympathetic recordings were observed and maintained. Satisfactory

recordings of MSNA were defined by spontaneous, pulse-synchronous bursts that do not change during tactile or auditory stimulation.

- **Limb Plethysmography**. Limb blood flow was measured from the forearm using venous occlusion plethysmography (D.E. Hokanson, Bellevue, WA, USA). A mercury-in-silastic strain gauges was placed around the maximal circumference of the forearm. A wrist cuff was inflated to 220 mmHg to arrest circulation to the hand. An arm cuff was inflated (~70mmHg) and deflated (0 mmHg) in 7-8 sec intervals (15 sec/cycle). This technique allows for occlusion of venous blood flow, but still allows arterial blood flow. A strain gauges was used to measure diameter changes during these inflation/deflation cycles. Vascular resistance was calculated as mean arterial pressure divided by limb blood flow, and vascular conductance was calculated as the reciprocal of vascular resistance. This technique is a time-honored, safe, and noninvasive method for estimating limb blood flow (M. J. Joyner, Dietz, & Shepherd, 2001).

- **Pneumobelt**: A strain gauge was secured around the lower ribcage to record respiratory rate.

3.1.2.1 Integrated Data Analysis

In addition to our primary measurements of interest, a series of secondary integrated analysis will be performed using two or more signals

- **Spontaneous Cardiovagal Baroreflex Sensitivity:** Spontaneous cardiovagal BRS was determined by beat-to-beat changes in R-R interval and systolic pressure using the sequence method (Blaber, Yamamoto, & Hughson, 1995). Within the time domain baroreflex was assessed by

identifying sequences of three or more consecutively increasing systolic pressures (SAP) that correspond to 3 or more consecutively lengthening R-R intervals (up-up sequences, vagal activation). Additionally, systolic pressures that exhibit 3 or more decreases and 3 or more consecutive shortenings of R-R intervals was identified (down-down sequences, vagal inhibition). Systolic pressures that changes by at least 1 mmHg per beat and R-R- intervals that change by at least 4 ms was identified as a sequence. Linear regression was used to determine the slope of the linear relationships between R-R intervals and SAP for each sequence.

- **Dynamic Baroreflex Sensitivity (Valsalva Maneuver):** The Valsalva maneuver provokes reproducible changes in arterial pressure which triggers autonomic responses to expiratory strain. Phases of the Valsalva strain were determined by the arterial pressure waveform. The beginning of phase 2 was defined as the highest systolic pressure reached before arterial pressure begins to fall. The end of phase 2 was defined as the first systolic pressure that increases after the falling period. Phase IV was identified by the first pressure value that increases after release from the strain and continues until the first noticeable drop after overshoot. Cardiovagal BRS was determined by calculating the slope between SAP and R-R interval during phase II and IV of strain (Yamazaki et al., 2003).

3.1.2.2 Identification of Fasting Compliance

Fasting for 24 hours can be difficult. To assure fasting compliance, and also to have a means to quantify the effect of the fast as a stimulus we measured blood glucose, blood ketones, and lipids. The lipid profile quantified total cholesterol, high-density lipoprotein, low-denisity lipoproteins, triglycerides, and glucose using an Alera Cholestech LDX analyzer. Blood ketones were measured using a Precision Xtra ketone monitor.

Measurements were taken before each autonomic test. Ketones are produced 8 to 12 hours after caloric cessation to levels around 0.2 to 0.5 mM which is maintained through 24 hours (Cahill Jr, 1978). Fasting for 24-hours decreases glucose by ~8 mg/dL, and triglyerides ~50 mg/dL while increasing total cholesterol by ~ 9 mg/dL (Horne et al., 2013). At least one of these criteria was met for a fast to be considered successful.

3.1.2.3 Standardized Meal

A standardized lunch (Appendix A.2 & A.3) was provided as the last meal consumed before the fed and fasted experimental protocols. Resting metabolic rate calculations were estimated using the Mifflin-St. Jeor equation (Frankenfield, Roth-Yousey, & Compher, 2005).

- Male: RMR = 9.99 x weight(kg) + 6.25 x height(cm) – 4.92 x age + 5
- Female: RMR= 9.99 x weight(kg) + 6.25 x height (cm) – 4.92 x age – 161

The RMR estimate was then multiplied by a Harris-Benedict physical activity factor to estimate total daily energy needs to maintain the participants given weight. Caloric content of the meal was estimated to be 1/3 of total caloric intake needed to maintain weight.

- Calories needed = RMR X Activity factor
- Activity Factor = Sedentary: 1.2; Mild: 1.375; Moderate: 1.55; Heavy: 1.725; Extreme: 1.9

3.1.2.4 Assessment of Hydration Status

Urine was collected before each autonomic testing session. To assess hydration status urine specific gravity was obtained using a PALS-10S urine refractometer (Atago, Tokyo, Japan).

3.2 Specific Aims, Experimental Protocol, and Power Analysis

3.2.1 Specific Aims

Specific Aim 1: Determine the influence of a 24-hour fast on hemodynamics, peripheral neural activity, and cardiovascular control at rest.

Hypothesis Aim 1: Acute fasting will reduce blood pressure, muscle sympathetic nerve activity, and heart rate. Fasting will also enhance parasympathetic modulation of the heart and enhance baroreflex sensitivity

Specific Aim 2: Determine the influence of a 24-hour fast on neural and cardiovascular responses to mental stress.

Hypothesis Aim 2: Acute fasting will reduce cardiovascular and neural reactivity to mental stress

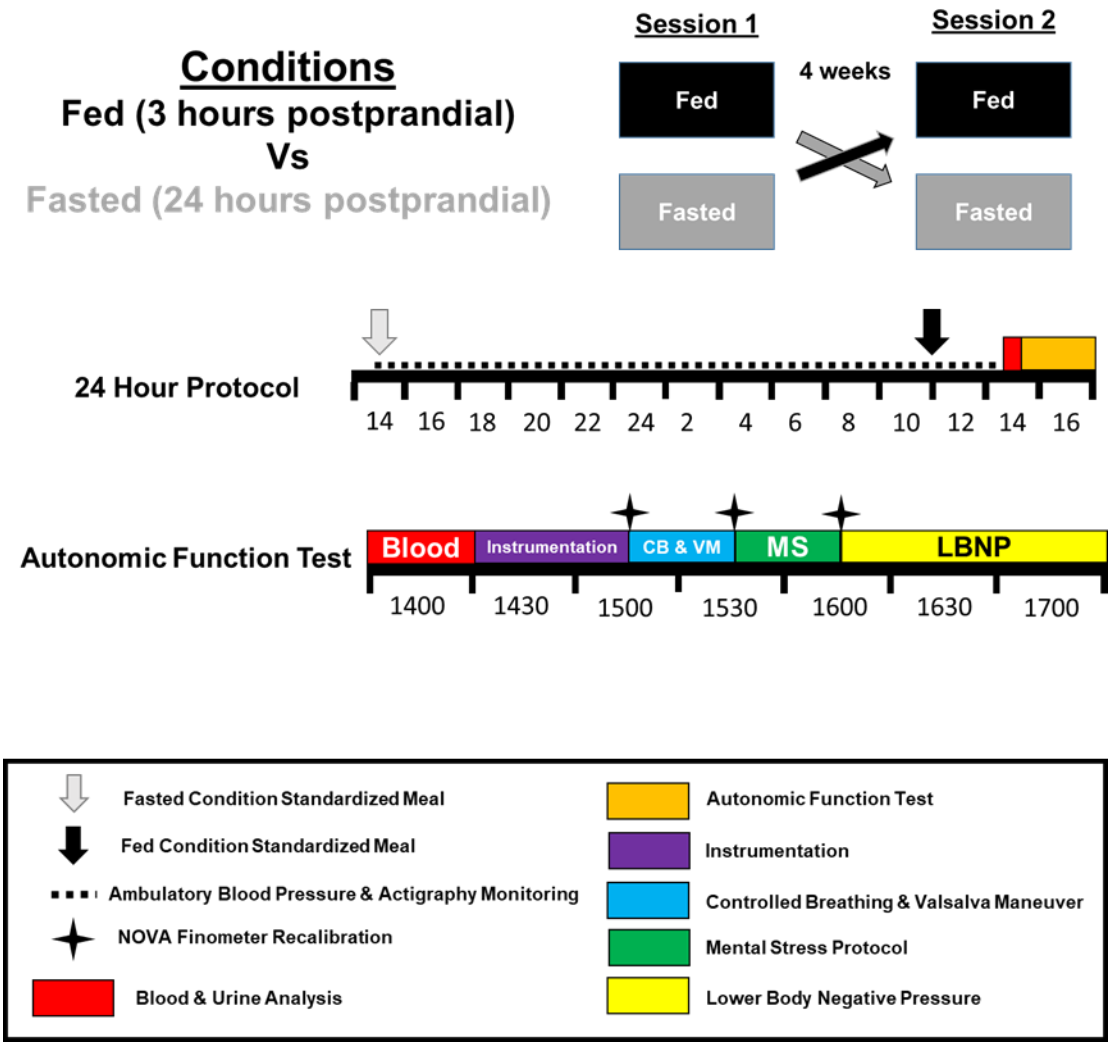
Specific Aim 3: Determine the influence of a 24-hour fast on neural and cardiovascular responses to a severe orthostatic stress.

Hypothesis Aim 3: Acute fasting will reduce tolerance to a severe orthostatic stress by reducing cardiovascular and neural reactivity to lower body negative pressure

3.2.2 Experimental Design

This study is a randomized controlled crossover design with repeated measures. The two conditions are the fed condition which is defined as 3 hours postprandial and the fasted condition which is defined as 24 hours post prandial. The conditions were randomized, and participants were informed 3 days before scheduled autonomic testing which condition, they were assigned. For example, if they were assigned to the fed condition for the first autonomic test 4 weeks later, they would be fasted for the autonomic function test. Participants would report to the lab 24 hours before their scheduled autonomic test to be fitted with an actigraphy watch and ambulatory blood pressure cuff. The experimental protocol is summarized in figure 3.

Figure 3: Experimental Design



3.2.3 Power Analysis

We used a two-tailed paired samples t-test to estimate power based on data from a recent publication that specifically investigated acute fasting time in humans using heart rate as the primary variable of interest (Ohara et al., 2015) Using the heart rate mean and SD (Fed = 65±5 & Fasted 61±5) from Ohara et al., we calculated an effect size of 0.8. With an $\alpha = 0.05$ and a $\beta = .95$ we estimated that a sample size of 23 participants would give us sufficient power. We tested a total of 25 participants.

4 RESULTS AND CONTROLS COMMON ACROSS AIM 1-3

4.1 Participant Characteristics

Twenty-five heathy young adults (14 men and 11 women) were recruited to participate in this study. All participants had no history of autonomic dysfunction, hypertension, respiratory disease, diabetes, or nicotine usage, and were not taking any prescription medications. Participant eligibility was evaluated through study orientation where they were informed of the purpose of the study, what would be asked of them, and the potential risks. Informed consent was obtained during the orientation visit.

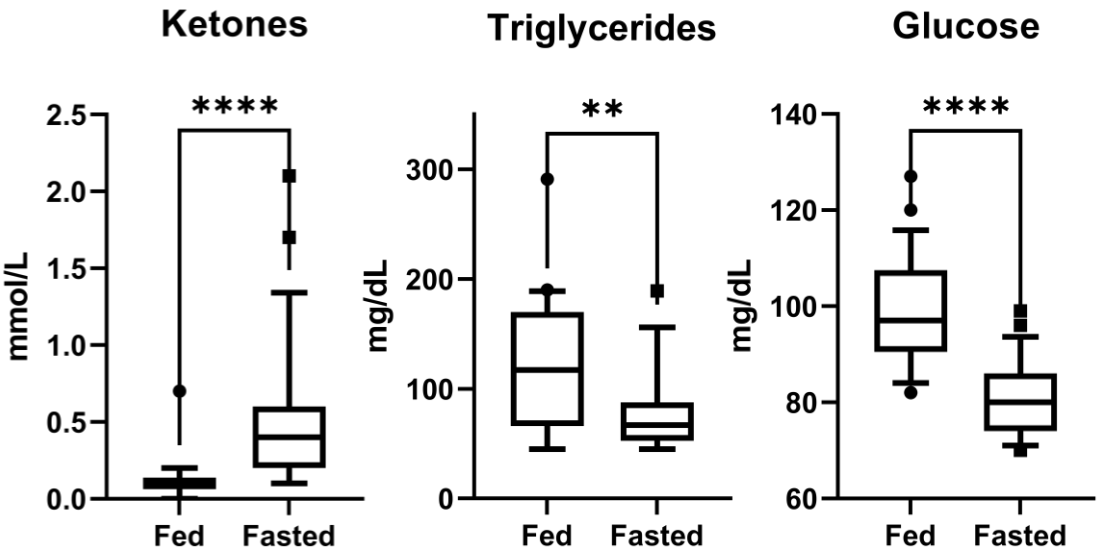
Table 1: Participant Characteristics

Baseline Participant Characteristics at Orientation	
N	25 (11F)
Age, yrs	23±3
Height, cm	176±16
Weight, kg	76±16
BMI, kg/m^2	24±4
Systolic, mmHg	110±12
Diastolic, mmHg	67±6
Heart Rate, BPM	71±14
Values are mean ± SD	

4.2 Blood Biomarkers

Blood biomarkers were collected to ensure fasting compliance and to ensure that a 24-hour fast was a sufficient stimulus to intiate ketone production. Participants were weight stable and similarly hydrated between conditions. Blood triglycerides and glucose were significantly decreased in the fasted condition compared to the fed condition. Blood ketones were significantly increased in the fasted condition compared to the fed condition. The magnitude of change in glucose, triglycerides, and ketones between the fed and fasted condition are similar to previously published literature investigating blood biomarkers after a 24 hour fast (Cahill Jr, 1978; Horne et al., 2013). Specific gravity was collected in 23 participants because 2 participants could not produce a urine sample at the start of the experimental session.

Figure 4: Pertinent Blood Biomarkers



Blood biomarkers represented as boxplots. The line in the boxplots represents the median and the box represents the interquartile range (IQR; the difference between the 25th and 75th percentile. The Whiskers extend from the upper and lower edge of the box to the 10th and 90th percentile values. *p<0.05; **p<0.01; ****p<0.0001

Table 2: Blood Biomarkers

Variable	Fed	Fasted	MD	t-test p-value	Wilcoxon p-value
Weight, kg	76.3±15.4	75.6±15.2	0.7±0.6	.271	
Urine Specific Gravity, A.U. (N=23)	1.015±.002	1.011±.002	.004±.002	.113	
Total Cholesterol, mg/dL	172.4±35.2	172.7±29.1	0.3±4	.937	
Low-Density Lipoprotein, mg/dL	95.6±29.9	105.5±21.5	5.8±5	.253	
High-Density Lipoprotein, mg/dL	58.8±18.9	56.2±20.3	2.4±1.6	.151	
Triglycerides, mg/dL	120.8±61.6	77.2±37.9**			.003
Glucose, mg/dL	98.8±11.7	80.9±8.0**	18±2.6	<.001	
Ketones, mmol/L (β-hydroxybutyrate)	.128±.13	.536±.49**			<.001

Values are mean ± SD. Paired t-tests were used to compare variables between the fed and fasted condition. Wilcoxon matched-pairs signed rank test used to compare variables not normally distributed. MD = mean difference between the fed and fasted condition Two-tailed p-values are displayed. (*) = $p \leq 0.05$ (**) = $p \leq 0.01$

4.3 Actigraphy

Participants wore Actiwatch Spectrum PRO watches for the 24 hours leading up to the autonomic testing session. Inclusion of actigraphy was primarily to ensure that participants were able to sleep while fasting and to

properly calibrate the ambulatory blood pressure data points to the wake and sleep times. Actigraphy was successfully collected in 22 participants. 3 Participants had unsuccessful readings during one of the conditions. 2 Participants removed the watch and forgot to replace it in a timely manner and 1 participant experienced equipment failure. Participants in the fasted condition experienced more awakenings, spent more time in bed and got an extra hour of sleep on average when compared to the fed condition. However, minutes active, wake after sleep onset (WASO) and sleep efficiency were not different between the conditions.

Table 3: Actigraphy Data

Variable	Fed	Fasted	MD	t-test p-value
Time in Bed, hours	7.7±1.7	8.9±1.9**	1.3±0.4	.005
Total Sleep Time, hours	6.6±1.5	7.6±1.6*	1.1±0.4	.011
Sleep Efficiency, %	84.7±6.7	85.4±4.4	0.7±1.3	.610
Sleep Latency, min	15.3±18.1	22.0±17.8	8.6±5.6	.140
Awakenings, #	30±12.0	35.8±14.3*	5.5±2.5	.037
WASO, #	35.2±15.8	37.9±17.9	1.7±3.7	.644
Activity Minutes	612.3±234.6	588.19±361.7	19.6±60.7	.750

Values are mean ± SD. Paired t-tests were used to compare variables between the fed and fasted condition. MD = mean difference between the fed and fasted condition Two-tailed p-values are displayed. (*) = $p \leq 0.05$ (**) = $p \leq 0.01$

5 THE INFLUENCE OF AN ACUTE FAST ON 24-HOUR AMBULUATORY BLOOD PRESSURE

5.1 Introduction

Cardiovascular disease remains the leading cause of death in the United States. Hypertension (SAP ≥130 mmHg or DAP ≥80 mmHg) is associated with an increased risk of ischemic heart disease and cardiac failure (Wilson, 1997). Hypertension is a treatable risk factor, that when controlled can mitigate the risk cardiovascular disease. Physicians have a variety of pharmaceuticals at their disposal that do assist in the management of hypertension and reduce the risk of cardiovascular disease (Glynn, Murphy, Smith, Schroeder, & Fahey, 2010). However, observational studies have reported that almost half of hypertensive patients taking medications do not meet their target blood pressure (Reid et al., 2008). Therefore, despite pharmaceutical treatment hypertension remains a major public health concern and risk for cardiovascular disease. It is important to understand which components of blood pressure influence risk.

The Framingham Heart Study contributed significantly to our understanding of the relationship between blood pressure and heart disease. Specifically, the Framingham Heart study was able to distinguish the contribution of systolic versus diastolic blood pressure to risk of coronary heart disease (CHD). The study reported that in those under the age of 45 diastolic was the dominant predictor of CHD risk. However, as people age systolic blood pressure becomes the more reliable predictor (Kannel, Gordon, & Schwartz, 1971). Later, pulse pressure was also identified as an important component of risk of CHD (Franklin, Khan, Wong, Larson, & Levy, 1999). Pulse pressure is the difference between systolic pressure and diastolic and correlates with arterial stiffness (Mitchell et al., 2010).

Lifestyle interventions such as diet and exercise are well known to assist in reducing blood pressure (Bruno, Amaradio, Pricoco, Marino, & Bruno, 2018) Recently, it has been reported that chronic intermittent fasting can potentially lower both systolic and diastolic blood pressure (Malinowski et al., 2019). However, how fasting influences blood pressure acutely is unknown. Therefore, the purpose of this study was to determine how fasting for 24 hours influences 24-hour ambulatory blood pressure. Specifically, we will test the hypothesis that acute fasting lowers systolic, diastolic, and pulse pressure compared to normal eating.

5.2 Methods

5.2.1 Data Collection

24-hour ambulatory blood pressure monitor (ABPM) data were collected twice once in the fed state (3-hours post prandial) and once in the fasted state (24-hours postprandial) separated by 4 weeks. Sampling frequency was set to collect brachial blood pressures every 20 min between 0700 and 2200 hours and every 30 min from 2200 to 0700 hours. Participants were instructed to immobilize their arm upon cuff inflation and to only remove the cuff for showering. Participants wore the cuff for a continuous 24-hour period. Daytime and nighttime blood pressure values were defined by the actigraphy watch. Participant characteristics and actigraphy data can be found in chapter 4.

5.2.2 Data Analysis

To be included in this data set a minimum of 20 daytime measurements and a minimum of 7 nighttime measurements were needed for each condition. 20 participants were included in this data set. Two participants were eliminated for device failure and 3 participants were eliminated for not meeting the minimum requirement for successful readings for both conditions. Daytime and nighttime blood pressure values were adjusted to reflect

actigraphy wake time, sleep time, and sleep latency. Fed vs. fasted conditions were compared using a paired t-test. Normality was assessed using a Shapiro-Wilk test. If data were found to be not normally distributed a Wilcoxon matched pairs signed rank test was used to assess dependent variables. Two-tailed p-value <0.05 was considered statistically significant for all tests. The table below shows quality and quantity of ABPM readings per condition.

Table 4: Ambulatory Blood Pressure Data Quality

	Fed	**Fasted**
Reading Success, %	74±12	78±13
Average Wake Recordings, #	32±7	34±8
Average Sleep Recordings, #	13±3	15±5
Total Brachial Recordings, #	902	970

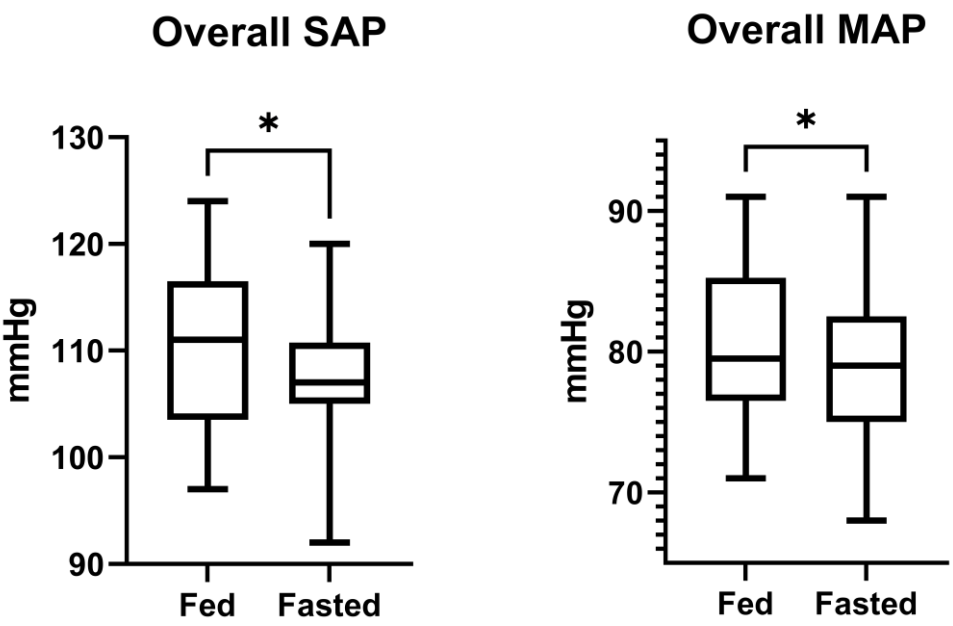
Data presented are mean ± SD

5.3 Results

5.3.1 Overall Blood Pressure

Fasting significantly reduced overall systolic and mean arterial pressure when compared to the fed condition. In total 902 brachial blood pressures were averaged from the fed condition and 970 brachial blood pressures were averaged from the fasted condition. Heart rate was also significantly reduced during the fasted state. However, heart rate was estimated from the blood pressure cuff and not an ECG. Overall pulse pressure and diastolic blood pressure were not statistically different between the two conditions but tended to decrease.

Figure 5: Overall Ambulatory Systolic and Mean Blood Pressure



Overall Systolic (SAP) and mean arterial pressure (MAP) represented as boxplots. The line in the boxplots represents the median and the box represents the interquartile range (IQR; the difference between the 25th and 75th percentile). The whiskers extend from the upper and lower edge of the box to the highest and lowest values. *p<0.05

Table 5: Overall Ambulatory Blood Pressure Data

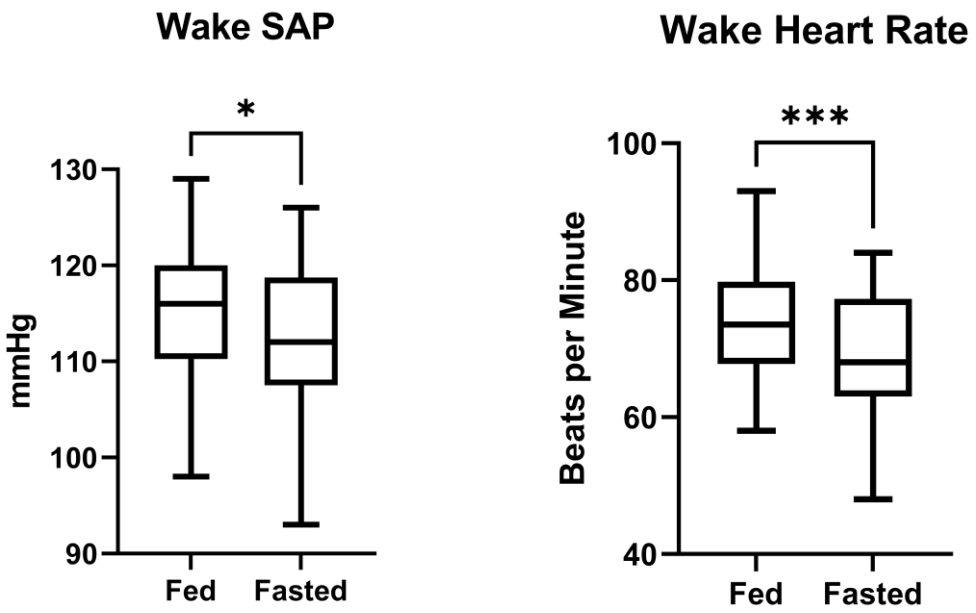
Variable	Fed	Fasted	MD	t-test p-value
Overall SAP	110±1.7	108±1.5*	2.2±1	.045
Overall DAP	64±1.3	63±1.3	1.6±1	.074
Overall MAP	81±1.2	78±1.2*	2.1±1	.039
Overall Pulse Pressure	47±1.4	46±1.2	1±0.5	.106
Overall Heart Rate	69±1.9	65±2.2**	3.9±1	.003

Values are mean ± SE. Paired t-tests were used to compare variables between the fed and fasted condition. MD = mean difference between the fed and fasted condition Two-tailed p-values are displayed. (*) = p ≤ 0.05 (**) = p ≤ 0.01

5.3.2 Wake Blood Pressure

Wake blood pressure readings were verified via actigraphy watch. Fasting significantly reduced wake systolic blood pressure and wake heart rate. Diastolic and mean arterial pressure were similar between conditions.

Figure 6: Wake Ambulatory Systolic and Heart Rate



Wake Systolic (SAP) and heart rate (MAP) represented as boxplots. The line in the boxplots represents the median and the box represents the interquartile range (IQR; the difference between the 25th and 75th percentile). The whiskers extend from the upper and lower edge of the box to the highest and lowest values. *p<0.05; ***p<.0001

Table 6: Wake Ambulatory Blood Pressure Data

Variable	Fed	Fasted	MD	t-test p-value	Wilcoxon p-value
Wake SAP	115±2.0	113±1.8*			.018
Wake DAP	69±1.5	68±1.6	1.5±1	.270	
Wake MAP	85±1.5	83±1.5*	1.7±1	.145	
Wake Pulse Pressure	46±1.3	43±2.1			.371
Wake Heart Rate	74±2.0	68±2.4**	5.4±1	<.001	

Values are mean ± SE. Paired t-tests were used to compare variables between the fed and fasted condition. Wilcoxin matched-pairs signed rank test was used to test variables not normally distributed. MD = mean difference between the fed and fasted condition Two-tailed p-values are displayed. (*) = $p \leq 0.05$ (**) = $p \leq 0.01$

5.3.3 Sleep Blood Pressure

Fasting did not affect nighttime blood pressure values or nighttime dipping patterns.

Table 7: Sleep Ambulatory Blood Pressure Data

Variable	Fed	Fasted	MD	p-value
Sleep SAP, mmHg	100±1.5	99±1.2	1±1.4	.550
SAP Dip, %	13±1.4	12±1.1	0.4±1.1	.737
Sleep DAP, mmHg	54±1.2	53±1.0	1±1.1	.346
DAP Dip, %	21±1.5	20±1.5	0.8±2	.704
Sleep MAP	72±1.1	70±1.0	2±1.2	.163
MAP Dip, %	15±1.3	16±1.2	0.7±1.6	.669
Sleep Pulse Pressure	46±1.2	45±0.9	0.3±1	.822
Sleep Heart Rate	59±1.9	57±2.3	2±1.4	.271

Values are mean ± SE. Paired t-tests were used to compare variables between the fed and fasted condition. MD = mean difference between the fed and fasted condition Two-tailed p-values are displayed. (*) = $p \leq 0.05$ (**) = $p \leq 0.01$

5.4 Discussion

In young healthy normotensive individuals, acute fasting reduces overall 24-hour ambulatory blood pressure and heart rate. Additionally, fasting tends to reduce wake pulse pressure. Blood pressure is a functional product of cardiac output and peripheral resistance. Heart rate was significantly reduced while fasting, suggesting that decreased cardiac output may potentially be causing the measured reduction in blood pressure. The mechanism behind this reduction in blood pressure warrants further exploration.

The reductions in arterial pressure we measured are mild and acute (Δ2 mmHg SAP, Δ3 mmHg MAP). However, reductions in arterial pressure associated with fasting are similar in magnitude to the 24-hour post exercise hypotension measured after a single bout of resistance exercise (Δ1.7 mmHg SAP) (Casonatto, Goessler, Cornelissen, Cardoso, & Polito, 2020). Fasting may be an alternative for those who still want the acute hypotensive effects of exercise but are unable to make it to the gym. In a majority of individuals seeking to lower their blood pressure, reducing systolic blood pressure is particularly difficult (Chobanian et al., 2003). Thus, it is important to recognize that acute fasting primarily reduces the systolic component of blood pressure. Controlling systolic blood pressure is the most crucial component when assessing risk of future cardiovascular disease (Kannel et al., 1971).

Chronic intermittent fasting regimens of 8 to 12 weeks have been reported to reduce blood pressure (Malinowski et al., 2019). Our study is the first to report the acute hypotensive effects of fasting over a 24-hour period. Recently, a study demonstrated that medically supervised fasting lasting 4 to 41 days (average 10 days) can significantly reduce blood pressure (Grundler, Mesnage, Michalsen, & Toledo, 2020). However, fasting for multiple days is difficult and can interfere with individuals' day-to-day lives. Crucially, normotensive individuals in the study only exhibited a blood pressure reduction of 3 mmHg systolic and 1.9 mmHg diastolic, suggesting that there may be a floor effect to reductions of blood pressure seen during fasting in normotensive individuals. However, a 24 hour fast does appear to be sufficient to elicit beneficial blood pressure reductions.

In conclusion, an acute 24-hour fast reduces blood pressure and heart rate in young, healthy, normotensive individuals. Fasting does not influence blood pressure dipping or sleep blood pressure. Acute fasting may be a

useful non-pharmacological intervention for those seeking to reduce their blood pressure.

6 THE INFLUENCE OF AN ACUTE FAST ON NEURAL AND CARDIOVASCULAR CONTROL AT REST

6.1 Introduction

The autonomic nervous system plays a key role in the regulation of blood pressure and is a primary contributor to cardiovascular homeostasis in humans. Chronic intermittent fasting has been reported to improve multiple indicators of cardiovascular health. Intermittent fasting reduces systemic inflammation, oxidative stress, blood pressure, and resting heart rate (de Cabo & Mattson, 2019). Additionally, routine periodic fasting (1 day per month) has been associated with lower prevalence of coronary artery disease (Horne et al., 2008). However, few studies have investigated how fasting influences the autonomic nervous system and thus the mechanisms behind the beneficial cardiovascular outcomes reported from intermittent fasting are unknown.

An overwhelming majority of studies investigating the influence of fasting on autonomic control have used spectral analysis of heart rate variability. Short 12 hour fasts increase vagal activity (Kuwahara et al., 2011; Ohara et al., 2015) and longer periods of food deprivation ≥24 hours decrease vagal modulation of the heart (S. J. Brown et al., 2012; Mazurak et al., 2013). The only study that has utilized muscle sympathetic nerve activity to date is from Andersson et al., who measured MSNA after a 48-hour fast. While the study reported a slight increase in sympathetic nerve activity, the sample population were obese women, aged 46-62, and MSNA was only recorded in 6 participants (Andersson et al., 1988). How a 24-hour fast influences peripheral neural activity in young, healthy individuals remains unknown. Multiple studies have demonstrated that 48-hours of fasting reduces arterial pressure in lean and obese populations (Andersson et al., 1988; Solianik, Sujeta, Terentjeviene, & Skurvydas, 2016). However, the mechanism behind the observed reductions in blood pressure are unknown.

Additionally, 48-hours of complete caloric cessation is not typical for intermittent fasting that typically utilize 18-24-hour caloric cessation time periods (Anton et al., 2018; de Cabo & Mattson, 2019; Varady & Hellerstein, 2007).

The purpose of this aim is to assess how 24-hours of fasting influences hemodynamics, peripheral efferent nerve traffic, and cardiovascular control at rest. To assess how fasting influences the arterial baroreflex, secondary analyses will be conducted on spontaneous cardiovagal baroreflex sensitivity. Additionally, dynamic baroreflex responses will be assessed by correlating changes in arterial pressure and R-R interval during the Valsalva maneuver.

6.2 Methods

6.2.1 Experimental Design

After blood and urine samples were collected participants laid supine on a laboratory table. The participants were then instrumented with an ECG, finger photoplethysmography, microneurography, venous occlusion plethysmography and a pneumobelt. After stable hemodynamic and microneurographic signals were established, participants were asked to breath in time to a computer display set at 15 breaths per minute (0.25 Hz) for 10 minutes (figure below). After the 10 minutes of controlled breathing, participants were asked to perform 3 Valsalva maneuvers. During each Valsalva maneuver participants forcefully exhaled to 40 mmHg for 15 seconds into a modified pressure manometer. Each of the 3 Valsalva maneuvers was separated by a 1-min recovery with normal uncontrolled breathing. A nose clip was used to seal the nose and a small leak was allowed in the manometer to keep the glottis open during each strain.

6.2.2 Data Analysis

Data were sampled at 500 Hz (WINDAQ, Dataq Instruments, Akron OH) and analyzed with specialized software (WINCPRS, Absolute Aliens, Turku Finland). R-waves measured from the ECG were automatically detected, visually inspected, and marked. Systolic and diastolic blood pressures were marked from the Finometer tracings. Muscle sympathetic nerve burst were automatically detected and manually verified based on their amplitude and a 1.3 second expected burst peak latency from the previous R-wave. Data were averaged from the last 8 minutes of the 10 min controlled breathing time.

Heart rate variability was assessed in the frequency domain from R-R interval spectral power. To obtain power spectrums, the last 8-minutes of controlled breathing were fast Fourier transformed with a Hanning window. The high frequency (0.15-0.40 Hz) and low frequency (0.04-0.15 Hz) components were then normalized to total power (ex. HF/Total power). Using the arterial pressure waveform as an input stroke volume was automatically estimated on a beat-to-beat basis using the pulse contour method (Jansen & Lipsitz, 1995). Forearm blood flow was measured using the limb occlusion plethysmography method described in section 3.1.1.2.

Spontaneous cardiovagal baroreflex was calculated using the method explained in section 3.1.1.3. To calculate dynamic baroreflex sensitivity to the Valsalva maneuver the slope method was utilized. Dynamic baroreflex sensitivity was determined by the linear relationship of SAP and RRI during the hypotensive phase II and hypertensive phase IV of the Valsalva maneuver. To be considered a valid sequence, correlation coefficients were set at >0.70, and only Valsalva maneuvers that demonstrated morphological and temporal consistency were included in the analysis. Additionally, a minimum reduction of 15 mmHg during phase II was required for inclusion.

6.2.3 Statistical Analysis

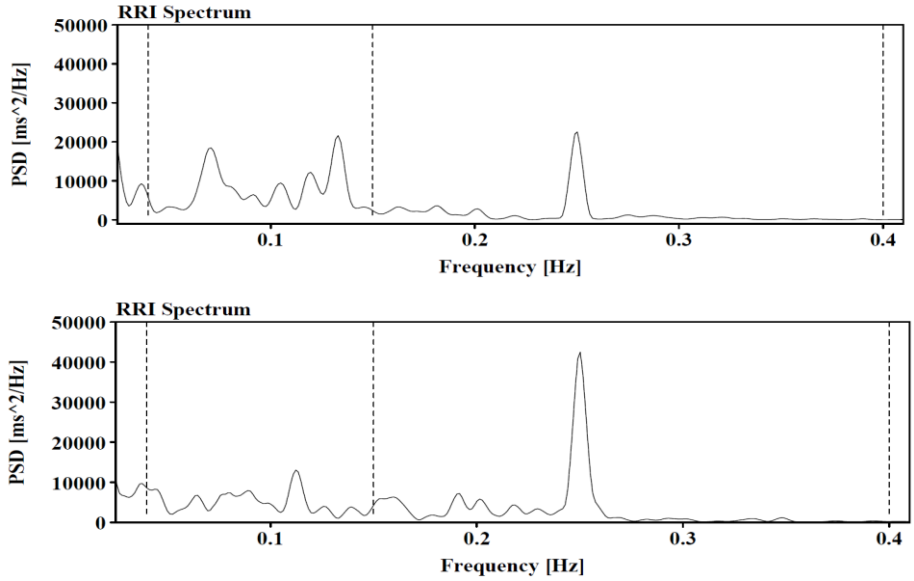
All data were analyzed statistically using the commercial software Sigmaplot 14.0 and Prism GraghPad. Dependent variables of interest were assessed using a paired t-test. Normality was assessed using a Shapiro-Wilk test. If the data failed the Shapiro-Wilk normality test a non-parametric Wilcoxon signed-rank test was used to assess the dependent variables.

6.3 Results

6.3.1 Influence of Fasting on Vagal Modulation and Hemodynamics

Fasting increased R-R interval and heart rate variability measured via spectral analysis. The increase in R-R interval paired with the increase in high frequency power suggests that fasting enhances cardiac vagal tone. A representative power spectral density is shown for a subject in the fed and fasted condition is displayed in figure 7. The fasted condition demonstrates a

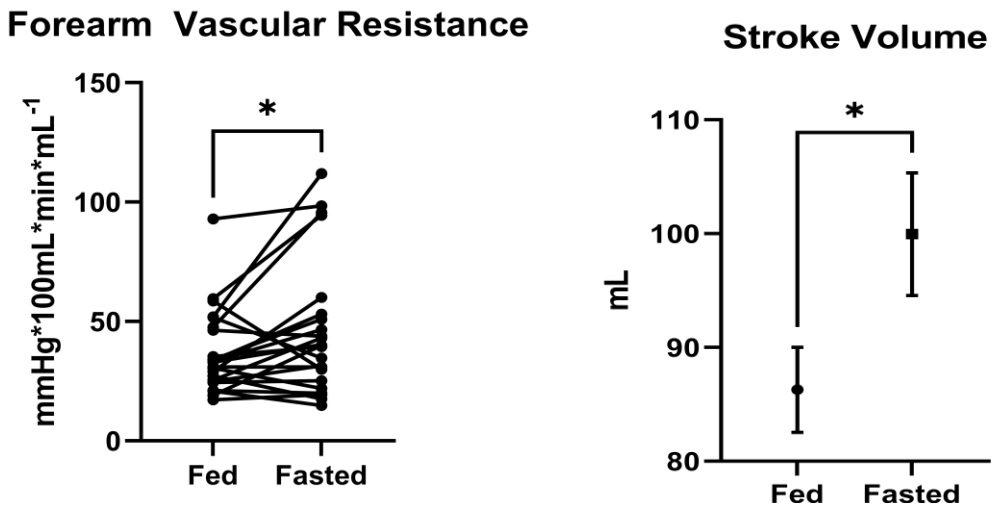
Figure 7: Spectral Power Fed vs. Fasted Representative Subject



Raw spectral power density for one subject during controlled breathing at 0.25 Hz in both the fed (top) and fasted (bottom) condition)

quiescence of spectral power in the low frequency range (0.04-0.15Hz) and an obvious increase in power in the high frequency range (0.15-0.40 Hz). Blood pressure was similar during controlled breathing between conditions. Stroke volume and forearm vascular resistance significantly increased in the fasted condition compared to the fed condition. Muscle sympathetic burst frequency and burst incidence did not change between the fed and fasted conditions (N=13; 3F) as shown in Table 8.

Figure 8: Forearm Vascular Resistance and Stroke Volume



Forearm Vascular Resistance (FVR) represented as individual data points and connecting lines. Stroke volume (MAP) represented as mean with standard error. *p<0.05

Table 8: Fed vs. Fasted Controlled Breathing Data

Variable	Fed	Fasted	MD	t-test p-value
R-R Interval, ms	992±30	1059±37*	67±32	.024
SAP, mmHg	107±2.5	107±2.2	0.1±2.5	.481
DAP, mmHg	57±1.9	58±2.0	0.3±1.8	.431
Burst Frequency, b/min	16±3.1	15±2.2	1.6±1.7	.180
Burst Incidence, b/100hb	23±4.4	26±3.8	0.4±2.2	.426
RRI HFnu, A.U.	54.5±2.6	61.8±3.2*	.07±.04	.022
RRI LFnu, A.U.	45.5±2.6	38.2±3.2*	.07±.04	.022
Stroke Volume, mL	88±4.0	100±5.4*	13.7±6	.017
Forearm Blood Flow, $ml*100ml^{-1}*min^{-1}$	2.4±.19	2.2±.22	0.2±0.2	.143
Forearm Vascular Resistance, $mmHg*100mL*min*mL^{-1}$	36.9±3.6	46.2±5.9*	9.4±4.3	.019

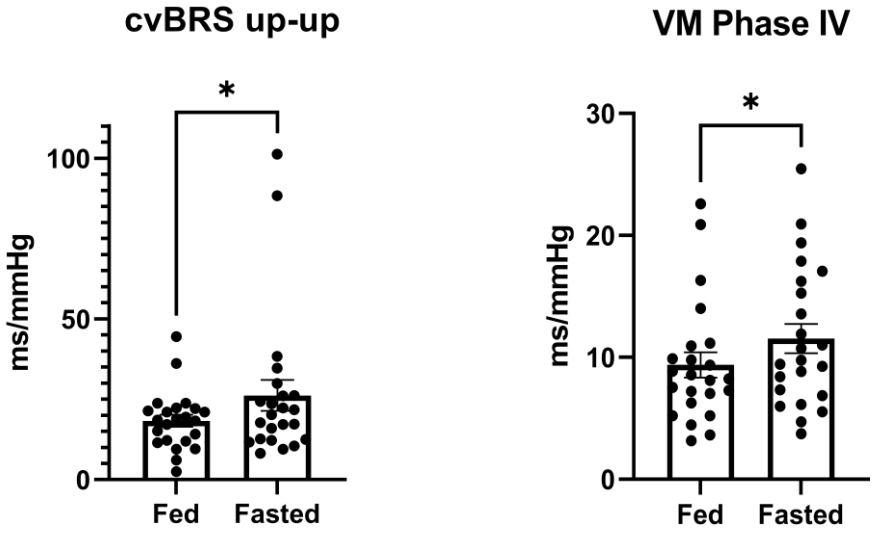
Values are mean ± SE. Paired t-tests were used to compare variables between the fed and fasted condition. MD = mean difference between the fed and fasted condition one-tailed p-values are displayed. (*) = $p \leq 0.05$ (**) = $p \leq 0.01$

6.3.2 Influence of Fasting on Spontaneous and Dynamic Cardiovagal Baroreflex Sensitivity

Spontaneous cardiovagal baroreflex sensitivity was enhanced in the fasted condition. Specifically, cvBRS up-up sequences were significantly increased and cvBRS down-down sequences exhibited a strong trend to

increase in the fasted condition. An example of the Valsalva maneuver phases is depicted in figure 8. Enhanced cardiovagal baroreflex sensitivity was also measured during Phase IV, or the hypertensive component of the Valsalva maneuver. Increases in RRI were also augmented during the Phase IV overshoot in the fasted condition compared to the fed condition. Twenty-two participants were included in the Valsalva analysis. Three participants were excluded because they had no SAP-RRI correlations above .70. Average SAP-RRI correlation coefficients for included participants were 0.88 for Phase II and 0.81 for Phase IV.

Figure 9: Influence of Fasting on Baroreflex Sensitivity



Cardiovagal Baroreflex sensitivity up-up (cvBRS up-up) represented as individual data points and bar graph (mean ± SE). Valsalva phase IV SAP-RRI (VM Phase IV) slope represented as individual data points and bar graph (mean ± SE) *p<0.05;

Figure 10: Representative Valsalva Response for One Subject

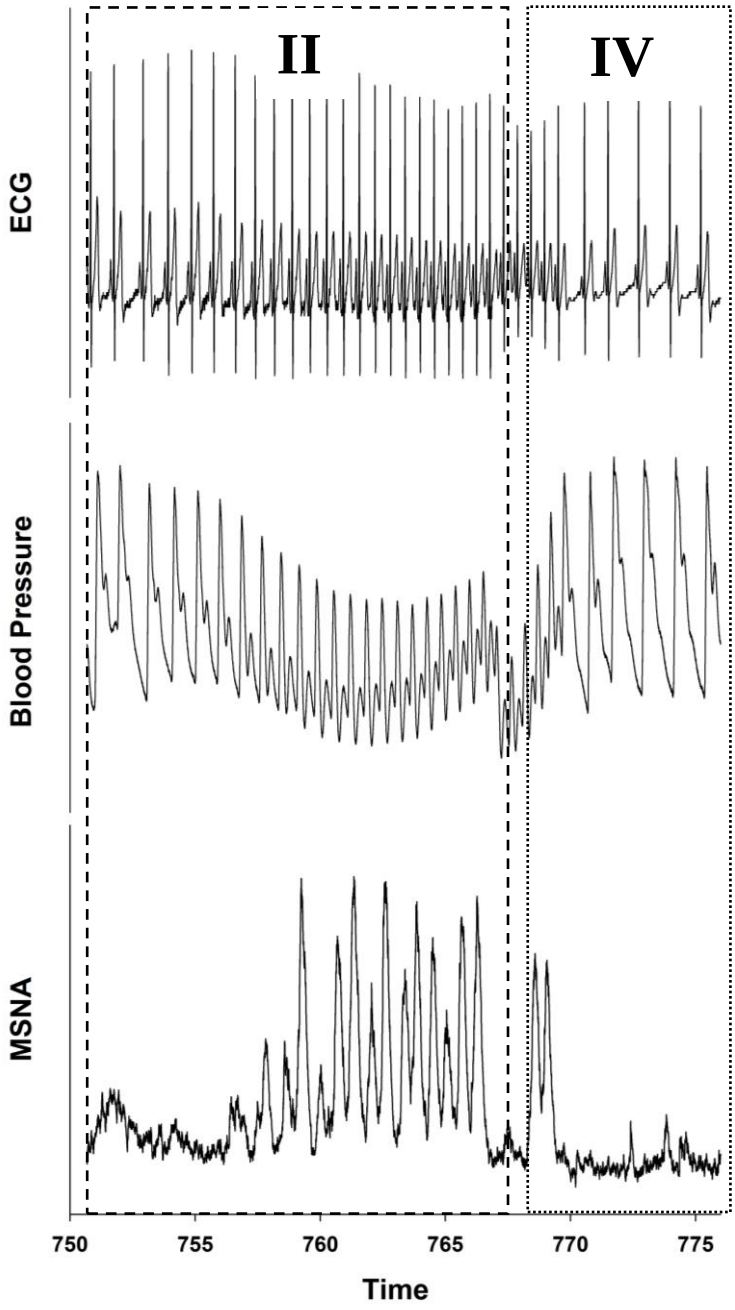


Table 9: Influence of Fasting on Baroreflex Sensitivity

Variable	Fed	Fasted	MD	t-test p-value	Wilcoxon p-value
SP cvBRS up-up, ms/mmHg	20±2.3	26±4.8*			.029
SP cvBRS down-down, ms/mmHg	17±1.6	21±2.4			.076
VM cvBRS Phase II, ms/mmHg	11±1	12±1.4			.474
VM cvBRS Phase IV, ms/mmHg	9±1.0	12±1.2**	2.2±0.9	.010	
ΔRRI Phase IV, ms	414±42.2	484±40.6*	69.7±33.9	.026	

Values are mean ± SE. Paired t-tests were used to compare variables between the fed and fasted condition. SP = Spontaneous VM = Valsalva Maneuver. MD = mean difference between the fed and fasted condition. One-tailed p-values are displayed. (*) = $p \leq 0.05$ (**) = $p \leq 0.01$

6.4 Discussion

We tested young, healthy, normotensive individuals twice, once 24-hours postprandial (fasted) and 3-hours postprandial (Fed) separated by a month. Our purpose was to determine how acute fasting influences cardiovascular control mechanisms, hemodynamics, and peripheral sympathetic activity. We report three novel findings. First, acute fasting increases vagal modulation at the heart without altering peripheral sympathetic outflow. Second, fasting enhances cardiovagal baroreflex

sensitivity measured both spontaneously and when baroreceptors are challenged with a naturally induced hypertensive stimulus. Third, forearm vascular resistance and stroke volume were increased in the fasted state.

Previous studies have reported mixed results on the influence of acute fasting on cardiovagal activity. Herbert et al., reported that 24 hours of fasting reduced the high frequency component of HRV but did not report an increase in heart rate that would be expected with reduced vagal activity at the heart (Herbert et al., 2012; Mazurak et al., 2013). Mazurak et al., reported that after 24 hours of fasting participants tended to demonstrate increases in HRV and RRI that abated after 48 hours of fasting (Mazurak et al., 2013). However, both these studies were done soley in women and only reported heart rate varibility and not cardiovagal baroreflex sensitivity. In contrast, our study provides the following evidence to suggest that acute fasting enhances vagal modulation of the heart. 1) fasting increases R-R interval; 2) concomitanly, HFnu is significantly increased and LFnu is decreased; 3) spontanoeous and dynamic cardiovagal baroreflex sensitvity is increased indicating enhanced reflexive vagal activation. Additonally, the enhanced cardiovagal baroreflex sensitvity provides a potenital explanatory mechanism for the overall reduction in ambulatory blood pressure recorded while particpants were fasting (chapter 5). Increased cardiovagal baroreflex sensitvity and enhanced dampening of increases in blood pressure, as measured during the Valsalva, could effectivly lower average 24-hour arterial pressure. Additonally, increased RRI indicates a reduction in heart rate in agreeance with the reduction in 24-hour heart rate measured from the ABPM monitor (chapter 5). This evidence supports the hypothesis that fasting increases parasympathetic activity at the heart.

We did not observe a change in peripheral sympathetic outflow between the fed and fasting condition. At first a lack of change in sympathetic outflow

is counter-intuitive as previous studies have suggested that MSNA and cardiac sympathetic markers of HRV change inparallel in response to autonomic challeneges (DeBeck, Petersen, Jones, & Stickland, 2010; Pagani et al., 1997) Additonally, MSNA correlates strongly with cardiac norepinephrine spillover at rest (B.G. Wallin et al., 1992). However, previous animal studies have reported that fasting induces a disassociation between symapthetic actvitiy at the heart and sympahetic stimulation of the adrenal medulla (J. B. Young et al., 1984). Therefore, it is resonable to speculate that in the fasted state, parasympathetically mediated cardiovascular control is enhanced and peripheral sympathetic outflow is unaffected and/or disassociated from cardiovascular oscilations.

Lastly, fasting reduces overall 24-hour blood pressure (Chapter 5) but a reduction in blood pressure was not observed acutly during the autonomic function test. Blood pressure between the conditions was similar during the autonomic test despite a measured reduction in heart rate in the fasted condition. Two separate measures explain how blood pressure is maintained in the fasted condition even with reduction in heart rate and no change in MSNA; 1) forearm vascular resistance is increased in the fasted condition; and 2) stroke volume is increased in the fasted condition. This study does not have a measured mechanism to explain the descriptive increases in vascular resistance or stroke volume. However, previous studies have measured that after 24 hours of fasting urine norepineprine increases (Chan, Mietus, Raciti, Goldberger, & Mantzoros, 2007) and as fasting time prolongs (48-72 hours) blood epinephrine, norepineprine, and cortisol increase (Boyle, Shah, & Cryer, 1989). We did not measure blood catecholamines but it is possible that after 24-hours of fasting circulating epinephrine and norepinephrine were elevated increasing peripheral vascular resistance and cardiac inotropy.

In conclusion, acute fasting increases vagal modulation of the heart and enhances cardiovagal baroreflex sensitivity. Increased baroreflex sensitivity and vagal modulation of the heart suggest that fasting may convey acute cardioprotective benefits. Additonally, enhanced baroerflex sensitivty potentially explains the reductions in 24-hour blood pressure reported in chapter 5. Heart rate was reduced in the fasted condition but blood pressure was similar during the autonomic test between conditions. The reduction in heart rate seems to have been compenstated by increased peripheral resistance and stroke volume. However, the creation of peripheral resistance is not from increased peripheral sympahetic activation and is beyond the scope of this study to discuss. Collectivly, the AMPB data from chapter 5 and these data support the idea that acute fasting may be an effective dietary intervention for lowering blood pressure and improving cardiovascular health. Nevertheless, future chronic studies are warrarented to eclucidate whether the cardiovascular benefits of fasting are sustained in the long term.

7 THE INFLUENCE OF AN ACUTE FAST ON NEURAL AND CARDIOVASCULAR REACTIVITY TO MENTAL STRESS

7.1 Introduction

Mental stress is associated with the development of myocardial ischemia and hypertension (Deanfield et al., 1984; Yan et al., 2003). There is evidence suggesting that short-term fasting can alter adrenocortical responses to a mental stressor (Kirschbaum et al., 1997). Short-term fasting (8-10 hours) can blunt cortisol release following a mental stressor in healthy young men (Kirschbaum et al., 1997). Additionally, short-term fasting (12 hours) influences splanchnic vascular responses to a mental stress (mental arithmetic). Performing a mental task while fasted induces vasoconstriction in the superior mesenteric artery, a response not exhibited under post-prandial conditions (Someya, Endo, Fukuba, Hirooka, & Hayashi, 2010). Vasoconstriction of the superior mesenteric artery suggests fasting alters vascular responses to mental stress to visceral organs and thus could modulate cardiac output and arterial pressure. A common experimental mental stressor is mental arithmetic. During mental arithmetic participants repeatedly subtract the number six or seven from a two- or three-digit number. This type of mental stress produces reproducible reactivity in mean arterial pressure, heart rate, and muscle sympathetic nerve activity across laboratory sessions (Fonkoue & Carter, 2015). The purpose of this aim is to investigate if fasting influences neural and vascular responsiveness to mental stress. We tested the hypothesis that fasting will reduce the cardiovascular and neural reactivity to mental stress.

7.2 Methods

7.2.1 Experimental Design

After the controlled breathing protocol three brachial blood pressures were taken and the NOVA Finometer was recalibrated to the averaged brachial pressure. Participants were then asked to rest quietly for 5 minutes,

and a second baseline was recorded. After the 5-minute baseline participants performed 5 minutes of mental stress via mental arithmetic. The mental arithmetic involved continuous and rapid subtraction of the number 6 or 7 from a two- or three-digit number. Participants were encouraged by investigators to answer as quickly and as accurately as possible. Participants answered verbally and were corrected if they answered incorrectly. ECG, beat-to-beat blood pressure, muscle sympathetic nerve activity, and forearm blood flow were recorded at rest and during the mental stress task. After the mental stress task participants were asked to rate their perceived stress using a five-point scale. 0, not stressful; 1 somewhat stressful; 2, stressful; 3, very stressful; and 4, very, very stressful.

7.2.2 Data Analysis

All data were analyzed statistically using the commercial software Sigmaplot 14. The stress reactivity was calculated as the mean stress response to each min of mental stress minus the 5-min mean baseline. The minute-by-minute reactivity to mental stress was analyzed using a 2-way repeated measures ANOVA (Condition X Time). Data are presented as means ± SE. A probability value of ≤ 0.05 was considered statistically significant.

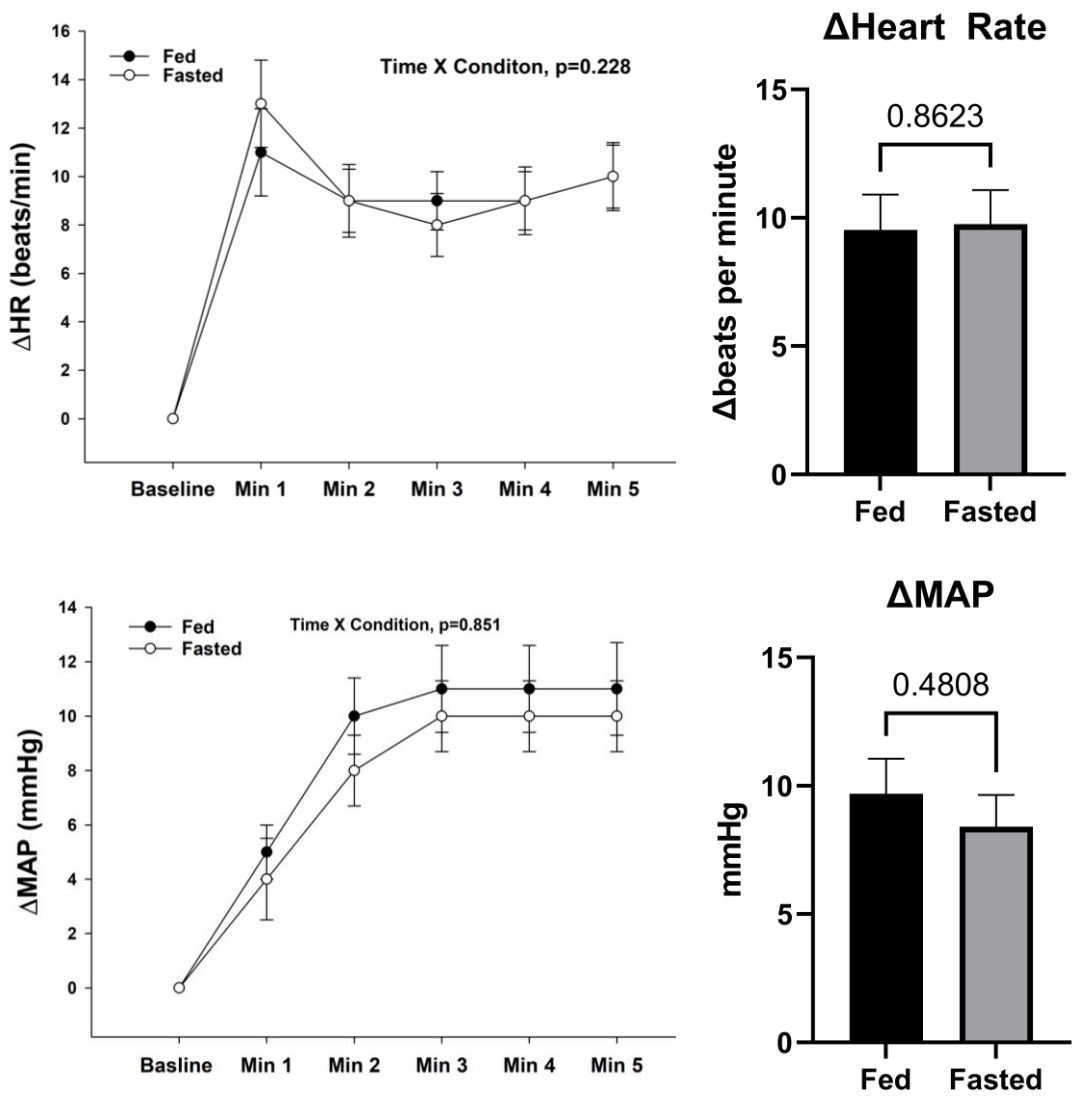
7.3 Results

7.3.1 Cardiovascular Reactivity to Mental Stress

During mental stress minute by minute changes (Δ) in HR, SAP, DAP, and MAP were remarkably comparable between fed and fasted condition. No condition by time effect was detected for any of the primary outcome variables. Additionally, perceived stress during mental arithmetic was similar between the fed and fasted condition (Fed vs. Fasted; 2.2±0.2 vs 2.3±0.2;

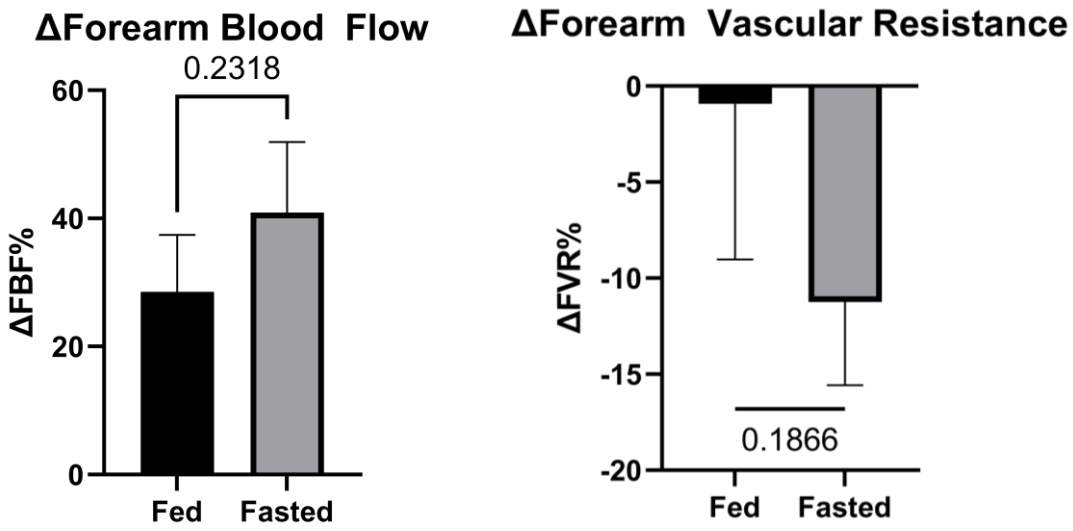
p=.737). Additionally, forearm blood flow and forearm vascular resistance was not altered between condition (N=14).

Figure 11: Mean Arterial Pressure and Heart Rate Responses to Mental Stress



Line graphs are minute by minute mean changes (Δ) in heart rate (HR) and mean arterial pressure (MAP). Bar graphs display mean ± SE changes in HR and MAP during 5 min of mental stress for both the fed and fasted condition.

Figure 12: Forearm Blood Flow Responses to Mental Stress

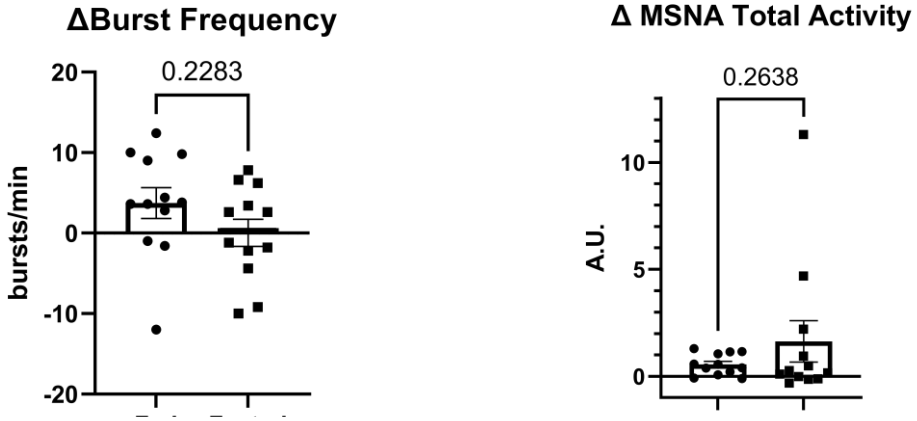


Bar graphs display percentage changes (Δ) form baseline in forearm blood flow (FBF) and forearm vascular resistance (FVR) during 5 min of mental stress for both the fed and fasted condition.

7.3.2 Neural Reactivity to Mental Stress

Burst frequency and MSNA total activity did not change between conditions at baseline or upon exposure to mental stress (N=12).

Figure 13: Muscle Sympathetic Activity Responses to Mental Stress



Bar graphs display changes (Δ) form baseline in burst frequency and MSNA total Activity during 5 min of mental stress for both the fed and fasted condition.

7.4 Discussion

This study is the first to investigate the influence of fasting on neural and cardiovascular reactivity to mental stress. In contrast to our hypothesis, fasting did not reduce neural or cardiovascular responses to a mental stress challenge. Changes in heart rate, blood pressure, forearm blood flow, and muscle sympathetic nerve activity were remarkably similar during a mental stress challenge between the fed and fasted condition, suggesting that the pressor response to mental stress and mechanisms that drive it are unaffected by a short-term negative energy balance.

Short-term fasting has been reported to increase irritability and psychological stress for some individuals (Watkins & Serpell, 2016). Our study demonstrates that fasting does not appear to act as a compounding stressor and does not increase the severity of the pressor response upon exposure to a mental stress. This is important to consider as greater cardiovascular responses to mental stress are associated with greater cardiovascular risk (Chida & Steptoe, 2010). Perceived stress measured after the mental stress task did not differ between the fed and fasted condition. Callister et al., reported that during a cognitive challenge perceived stress is an important determinant of the MSNA response (Callister, Suwarno, & Seals, 1992). Thus, the consistency in perceived stress between conditions could potentially influence the observed similarities in MSNA reactivity. However, more recent studies by Carter et al., have not found an association between perceived stress and MSNA reactivity (Carter, Durocher, & Kern, 2008; Carter & Ray, 2009).

Reactivity to mental stress is highly reproducible. MAP, HR, and MSNA reactivity to mental stress are reproducible within a study and across laboratory visits (Fonkoue & Carter, 2015) In addition, altering neural and

cardiovascular responses to mental stress within an individual is difficult. Aerobic exercise training for 8-weeks and even a 12-week weight loss program (5% loss of weight) do not alter neural or hemodynamic reactivity to mental stress (Ray & Carter, 2010; Torres & Nowson, 2007). A study in obese women found that weight loss acquired through the combination of diet and exercise reduces MSNA reactivity to mental stress (Tonacio et al., 2006). Even total sleep deprivation does not alter blood pressure of MSNA reactivity to mental stress, although sleep deprivation does increase heart rate reactivity (Yang, Durocher, Larson, Dellavalla, & Carter, 2012). Our measured cardiovascular and neural reactivity to mental stress are similar to other studies that have utilized mental arithmetic (Carter & Goldstein, 2015).

In conclusion, fasting does not alter cardiovascular or neural reactivity to mental stress or influence forearm blood flow reactivity to mental stress. Fasting does not act as a compounding stressor as perceived stress after mental arithmetic is also not augmented by fasting. This study provides insight into human cardiovascular reactivity to a mental stressor while in a negative energy balance.

8 THE INFLUENCE OF AN ACUTE FAST ON NEURAL AND CARDIOVASCULAR RESPONSES TO LOWER BODY NEGATIVE PRESSURE

8.1 Introduction

Tilt-table studies have demonstrated that participants who have fasted for 24, 48, and 72 hours exhibit greater vagal withdrawal when exposed to tilt compared to the fed state (S. J. Brown et al., 2012; Mazurak et al., 2013). Reduced parasympathetic activity at the heart during tilt suggests greater sympatho-excitation in response to an orthostatic challenge. The only lower body negative pressure (LBNP) study conducted in fasted humans was performed by Bennet et al. In their study they exposed 9 male participants to progressive LBNP of negative 10, 20, 30, 40 and 50 mmHg for periods of 1 min separated by 1 min rest. They compared 12-hours of fasting to 48-hours of fasting. After 48-hours of fasting systolic pressure decreased during LBNP and heart rate had increased to a greater extent when compared to the fed state (Bennett, MacDonald, & Sainsbury, 1984). 48-hours of fasting also increased forearm blood flow in Bennet's sample population at rest and impaired vasoconstriction during LBNP (Bennett et al., 1984). The tilt-table studies and LBNP study seem to provide converging evidence suggesting that fasting reduces tolerance to an orthostatic challenge. However, direct evidence of a reduced tolerance to an orthostatic challenge has not been established. This is important because while LBNP not only presents an orthostatic challenge it is also an effective simulation of hemorrhage (W. H. Cooke et al., 2004). Fulfillment of specific aim 3 could provide valuable information regarding the relationship between fasting and tolerance to hemorrhage that has application for preoperative fasting as well as military medicine. Therefore, the purpose of this study was to determine how fasting for 24 hours influences orthostatic tolerance to intense LBNP. Specifically, we

tested the hypothesis that acute fasting reduces orthostatic tolerance to intense LBNP

8.2 Methods

8.2.1 Experimental Design

After the mental stress protocol, a three-minute recovery period, and ten minutes of non-recorded rest time, three brachial blood pressures were taken and the NOVA Finometer recalibrated to the averaged brachial pressure. An airtight seal was then secured around participants' waists at the level of the iliac crest. Participants were then asked to rest quietly for 5 minutes and a third baseline was recorded. Following the 5-minute baseline lower body negative pressure was progressively applied in a stepwise manner. Lower body negative pressure was increased every 5 minutes in increments of 15 until – 60 mmHg (ex: -15, -30, -45, -60). After -60 mmHg LBNP was increased in increments of 10 mmHg until onset of pre-syncope or until voluntary participant termination of LBNP. Termination criteria for presyncope were; 1) a drop in systolic pressure of 15 mmHg or greater for 10 seconds or greater; 2) sudden bradycardia; and/or 3) participant-initiated termination. The chamber pressure was released immediately at the onset of presyncope or upon participant-initiated termination of LBNP.

8.2.2 Data Analysis

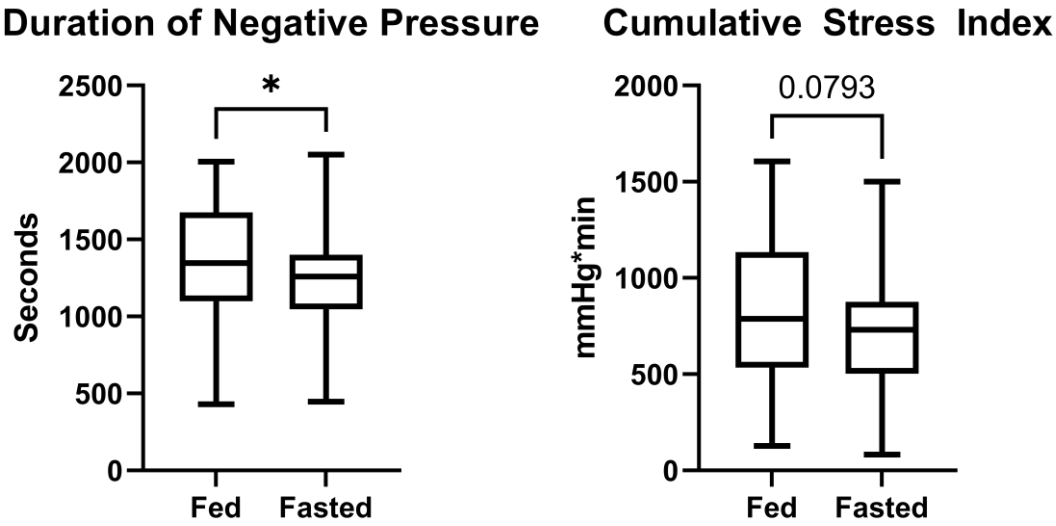
Tolerance to LBNP was assessed by total duration of negative pressure (DNP) in seconds and the cumulative stress index (CSI; pressure X time). ECG, beat-to-beat blood pressure, muscle sympathetic nerve activity, and forearm blood flow were measured continuously throughout the protocol. Data were averaged by taking the last 3 minutes of every 5-minute pressure stage. The last minute just prior to presyncope was also averaged for consistency despite variable termination times. Due to individual and conditional variability

in time to presyncope, we normalized time to presyncope for comparison between participants (appendix A.4). Quantifying percent to presyncope as 0, 40, 80, and 100% for each participant. Using this approach every participant is represented within the percentages to presyncope. A paired t-test was used to assess duration of negative pressure, cumulative stress index, and overall changes in forearm vascular resistance. A 2-way repeated measures ANOVA (% to presyncope by Condition) was used to assess primary outcome variables. Data are presented as means ± SE. A probability value of ≤ 0.05 was considered statistically significant.

8.3 Results

8.3.1 Fasting and Lower Body Negative Pressure Tolerance

Figure 14: Tolerance to Lower Body Negative Pressure



Duration of negative pressure and cumulative stress index represented as boxplots. The line in the boxplots represents the median and the box represents the interquartile range (IQR; the difference between the 25th and 75th percentile). The whiskers extend from the upper and lower edge of the box to the highest and lowest values. *p<0.05

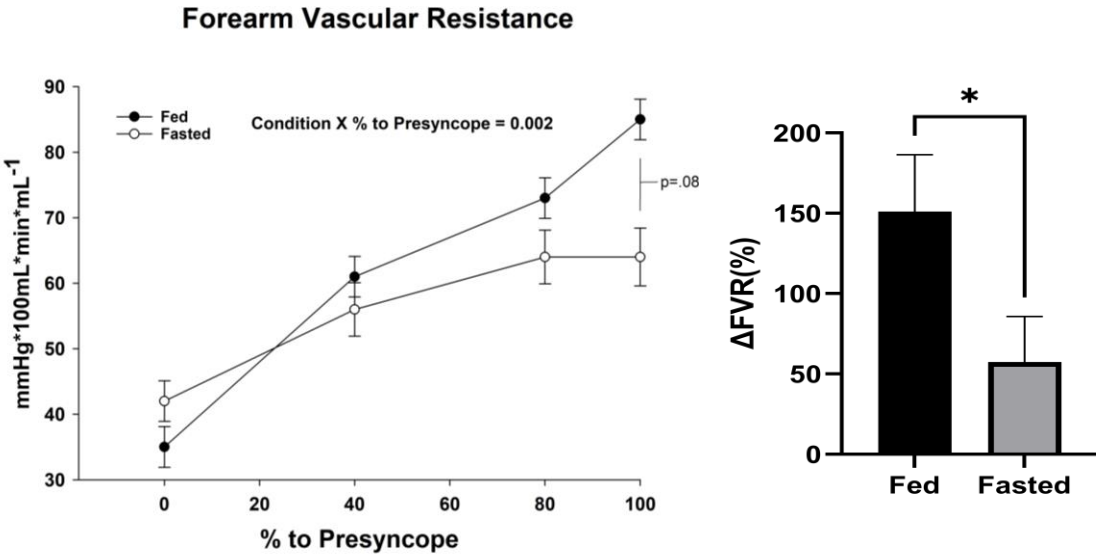
Fasting moderately reduced tolerance to the orthostatic stress of LBNP. Duration of negative pressure was significantly reduced in the fasted condition and cumulative stress index tended to be lower in the fasted

condition. Only participants for which the investigator-initiated termination of LBNP were included in this data set (N=18).

8.3.2 Hemodynamic and Neural Responses to Presyncopal LBNP

During LBNP, heart rate increased similarly in both conditions. Systolic blood pressure was maintained in both conditions up until presyncope. An example of a presyncopal drop in blood pressure and concomitant MSNA activity can be seen in figure 17. Maintaining stable nerve recordings throughout LBNP was difficult and was only measured in 7 participants. However, while there was no significant condition by percent to presyncope interaction, the fasted group was slower to ramp up nerve activity. The fasted condition significantly increased burst frequency starting at the 80% to

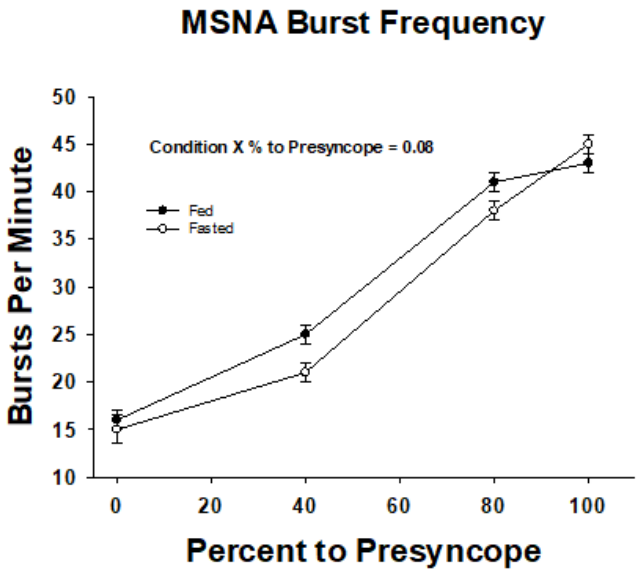
Figure 15: Forearm Vascular Resistance During Lower Body Negative Pressure



Line graph is mean ± SE changes in forearm vascular resistance (FVR) for a given percent to presyncope for both the fed and fasted condition. Bar graphs display percentage (mean ± SE) change in FVR from baseline to 100% presyncope for both the fed and fasted condition

presyncope time point. This is in contrast to the fed condition which significantly increased burst frequency from baseline at 40% to presyncope. Stroke volume and forearm blood flow decreased similarly between the conditions. The fed condition continuously increased calculated total peripheral resistance as LBNP increased. In contrast the fasted condition did not significantly increase calculated TPR during the LBNP protocol. Concurrently, while forearm vascular resistance increased in both condition when compared to baseline, only the fed condition significantly increased FVR at each time point. Additionally, percent change in FVR from baseline to presyncope was greater in the fed condition compared to the fasted condition.

Figure 16: Muscle Sympathetic Nerve Activity During Lower Body Negative Pressure



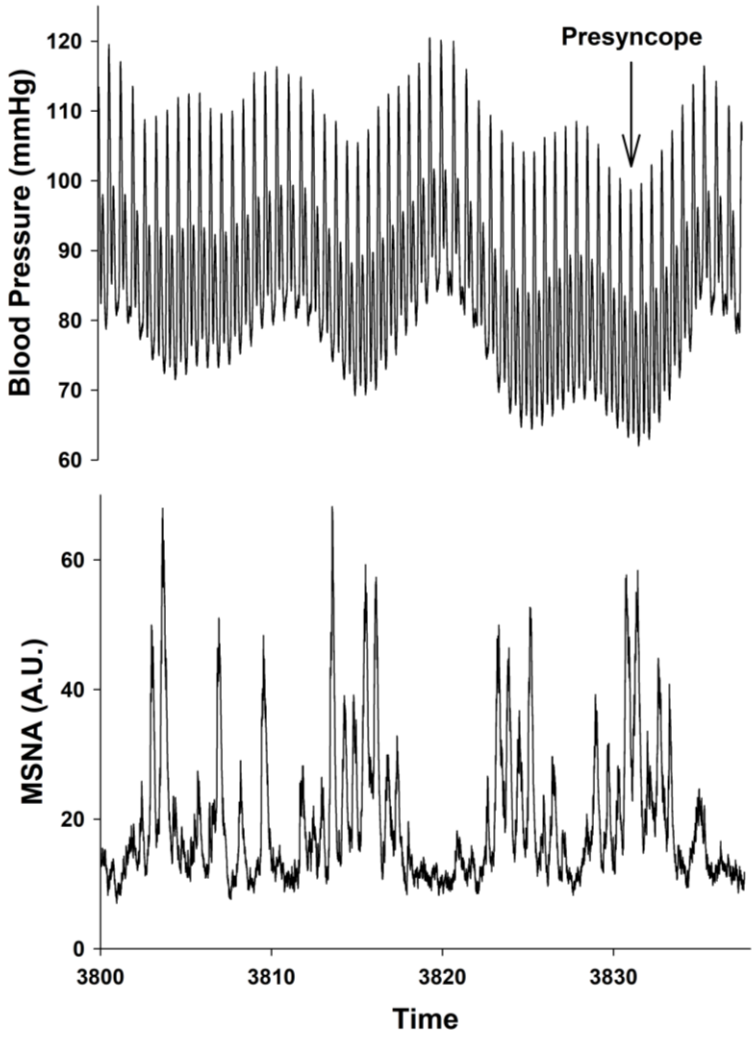
Line graph is mean±SE changes in muscle sympathetic nerve activity (MSNA; N = 7) for a given percent to presyncope for both the fed and fasted condition.

Table 10: Percent to Presyncope LBNP Data

Fed	**0**	**40%**	**80%**	**100%**	**%**	**Cond X %**
Heart Rate, beat per minute	63±1	65±1	85±1*	96±1*	<.01	.86
SAP, mmHg	115±1	116±1	114±1	110±1*	.01	.61
Burst Frequency, b/min (N=7)	15±1	25±1*	41±1*	43±1*	<.01	.08
Stroke Volume, mL	84±2	74±2*	55±2*	51±2*	<.01	<.01
TPR	17±1	19±1*	20±1*	21±1*	<.01	.41
Forearm Blood Flow, ml*100ml^{-1}*min^{-1}	2.4±.1	1.7±.1*	1.5±.1*	1.2±.1*	<.01	<.01
Forearm Vascular Resistance, mmHg*100mL*min*mL^{-1}	35±3	61±3*	73±3*	85±3*	<.01	<.01
Fasted	**0**	**40%**	**80%**	**100%**		
Heart Rate, beat per minute	59±1	61±1	80±1*	94±1*		
SAP, mmHg	113±1	114±1	114±1	105±1*		
Burst Frequency, b/min (N=7)	15±1	21±1	38±1*	45±1*		
Stroke Volume, mL	96±2†	83±2*	57±2*	50±2*		
TPR	17±1	18±1	20±1	17±1		
Forearm Blood Flow, ml*100ml^{-1}*min^{-1}	2.1±.1	1.7±.1*	1.6±.1*	1.5±.1*		
Forearm Vascular Resistance, mmHg*100mL*min*mL^{-1}	42±3	56±4*	64±4*	63±4*		

Values are mean ± SE. A 2-way repeated measures ANOVA was used to compare variables (% to presyncope X Condition). Fisher LSD post-hoc test was used to compare variables that had a significant percent to presyncope (%) or condition interaction (*) = significant differences within a condition from baseline (†) = significant difference between % X condition

Figure 17: Blood Pressure and MSNA at Presyncope in a Representative Subject



8.4 Discussion

Trauma is a leading cause of death worldwide and approximately half of the deaths can be attributed to hemorrhage. In both civilian and military

patients, hemorrhage is the primary cause of death within the first hour of traumatic injury. LBNP is an effective simulation of the central hypovolemia that occurs during hemorrhagic shock (William H. Cooke, Rickards, Ryan, Kuusela, & Convertino, 2009; William H Cooke, Kathy L Ryan, & Victor A Convertino, 2004). The purpose of this study was to investigate if fasting affected tolerance to a severe orthostatic stress. This study has two novel findings. First, acute fasting reduces participants orthostatic tolerance. The amount of time participants could tolerate negative pressure was reduced in the fasted state by approximately 10%. Additionally, the calculated cumulative stress index tended to be lower in the fasted condition. Second, the cause for the decrease in orthostatic tolerance appears to be an impaired ability to increase peripheral resistance.

In the fasted condition, forearm vascular resistance plateaus at 80% of presyncope and does not increase any further at presyncope. Furthermore, the total change in forearm vascular resistance from baseline to presyncope is significantly lower in the fasted condition compared to the fed condition. Concurrently, calculated peripheral resistance did not change in the fasted condition. These two separate estimates of peripheral resistance indicate that fasting impairs increases of vascular resistance to reduce tolerance to central hypovolemia. Heart rate and arterial pressure were similar between conditions when exposed to LBNP. MSNA data are limited, as maintaining a stable nerve signal for both conditions throughout LBNP is difficult. However, the fasted condition exhibited a slower increase in MSNA. In the fasted condition MSNA did not significantly increase from baseline until 80% to presyncope. In contrast, MSNA increased significantly in the fed condition at 40% to presyncope. Enhanced stroke volume in the fasted condition may account for this slower ramp in muscle sympathetic nerve activity. Higher intensity LBNP may be needed to overcome the enhanced stroke volume in the fasted condition to unload the baroreceptors and initiate vasoconstriction

via MSNA. Impaired vascular resistance has been reported before during LBNP after 48 hours of fasting (Bennett et al., 1984). However, the study by Bennet et al. was limited in its scope as exposure to LBNP was brief (1 min per stage) and did not test tolerance (max pressure -50 mmHg). Our study is the first to report that fasting in humans reduces tolerance to induced central hypovolemia and that this reduction in tolerance is likely caused by impaired vascular resistance.

Our results have clinical implications in regard to preoperative fasting. A recent study reported that hemorrhage is the leading cause of death post non-cardiac surgery (15.6%) (Spence et al., 2019). A majority of the post-surgery deaths occurred shortly after operating during the post-surgery stay. To mitigate the risk of aspiration, the American Society of Anesthesiologists recommends cessation of clear liquids 2 hours before surgery and solid food 6-8 hours before surgery ("Practice Guidelines for Preoperative Fasting and the Use of Pharmacologic Agents to Reduce the Risk of Pulmonary Aspiration: Application to Healthy Patients Undergoing Elective Procedures: An Updated Report by the American Society of Anesthesiologists Task Force on Preoperative Fasting and the Use of Pharmacologic Agents to Reduce the Risk of Pulmonary Aspiration*," 2017). However, due to the unpredictable nature of operating rooms fasting is typically prolonged up to 14 hours (Chon, Ma, & Mun-Price, 2017) and can even extend to 1 or 2 days (Diks et al., 2005). Animal studies have reported that 24 hours of fasting reduces the survival rate of hemorrhage (Ljungqvist, Jansson, & Ware, 1987). In rats subjected to hemorrhagic hypotension, all postprandial rats survived, and all rats deprived of food for 24 hours died. Saline infusion did not increase survival in the food deprived rats (Ljungqvist et al., 1987). Our study suggests that extended fasting (≥24 hours) may reduce humans' ability to tolerate central hypovolemia by approximately 10%. Although fasting moderately reduced tolerance to central hypovolemia, preoperative fasting duration may

be an important risk factor for physicians to consider in mitigating the risk of hemorrhagic shock.

In conclusion, fasting (24 hours postprandial) reduced participants tolerance to a severe orthostatic stress likely by blunting the increases of vascular resistance. An impaired peripheral vascular resistance response in the fasted state was measured by both venous occlusion plethysmography and by calculated total peripheral resistance. Further studies are warranted to understand the mechanisms underlying the reduced tolerance to central hypovolemia while fasted.

9 CONCLUSIONS AND FUTURE DIRECTIONS

9.1 Conclusion

Obesity is a major risk factor for the development of cardiovascular disease (Poirier et al., 2006). Contemporary humans' intake more and expend less energy than their ancestors. This energy imbalance has contributed to an alarming increase in prevalence of obesity in the United States (31% 2000 vs. 42% 2020) (Hales et al., 2020). Accessible and effective countermeasures are needed to abate the rise and obesity and improve cardiovascular health outcomes. Evidence suggests that chronic intermittent fasting can help individuals lose weight, reduce blood pressure, and improve cardiometabolic health (Stekovic et al., 2019; Varady et al., 2013). However, the mechanism behind these reported reductions in arterial pressure caused by chronic intermittent fasting has not been elucidated in humans. Therefore, the purpose of this dissertation was to investigate how acute fasting influences autonomic cardiovascular control of arterial pressure at rest and during stress.

This dissertation employed continuous 24-hour hemodynamic recordings while participants were fasted and fed to ascertain how acute food deprivation influences arterial pressure. Additionally, this dissertation utilized an autonomic battery to investigate how fasting influences the balance between the sympathetic and parasympathetic branches. The balance between the autonomic branches was investigated using indirect measures of autonomic activity at the heart and direct peripheral sympathetic neural recordings to vascular beds. **Aim 1** of this dissertation was to determine the influences of an acute fast on hemodynamics, sympathetic neural activity, and cardiovascular control at rest. **Aim 2** employed a mental arithmetic task to investigate how fasting influences neural and cardiovascular reactivity to a mental stressor. **Aim 3** investigated how an acute fast influenced tolerance

and cardiovascular reactivity to central hypovolemia. Broadly speaking, the scientific rationale for **Aim 1** was to elucidate if acute fasting conveyed any acute cardiovascular benefit and to elucidate the potential mechanisms behind the observed cardiometabolic benefits of chronic fasting. The scientific rationale behind **Aim 2** was to determine if acute fasting altered cardiovascular or neural reactivity to a mental stress task. Lastly, the rationale for **Aim 3** was to assess if fasting reduced tolerance to induced central hypovolemia. **Aim 3** is of clinically relevant as fasting is a common practice for those undergoing surgical procedures and hemorrhage is a serious risk during and after surgery.

From **Aim 1** we were able to demonstrate that acute fasting may convey cardioprotective benefits through enhanced vagal tone and reduced 24-hour blood pressure. Specifically, acute fasting increased heart rate variability and enhances cardiovagal baroreflex sensitivity at rest and during a naturally induced hypertensive stimulus. Improved cardiovagal baroreflex sensitivity potentially explains the measured reduction in overall 24-hour blood pressure. We did not measure any change in peripheral sympathetic activity indicating that fasting primary influences sympathetic modulation at the level of the heart. This aim provides mechanistic evidence to support the known cardiovascular benefits of chronic intermittent fasting.

Aim 2 demonstrated that fasting does not affect cardiovascular or neural reactivity to a mental stress task. Indicating that an acute fast does alter or act as a compounding stressor during a mental stress task. From **Aim 3** we report evidence to suggest that fasting reduces tolerance to a severe orthostatic stress by potentially reducing the ability to increase vascular resistance in the periphery. Additionally, **Aim 3** provides marginal evidence from neural recordings that sympathetic burst frequency is not the cause of this augmented peripheral resistance.

This dissertation provides several novel findings that contribute mechanistic and descriptive insights into how systemic energy balance influence the autonomic nervous systems control of arterial pressure at rest and during stress. Acute fasting appears to convey cardioprotective benefits by reducing blood pressure, increasing heart rate variability, and enhancing baroreflex sensitivity. However, the cardioprotective benefits of fasting should be balanced with the reduction in orthostatic tolerance. The reduction in orthostatic tolerance suggests that individuals who decide to acutely fast should do so if they are 1) not susceptible to postural orthostatic intolerance 2) not at risk for severe hemorrhage.

9.2 Future Directions

This dissertation provides evidence that suggests that acute fasting reduces blood pressure, increases vagal modulation of the heart, and enhances baroreflex sensitivity. However, the temporal range of these cardiovascular benefits when energy consumption resumes is unknown. Additionally, the sample population for this study was young, healthy, normotensive, normal weight individuals. Future studies should expand into populations of clinical interest, such as individuals with hypertension. Studying fasting in individuals with hypertension would provide valuable information regarding fasting as a viable intervention to decrease blood pressure and enhance baroreflex sensitivity in these individuals.

In animals, it is well established that food deprivation decreases survival to hemorrhagic hypotension (Ljungqvist et al., 1987). In rats, two separate studies have reported that food deprivation (20-24 hours) results in 100% mortality to hemorrhagic hypotension, compared to a 100% survival rate in the postprandial rats (DARLINGTON, NEVES, HA, CHEW, & DALLMAN, 1990; Ljungqvist et al., 1987) However, when food deprived (24 hours) rats are infused with glucose just prior to standardized hemorrhage all survive

(Alibegovic & Ljungqvist, 1993). The glucose infused rats were compared to food deprived rats that were given a saline infusion of equal volume. This study in animals provides evidence to suggest that glucose infusion just prior to simulated central hypovolemia in fasted humans may potentially increase their orthostatic tolerance. Future studies should employ oral or infused glucose to investigate if a rapid energy infusion could increase tolerance to central hypovolemia in fasted participants. Such a study could provide insightful knowledge to physicians wanting to mitigate the risk of hemorrhagic shock during and after surgery.

REFERENCES

Acheson, K., Jéquier, E., & Wahren, J. (1983). Influence of beta-adrenergic blockade on glucose-induced thermogenesis in man. *J Clin Invest, 72*(3), 981-986. doi:10.1172/jci111070

Acheson, K. J., Ravussin, E., Wahren, J., & Jéquier, E. (1984). Thermic effect of glucose in man. Obligatory and facultative thermogenesis. *J Clin Invest, 74*(5), 1572-1580. doi:10.1172/jci111573

Ackerknecht, E. H. (1974). The history of the discovery of the vegatative (autonomic) nervous system. *Medical history, 18*(1), 1-8.

Agoston, D. V. (2017). How to Translate Time? The Temporal Aspect of Human and Rodent Biology. *Frontiers in neurology, 8*, 92-92. doi:10.3389/fneur.2017.00092

Ali, Y. S., Daamen, N., Jacob, G., Jordan, J., Shannon, J. R., Biaggioni, I., & Robertson, D. (2000). Orthostatic intolerance: a disorder of young women. *Obstetrical & gynecological survey, 55*(4), 251-259.

Alibegovic, A., & Ljungqvist, O. (1993). Pretreatment with glucose infusion prevents fatal outcome after hemorrhage in food deprived rats. *Circulatory shock, 39*(1), 1-6. Retrieved from http://europepmc.org/abstract/MED/8481972

Andersson, B., Wallin, G., Hedner, T., Ahlberg, A. C., & Andersson, O. K. (1988). Acute effects of short-term fasting on blood pressure, circulating noradrenaline and efferent sympathetic nerve activity. *Acta Med Scand, 223*(6), 485-490. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/3389203

Anton, S. D., Moehl, K., Donahoo, W. T., Marosi, K., Lee, S. A., Mainous, A. G., 3rd, . . . Mattson, M. P. (2018). Flipping the Metabolic Switch: Understanding and Applying the Health Benefits of Fasting. *Obesity (Silver Spring), 26*(2), 254-268. doi:10.1002/oby.22065

Ariyasu, H., Takaya, K., Tagami, T., Ogawa, Y., Hosoda, K., Akamizu, T., . . . Nakao, K. (2001). Stomach Is a Major Source of Circulating Ghrelin, and Feeding State Determines Plasma Ghrelin-Like Immunoreactivity Levels in Humans. *The Journal of Clinical Endocrinology & Metabolism, 86*(10), 4753-4758. doi:10.1210/jcem.86.10.7885

Aronow, W. S., & Ahn, C. (1997). Association of postprandial hypotension with incidence of falls, syncope, coronary events, stroke, and total mortality at 29-month follow-up in 499 older nursing home residents. *J Am Geriatr Soc, 45*(9), 1051-1053. doi:10.1111/j.1532-5415.1997.tb05965.x

Astrup, A., Simonsen, L., Bulow, J., Madsen, J., & Christensen, N. J. (1989). Epinephrine mediates facultative carbohydrate-induced thermogenesis in human skeletal muscle. *American Journal of Physiology-*

Endocrinology and Metabolism, 257(3), E340-E345. doi:10.1152/ajpendo.1989.257.3.E340
Benjamin, N., Calver, A., Collier, J., Robinson, B., Vallance, P., & Webb, D. (1995). Measuring Forearm Blood Flow and Interpreting the Responses to Drugs and Mediators. *Hypertension, 25*(5), 918-923. doi:doi:10.1161/01.HYP.25.5.918
Bennett, T., MacDonald, I. A., & Sainsbury, R. (1984). The Influence of Acute Starvation on the Cardiovascular Responses to Lower Body Subatmospheric Pressure or to Standing in Man. *Clinical Science, 66*(2), 141-146. doi:10.1042/cs0660141
Berne, C., Fagius, J., & Niklasson, F. (1989). Sympathetic response to oral carbohydrate administration. Evidence from microelectrode nerve recordings. *J Clin Invest, 84*(5), 1403-1409. doi:10.1172/jci114313
Blaber, A., Yamamoto, Y., & Hughson, R. (1995). Methodology of spontaneous baroreflex relationship assessed by surrogate data analysis. *Am. J. Physiol, 268*, H1682-H1687.
Blackwell, D. L., & Clarke, T. C. (2018). State variation in meeting the 2008 federal guidelines for both aerobic and muscle-strengthening activities through leisure-time physical activity among adults aged 18-64: United States, 2010-2015. *National health statistics reports*(112), 1-22.
Boden, G., Chen, X., Mozzoli, M., & Ryan, I. (1996). Effect of fasting on serum leptin in normal human subjects. *The Journal of Clinical Endocrinology & Metabolism, 81*(9), 3419-3423.
Boyle, P. J., Shah, S. D., & Cryer, P. E. (1989). Insulin, glucagon, and catecholamines in prevention of hypoglycemia during fasting. *American Journal of Physiology-Endocrinology and Metabolism, 256*(5), E651-E661. doi:10.1152/ajpendo.1989.256.5.E651
Brodde, O.-E., Bruck, H., Leineweber, K., & Seyfarth, T. (2001). Presence, distribution and physiological function of adrenergic and muscarinic receptor subtypes in the human heart. *Basic research in cardiology, 96*(6), 528-538.
Brown, S. J., Bryant, M., Mündel, T., & Stannard, S. (2012). Human cardiac autonomic responses to head-up tilting during 72-h starvation. *European journal of applied physiology, 112*(6), 2331-2339.
Brown, T. E., Beightol, L. A., Koh, J., & Eckberg, D. L. (1993). Important influence of respiration on human R-R interval power spectra is largely ignored. *J Appl Physiol (1985), 75*(5), 2310-2317. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/8307890
Bruno, C. M., Amaradio, M. D., Pricoco, G., Marino, E., & Bruno, F. (2018). Lifestyle and hypertension: an evidence-based review. *J Hypertens Manag, 4*(1), 1-10.
Burt, V. L., Whelton, P., Roccella, E. J., Brown, C., Cutler, J. A., Higgins, M., . . . Labarthe, D. (1995). Prevalence of hypertension in the US adult population. Results from the Third National Health and Nutrition

Examination Survey, 1988-1991. *Hypertension, 25*(3), 305-313. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/7875754
Cahill Jr, G. F. (1978). Physiology of acute starvation in man.
Callister, R., Suwarno, N. O., & Seals, D. R. (1992). Sympathetic activity is influenced by task difficulty and stress perception during mental challenge in humans. *The Journal of Physiology, 454*(1), 373-387. doi:https://doi.org/10.1113/jphysiol.1992.sp019269
Cannon, W. B. (1922). *Bodily changes in pain, hunger, fear and rage: An account of recent researches into the function of emotional excitement*: D. Appleton.
Cannon, W. B. (1929). Organization for physiological homeostasis. *Physiological reviews, 9*(3), 399-431.
Carroll, J. F., Kaiser, K. A., Franks, S. F., Deere, C., & Caffrey, J. L. (2007). Influence of BMI and Gender on Postprandial Hormone Responses. *Obesity, 15*(12), 2974-2983. doi:10.1038/oby.2007.355
Carter, J. R., Durocher, J. J., & Kern, R. P. (2008). Neural and cardiovascular responses to emotional stress in humans. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 295*(6), R1898-R1903. doi:10.1152/ajpregu.90646.2008
Carter, J. R., Fu, Q., Minson, C. T., & Joyner, M. J. (2013). Ovarian Cycle and Sympathoexcitation in Premenopausal Women. *Hypertension, 61*(2), 395-399. doi:doi:10.1161/HYPERTENSIONAHA.112.202598
Carter, J. R., & Goldstein, D. S. (2015). Sympathoneural and adrenomedullary responses to mental stress. *Comprehensive Physiology, 5*(1), 119-146. doi:10.1002/cphy.c140030
Carter, J. R., Kupiers, N. T., & Ray, C. A. (2005). Neurovascular responses to mental stress. *The Journal of Physiology, 564*(1), 321-327. doi:10.1113/jphysiol.2004.079665
Carter, J. R., & Lawrence, J. E. (2007). Effects of the menstrual cycle on sympathetic neural responses to mental stress in humans. *The Journal of Physiology, 585*(Pt 2), 635-641. doi:10.1113/jphysiol.2007.141051
Carter, J. R., & Ray, C. A. (2009). Sympathetic neural responses to mental stress: responders, nonresponders and sex differences. *American journal of physiology. Heart and circulatory physiology, 296*(3), H847-H853. doi:10.1152/ajpheart.01234.2008
Casolo, G. C., Stroder, P., Signorini, C., Calzolari, F., Zucchini, M., Balli, E., . . . Lazzerini, S. (1992). Heart rate variability during the acute phase of myocardial infarction. *Circulation, 85*(6), 2073-2079. doi:doi:10.1161/01.CIR.85.6.2073
Casonatto, J., Goessler, K. F., Cornelissen, V. A., Cardoso, J. R., & Polito, M. D. (2020). The blood pressure-lowering effect of a single bout of resistance exercise: A systematic review and meta-analysis of randomised controlled trials. *European Journal of Preventive Cardiology, 23*(16), 1700-1714. doi:10.1177/2047487316664147

Chan, J. L., Mietus, J. E., Raciti, P. M., Goldberger, A. L., & Mantzoros, C. S. (2007). Short-term fasting-induced autonomic activation and changes in catecholamine levels are not mediated by changes in leptin levels in healthy humans. *Clinical Endocrinology, 66*(1), 49-57. doi:https://doi.org/10.1111/j.1365-2265.2006.02684.x

Chida, Y., & Steptoe, A. (2010). Greater Cardiovascular Responses to Laboratory Mental Stress Are Associated With Poor Subsequent Cardiovascular Risk Status. *Hypertension, 55*(4), 1026-1032. doi:doi:10.1161/HYPERTENSIONAHA.109.146621

Chobanian, A. V., Bakris, G. L., Black, H. R., Cushman, W. C., Green, L. A., Izzo Jr, J. L., . . . Wright Jr, J. T. (2003). Seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure. *Hypertension, 42*(6), 1206-1252.

Chon, T., Ma, A., & Mun-Price, C. (2017). Perioperative Fasting and the Patient Experience. *Cureus, 9*(5), e1272. doi:10.7759/cureus.1272

Church, T. S., Thomas, D. M., Tudor-Locke, C., Katzmarzyk, P. T., Earnest, C. P., Rodarte, R. Q., . . . Bouchard, C. (2011). Trends over 5 Decades in U.S. Occupation-Related Physical Activity and Their Associations with Obesity. *PLOS ONE, 6*(5), e19657. doi:10.1371/journal.pone.0019657

Colman, R. J., Beasley, T. M., Kemnitz, J. W., Johnson, S. C., Weindruch, R., & Anderson, R. M. (2014). Caloric restriction reduces age-related and all-cause mortality in rhesus monkeys. *Nature communications, 5*(1), 3557. doi:10.1038/ncomms4557

Cooke, W. H. (1998). Heart rate variability and baroreceptor responsiveness to evaluate autonomic cardiovascular adaptations to exercise. *Journal of Exercise Physiology, 1*(http://www.css.edu/users/tboone2/asep/jan13b.htm), 1-7.

Cooke, W. H., Cox, J. F., Diedrich, A. M., Taylor, J. A., Beightol, L. A., Ames, J. E. t., . . . Eckberg, D. L. (1998). Controlled breathing protocols probe human autonomic cardiovascular rhythms. *Am J Physiol, 274*(2 Pt 2), H709-718. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/9486278

Cooke, W. H., Rickards, C. A., Ryan, K. L., Kuusela, T. A., & Convertino, V. A. (2009). Muscle sympathetic nerve activity during intense lower body negative pressure to presyncope in humans. *The Journal of Physiology, 587*(20), 4987-4999. doi:10.1113/jphysiol.2009.177352

Cooke, W. H., Ryan, K. L., & Convertino, V. A. (2004). Lower body negative pressure as a model to study progression to acute hemorrhagic shock in humans. *J Appl Physiol (1985), 96*(4), 1249-1261. doi:10.1152/japplphysiol.01155.2003

Cooke, W. H., Ryan, K. L., & Convertino, V. A. (2004). Lower body negative pressure as a model to study progression to acute hemorrhagic shock in humans. *Journal of Applied Physiology, 96*(4), 1249-1261.

Cooper, K., Edholm, O., & Mottram, R. (1955). The blood flow in skin and muscle of the human forearm. *The Journal of Physiology, 128*(2), 258-267.

Crittenden, A. N., & Schnorr, S. L. (2017). Current views on hunter-gatherer nutrition and the evolution of the human diet. *American Journal of Physical Anthropology, 162*, 84-109.

DARLINGTON, D. N., NEVES, R. B., HA, T., CHEW, G., & DALLMAN, M. F. (1990). Fed, but not Fasted, Adrenalectomized Rats Survive the Stress of Hemorrhage and Hypovolemia*. *Endocrinology, 127*(2), 759-765. doi:10.1210/endo-127-2-759

de Boer, R. W., Karemaker, J. M., & Strackee, J. (1985). Relationships between short-term blood-pressure fluctuations and heart-rate variability in resting subjects 1: a spectral analysis approach. *Med. Biol. Eng. Comput, 23*, 352-358.

de Cabo, R., & Mattson, M. P. (2019). Effects of Intermittent Fasting on Health, Aging, and Disease. *New England Journal of Medicine, 381*(26), 2541-2551. doi:10.1056/NEJMra1905136

Deanfield, J. E., Shea, M., Kensett, M., Horlock, P., Wilson, R. A., de Landsheere, C. M., & Selwyn, A. P. (1984). Silent myocardial ischaemia due to mental stress. *Lancet, 2*(8410), 1001-1005. doi:10.1016/s0140-6736(84)91106-1

DeBeck, L. D., Petersen, S. R., Jones, K. E., & Stickland, M. K. (2010). Heart rate variability and muscle sympathetic nerve activity response to acute stress: the effect of breathing. *American journal of physiology. Regulatory, integrative and comparative physiology, 299*(1), R80-R91. doi:10.1152/ajpregu.00246.2009

deBoer, R. W., Karemaker, J. M., & Strackee, J. (1987). Hemodynamic fluctuations and baroreflex sensitivity in humans: a beat-to-beat model. *American Journal of Physiology-Heart and Circulatory Physiology, 253*(3), H680-H689. doi:10.1152/ajpheart.1987.253.3.H680

Dekker, J. M., Schouten, E. G., Klootwijk, P., Pool, J., Swenne, C. A., & Kromhout, D. (1997). Heart Rate Variability from Short Electrocardiographic Recordings Predicts Mortality from All Causes in Middle-aged and Elderly Men: The Zutphen Study. *American Journal of Epidemiology, 145*(10), 899-908. doi:10.1093/oxfordjournals.aje.a009049

Diks, J., van Hoorn, D. E., Nijveldt, R. J., Boelens, P. G., Hofman, Z., Bouritius, H., . . . van Leeuwen, P. A. (2005). Preoperative fasting: an outdated concept? *JPEN J Parenter Enteral Nutr, 29*(4), 298-304. doi:10.1177/0148607105029004298

Dolan, E., Stanton, A., Thijs, L., Hinedi, K., Atkins, N., McClory, S., . . . O'Brien, E. (2005). Superiority of Ambulatory Over Clinic Blood Pressure Measurement in Predicting Mortality. *Hypertension, 46*(1), 156-161. doi:doi:10.1161/01.HYP.0000170138.56903.7a

Eckberg, D. L. (1997). Sympathovagal balance: a critical appraisal. *Circulation, 96*, 3224-3232.

Einhorn, D., Young, J. B., & Landberg, L. (1982). Hypotensive effect of fasting: possible involvement of the sympathetic nervous system and endogenous opiates. *Science, 217*(4561), 727-729.

Electrophysiology, T. F. o. t. E. S. o. C. a. t. N. A. S. o. P. a. (1996). Heart rate variability: Standards of measurement, physiological interpretation, and clinical use. *Circulation, 93*, 1043-1065.

English, P. J., Ghatei, M. A., Malik, I. A., Bloom, S. R., & Wilding, J. P. H. (2002). Food Fails to Suppress Ghrelin Levels in Obese Humans. *The Journal of Clinical Endocrinology & Metabolism, 87*(6), 2984-2987. doi:10.1210/jcem.87.6.8738

Epstein, A. E., Hirschowitz, B. I., Kirklin, J. K., Kirk, K. A., Nay, G. N., & Plumb, V. J. (1990). Evidence for a central site of action to explain the negative chronotropic effect of atropine: studies on the human transplanted heart. *Journal of the American College of Cardiology, 15*, 1610-1617.

Espelund, U., Hansen, T. K., Hojlund, K., Beck-Nielsen, H., Clausen, J. T., Hansen, B. S., . . . Frystyk, J. (2005). Fasting unmasks a strong inverse association between ghrelin and cortisol in serum: Studies in obese and normal-weight subjects. *Journal of Clinical Endocrinology & Metabolism, 90*(2), 741-746. doi:10.1210/jc.2004-0604

Fagius, J., & Wallin, B. G. (1993). Long-term variability and reproducibility of resting human muscle nerve sympathetic activity at rest, as reassessed after a decade. *Clinical Autonomic Research, 3*(3), 201-205.

Farrell, T. G., Odemuyiwa, O., Bashir, Y., Cripps, T. R., Malik, M., & Ward, D. E. (1992). Prognostic value of baroreflex sensitivity testing after acute myocardial infarction. *Br. Heart. J, 67*, 129-137.

Finkelstein, E. A., Trogdon, J. G., Cohen, J. W., & Dietz, W. (2009). Annual Medical Spending Attributable To Obesity: Payer-And Service-Specific Estimates. *Health Affairs, 28*(Supplement 1), w822-w831. doi:10.1377/hlthaff.28.5.w822

Fonkoue, I. T., & Carter, J. R. (2015). Sympathetic neural reactivity to mental stress in humans: test-retest reproducibility. *American journal of physiology. Regulatory, integrative and comparative physiology, 309*(11), R1380-R1386. doi:10.1152/ajpregu.00344.2015

Fontana, L., Meyer, T. E., Klein, S., & Holloszy, J. O. (2004). Long-term calorie restriction is highly effective in reducing the risk for atherosclerosis in humans. *Proceedings of the National Academy of Sciences, 101*(17), 6659-6663.

Fontana, L., Partridge, L., & Longo, V. D. (2010). Extending healthy life span—from yeast to humans. *Science, 328*(5976), 321-326.

Frankenfield, D., Roth-Yousey, L., & Compher, C. (2005). Comparison of Predictive Equations for Resting Metabolic Rate in Healthy Nonobese and Obese Adults: A Systematic Review. *Journal of the American Dietetic Association, 105*(5), 775-789. doi:https://doi.org/10.1016/j.jada.2005.02.005

Franklin, S. S., Khan, S. A., Wong, N. D., Larson, M. G., & Levy, D. (1999). Is Pulse Pressure Useful in Predicting Risk for Coronary Heart Disease? *Circulation, 100*(4), 354-360. doi:doi:10.1161/01.CIR.100.4.354

Franklin, S. S., Thijs, L., Hansen, T. W., O'Brien, E., & Staessen, J. A. (2013). White-Coat Hypertension. *Hypertension, 62*(6), 982-987. doi:doi:10.1161/HYPERTENSIONAHA.113.01275

Glynn, L. G., Murphy, A. W., Smith, S. M., Schroeder, K., & Fahey, T. (2010). Interventions used to improve control of blood pressure in patients with hypertension. *Cochrane Database Syst Rev*(3), Cd005182. doi:10.1002/14651858.CD005182.pub4

Gonzalez, J. E., & Cooke, W. H. (2021). Acute effects of electronic cigarettes on arterial pressure and peripheral sympathetic activity in young nonsmokers. *American Journal of Physiology-Heart and Circulatory Physiology, 320*(1), H248-H255. doi:10.1152/ajpheart.00448.2020

Grassi, G., Bolla, G., Seravalle, G., Turri, C., Lanfranchi, A., & Mancia, G. (1997). Comparison between reproducibility and sensitivity of muscle sympathetic nerve traffic and plasma noradrenaline in man. *Clin Sci (Lond), 92*(3), 285-289. doi:10.1042/cs0920285

Grassi, G., Seravalle, G., Calhoun, D. A., Bolla, G. B., Giannattasio, C., Marabini, M., . . . Mancia, G. (1994). Mechanisms responsible for sympathetic activation by cigarette smoking in humans. *Circulation, 90*(1), 248-253. doi:10.1161/01.cir.90.1.248

Grassi, G., Seravalle, G., Cattaneo, B. M., Bolla, G. B., Lanfranchi, A., Colombo, M., . . . Mancia, G. (1995). Sympathetic activation in obese normotensive subjects. *Hypertension, 25*(4 Pt 1), 560-563. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/7721398

Grundler, F., Mesnage, R., Michalsen, A., & Toledo, F. W. d. (2020). Blood Pressure Changes in 1610 Subjects With and Without Antihypertensive Medication During Long‐Term Fasting. *journal of American Heart Association, 9*(23), e018649. doi:doi:10.1161/JAHA.120.018649

Guelen, I., Westerhof, B. E., van der Sar, G. L., van Montfrans, G. A., Kiemeneij, F., Wesseling, K. H., & Bos, W. J. (2008). Validation of brachial artery pressure reconstruction from finger arterial pressure. *J Hypertens, 26*(7), 1321-1327. doi:10.1097/HJH.0b013e3282fe1d28

Habito, R. C., & Ball, M. J. (2001). Postprandial changes in sex hormones after meals of different composition. *Metabolism-Clinical and Experimental, 50*(5), 505-511.

Hainsworth, R. (2004). Pathophysiology of syncope. *Clinical Autonomic Research, 14*(1), i18-i24.

Hales, C. M., Carroll, M. D., Fryar, C. D., & Ogden, C. L. (2020). Prevalence of Obesity and Severe Obesity Among Adults: United States, 2017-2018. *NCHS Data Brief*(360), 1-8. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/32487284

Hart, E. C., Charkoudian, N., Wallin, B. G., Curry, T. B., Eisenach, J., & Joyner, M. J. (2011). Sex and ageing differences in resting arterial pressure regulation: the role of the β-adrenergic receptors. *The Journal of Physiology, 589*(21), 5285-5297. doi:https://doi.org/10.1113/jphysiol.2011.212753

Hart, E. C., Charkoudian, N., Wallin, B. G., Curry, T. B., Eisenach, J. H., & Joyner, M. J. (2009). Sex differences in sympathetic neural-hemodynamic balance: implications for human blood pressure regulation. *Hypertension, 53*(3), 571-576.

Harvey, R. E., Hart, E. C., Charkoudian, N., Curry, T. B., Carter, J. R., Fu, Q., . . . Barnes, J. N. (2015). Oral Contraceptive Use, Muscle Sympathetic Nerve Activity, and Systemic Hemodynamics in Young Women. *Hypertension, 66*(3), 590-597. doi:doi:10.1161/HYPERTENSIONAHA.115.05179

Hayano, J., Sakakibara, Y., Yamada, A., Yamada, M., Mukai, S., Fujinami, T., . . . Takata, K. (1991). Accuracy of assessment of cardiac vagal tone by heart rate variability in normal subjects. *Am. J. Cardiol, 67*, 199-204.

Haymond, M. W., Karl, I. E., Clarke, W. L., Pagliara, A. S., & Santiago, J. V. (1982). Differences in circulating gluconeogenic substrates during short-term fasting in men, women, and children. *Metabolism, 31*(1), 33-42.

Heilbronn, L. K., Smith, S. R., Martin, C. K., Anton, S. D., & Ravussin, E. (2005). Alternate-day fasting in nonobese subjects: effects on body weight, body composition, and energy metabolism. *Am J Clin Nutr, 81*(1), 69-73. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/15640462

Herbert, B. M., Herbert, C., Pollatos, O., Weimer, K., Enck, P., Sauer, H., & Zipfel, S. (2012). Effects of short-term food deprivation on interoceptive awareness, feelings and autonomic cardiac activity. *Biol Psychol, 89*(1), 71-79. doi:10.1016/j.biopsycho.2011.09.004

Hirsch, J. A., & Bishop, B. (1981). Respiratory sinus arrhythmia in humans: how breathing pattern modulates heart rate. *Am. J. Physiol, 241*(10), H620-H629.

Højlund, K., Wildner-Christensen, M., Eshøj, O., Skjærbæk, C., Holst, J. J., Koldkjær, O., . . . Beck-Nielsen, H. (2001). Reference intervals for glucose, β-cell polypeptides, and counterregulatory factors during prolonged fasting. *American Journal of Physiology-Endocrinology and Metabolism, 280*(1), E50-E58. doi:10.1152/ajpendo.2001.280.1.E50

Høost, U., Kelbaek, H., Rasmusen, H., Court-Payen, M., Christensen, N. J., Pedersen-Bjergaard, U., & Lorenzen, T. (1996). Haemodynamic effects of eating: the role of meal composition. *Clin Sci (Lond), 90*(4), 269-276. doi:10.1042/cs0900269

Horne, B. D., May, H. T., Anderson, J. L., Kfoury, A. G., Bailey, B. M., McClure, B. S., . . . Intermountain Heart Collaborative, S. (2008). Usefulness of routine periodic fasting to lower risk of coronary artery disease in patients undergoing coronary angiography. *Am J Cardiol, 102*(7), 814-819. doi:10.1016/j.amjcard.2008.05.021

Horne, B. D., Muhlestein, J. B., Lappe, D. L., May, H. T., Carlquist, J. F., Galenko, O., . . . Anderson, J. L. (2013). Randomized cross-over trial of short-term water-only fasting: metabolic and cardiovascular consequences. *Nutr Metab Cardiovasc Dis, 23*(11), 1050-1057. doi:10.1016/j.numecd.2012.09.007

Horne, B. D., Muhlestein, J. B., May, H. T., Carlquist, J. F., Lappe, D. L., Bair, T. L., . . . Intermountain Heart Collaborative Study, G. (2012). Relation of routine, periodic fasting to risk of diabetes mellitus, and coronary artery disease in patients undergoing coronary angiography. *Am J Cardiol, 109*(11), 1558-1562. doi:10.1016/j.amjcard.2012.01.379

Imholz, B. P. M., Wieling, W., van Montfrans, G. A., & Wesseling, K. H. (1998). Fifteen years experience with finger arterial pressure monitoring: assessment of the technology. *Cardiovasc. Res, 38*, 605-616.

Jansen, R. W., & Lipsitz, L. A. (1995). Postprandial hypotension: epidemiology, pathophysiology, and clinical management. *Annals of internal medicine, 122*(4), 286-295.

Jones, P. P., Davy, K. P., Alexander, S., & Seals, D. (1997). Age-related increase in muscle sympathetic nerve activity is associated with abdominal adiposity. *American Journal of Physiology-Endocrinology and Metabolism, 272*(6), E976-E980.

Joyner, M. J., Charkoudian, N., & Wallin, B. G. (2010). Sympathetic Nervous System and Blood Pressure in Humans. *Hypertension, 56*(1), 10-16. doi:doi:10.1161/HYPERTENSIONAHA.109.140186

Joyner, M. J., Dietz, N. M., & Shepherd, J. T. (2001). From Belfast to Mayo and beyond: the use and future of plethysmography to study blood flow in human limbs. *J Appl Physiol (1985), 91*(6), 2431-2441. doi:10.1152/jappl.2001.91.6.2431

Kannel, W. B., Gordon, T., & Schwartz, M. J. (1971). Systolic versus diastolic blood pressure and risk of coronary heart disease: The Framingham study. *The American journal of cardiology, 27*(4), 335-346. doi:https://doi.org/10.1016/0002-9149(71)90428-0

Keir, D. A., Badrov, M. B., Tomlinson, G., Notarius, C. F., Kimmerly, D. S., Millar, P. J., . . . Floras, J. S. (2020). Influence of Sex and Age on Muscle Sympathetic Nerve Activity of Healthy Normotensive Adults.

Hypertension, 76(3), 997-1005. doi:doi:10.1161/HYPERTENSIONAHA.120.15208
Kimura, I., Inoue, D., Maeda, T., Hara, T., Ichimura, A., Miyauchi, S., . . . Tsujimoto, G. (2011). Short-chain fatty acids and ketones directly regulate sympathetic nervous system via G protein-coupled receptor 41 (GPR41). *Proceedings of the National Academy of Sciences, 108*(19), 8030. doi:10.1073/pnas.1016088108
Kirschbaum, C., Bono, E. G., Rohleder, N., Gessner, C., Pirke, K. M., Salvador, A., & Hellhammer, D. H. (1997). Effects of Fasting and Glucose Load on Free Cortisol Responses to Stress and Nicotine*. *The Journal of Clinical Endocrinology & Metabolism, 82*(4), 1101-1105. doi:10.1210/jcem.82.4.3882
Krakoff, L. R. (2013). Ambulatory Blood Pressure Improves Prediction of Cardiovascular Risk: Implications for Better Antihypertensive Management. *Current Atherosclerosis Reports, 15*(4), 317. doi:10.1007/s11883-013-0317-9
Kraus, W. E., Bhapkar, M., Huffman, K. M., Pieper, C. F., Das, S. K., Redman, L. M., . . . Ravussin, E. (2019). Two Years of Calorie Restriction and Cardiometabolic Risk Factors. *The lancet. Diabetes & endocrinology, 7*(9), 673.
Kück, J.-L., Bönhof, G. J., Strom, A., Zaharia, O.-P., Müssig, K., Szendroedi, J., . . . Ziegler, D. (2020). Impairment in Baroreflex Sensitivity in Recent-Onset Type 2 Diabetes Without Progression Over 5 Years. *Diabetes, 69*(5), 1011-1019. doi:10.2337/db19-0990
Kuwahara, K., Okita, Y., Kouda, K., & Nakamura, H. (2011). Effects of Modern Eating Patterns on the Cardiac Autonomic Nervous System in Young Japanese Males. *Journal of PHYSIOLOGICAL ANTHROPOLOGY, 30*(6), 223-231. doi:10.2114/jpa2.30.223
La Rovere, M. T., Bersano, C., Gnemmi, M., Specchia, G., & Schwartz, P. J. (2002). Exercise-induced increase in baroreflex sensitivity predicts improved prognosis after myocardial infarction. *Circulation, 106*(8), 945-949.
LANDSBERG, L. (1986). Diet, Obesity and Hypertension: An Hypothesis Involving Insulin, the Sympathetic Nervous System, and Adaptive Thermogenesis. *QJM: An International Journal of Medicine, 61*(3), 1081-1090. doi:10.1093/oxfordjournals.qjmed.a068066
Landsberg, L., & Young, J. B. (1978). Fasting, feeding and regulation of the sympathetic nervous system. *New England Journal of Medicine, 298*(23), 1295-1301.
Langewouters, G., Wesseling, K., & Goedhard, W. (1984). The static elastic properties of 45 human thoracic and 20 abdominal aortas in vitro and the parameters of a new model. *Journal of biomechanics, 17*(6), 425-435.

Ljungqvist, O., Jansson, E., & Ware, J. (1987). Effect of food deprivation on survival after hemorrhage in the rat. *Circ Shock, 22*(3), 251-260. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/3621480

Lombardi, F., Sandrone, G., Spinnler, M. T., Torzillo, D., Lavezzaro, G. C., Brusca, A., & Malliani, A. (1996). Heart rate variability in the early hours of an acute myocardial infarction. *The American journal of cardiology, 77*(12), 1037-1044.

Mager, D. E., Wan, R., Brown, M., Cheng, A., Wareski, P., Abernethy, D. R., & Mattson, M. P. (2006). Caloric restriction and intermittent fasting alter spectral measures of heart rate and blood pressure variability in rats. *The FASEB Journal, 20*(6), 631-637. doi:https://doi.org/10.1096/fj.05-5263com

Malinowski, B., Zalewska, K., Węsierska, A., Sokołowska, M. M., Socha, M., Liczner, G., . . . Wiciński, M. (2019). Intermittent Fasting in Cardiovascular Disorders-An Overview. *Nutrients, 11*(3), 673. doi:10.3390/nu11030673

Malliani, A., Pagani, M., Lombardi, F., & Cerutti, S. (1991). Cardiovascular neural regulation explored in the frequency domain. *Circulation, 84*, 482-492.

Mandsager, K., Harb, S., Cremer, P., Phelan, D., Nissen, S. E., & Jaber, W. (2018). Association of Cardiorespiratory Fitness With Long-term Mortality Among Adults Undergoing Exercise Treadmill Testing. *JAMA Network Open, 1*(6), e183605-e183605. doi:10.1001/jamanetworkopen.2018.3605

Manfrini, O., Pizzi, C., Trerè, D., Fontana, F., & Bugiardini, R. (2003). Parasympathetic failure and risk of subsequent coronary events in unstable angina and non-ST-segment elevation myocardial infarction. *European Heart Journal, 24*(17), 1560-1566. doi:10.1016/s0195-668x(03)00345-2

Mattison, J. A., Colman, R. J., Beasley, T. M., Allison, D. B., Kemnitz, J. W., Roth, G. S., . . . Anderson, R. M. (2017). Caloric restriction improves health and survival of rhesus monkeys. *Nature communications, 8*(1), 1-12.

Mazurak, N., Gunther, A., Grau, F. S., Muth, E. R., Pustovoyt, M., Bischoff, S. C., . . . Enck, P. (2013). Effects of a 48-h fast on heart rate variability and cortisol levels in healthy female subjects. *Eur J Clin Nutr, 67*(4), 401-406. doi:10.1038/ejcn.2013.32

McCorry, L. K. (2007). Physiology of the autonomic nervous system. *American journal of pharmaceutical education, 71*(4).

Merimee, T. J., Misbin, R. I., & Pulkkinen, A. J. (1978). Sex variations in free fatty acids and ketones during fasting: evidence for a role of glucagon. *J Clin Endocrinol Metab, 46*(3), 414-419. doi:10.1210/jcem-46-3-414

Minson, C. T., Halliwill, J. R., Young, T. M., & Joyner, M. J. (2000). Influence of the Menstrual Cycle on Sympathetic Activity, Baroreflex Sensitivity,

and Vascular Transduction in Young Women. *Circulation, 101*(8), 862-868. doi:doi:10.1161/01.CIR.101.8.862

Mitchell, G. F., Wang, N., Palmisano, J. N., Larson, M. G., Hamburg, N. M., Vita, J. A., . . . Vasan, R. S. (2010). Hemodynamic Correlates of Blood Pressure Across the Adult Age Spectrum. *Circulation, 122*(14), 1379-1386. doi:doi:10.1161/CIRCULATIONAHA.109.914507

Monahan, K. D., Dinenno, F. A., Tanaka, H., Clevenger, C. M., DeSouza, C. A., & Seals, D. R. (2000). Regular aerobic exercise modulates age-associated declines in cardiovagal baroreflex sensitivity in healthy men. *The Journal of Physiology, 529 Pt 1*(Pt 1), 263-271. doi:10.1111/j.1469-7793.2000.00263.x

Moore, S. C., Patel, A. V., Matthews, C. E., de Gonzalez, A. B., Park, Y., Katki, H. A., . . . Helzlsouer, K. J. (2012). Leisure time physical activity of moderate to vigorous intensity and mortality: a large pooled cohort analysis. *PLoS Med, 9*(11), e1001335.

Moro, T., Tinsley, G., Bianco, A., Marcolin, G., Pacelli, Q. F., Battaglia, G., . . . Paoli, A. (2016). Effects of eight weeks of time-restricted feeding (16/8) on basal metabolism, maximal strength, body composition, inflammation, and cardiovascular risk factors in resistance-trained males. *Journal of Translational Medicine, 14*(1), 290. doi:10.1186/s12967-016-1044-0

Mortara, A., La Rovere, M. T., Pinna, G. D., Prpa, A., Maestri, R., Febo, O., . . . Tavazzi, L. (1997). Arterial baroreflex modulation of heart rate in chronic heart failure: clinical and hemodynamic correlates and prognostic implications. *Circulation, 96*(10), 3450-3458.

Muller, M. D., Sauder, C. L., & Ray, C. A. (2013). Mental stress elicits sustained and reproducible increases in skin sympathetic nerve activity. *Physiological reports, 1*(1).

Narkiewicz, K., Phillips, B. G., Kato, M., Hering, D., Bieniaszewski, L., & Somers, V. K. (2005). Gender-Selective Interaction Between Aging, Blood Pressure, and Sympathetic Nerve Activity. *Hypertension, 45*(4), 522-525. doi:doi:10.1161/01.HYP.0000160318.46725.46

Natalucci, G., Riedl, S., Gleiss, A., Zidek, T., & Frisch, H. (2005). Spontaneous 24-h ghrelin secretion pattern in fasting subjects: maintenance of a meal-related pattern. *Eur J Endocrinol, 152*(6), 845-850. doi:10.1530/eje.1.01919

Nunan, D., Sandercock, G. R., & Brodie, D. A. (2010). A quantitative systematic review of normal values for short-term heart rate variability in healthy adults. *Pacing and clinical electrophysiology, 33*(11), 1407-1417.

O'Dea, K., Esler, M., Leonard, P., Stockigt, J., & Nestel, P. (1982). Noradrenaline turnover during under-and over-eating in normal weight subjects. *Metabolism, 31*(9), 896-899.

O'Brien, E., Parati, G., & Stergiou, G. (2013). Ambulatory Blood Pressure Measurement. *Hypertension, 62*(6), 988-994. doi:doi:10.1161/HYPERTENSIONAHA.113.02148

Ogden, C. L., Fakhouri, T. H., Carroll, M. D., Hales, C. M., Fryar, C. D., Li, X., & Freedman, D. S. (2017). Prevalence of Obesity Among Adults, by Household Income and Education - United States, 2011-2014. *MMWR Morb Mortal Wkly Rep, 66*(50), 1369-1373. doi:10.15585/mmwr.mm6650a1

Ohara, K., Okita, Y., Kouda, K., Mase, T., Miyawaki, C., & Nakamura, H. (2015). Cardiovascular response to short-term fasting in menstrual phases in young women: an observational study. *BMC women's health, 15*, 67-67. doi:10.1186/s12905-015-0224-z

Pagani, M., Montano, N., Porta, A., Malliani, A., Abboud, F. M., Birkett, C., & Somers, V. K. (1997). Relationship Between Spectral Components of Cardiovascular Variabilities and Direct Measures of Muscle Sympathetic Nerve Activity in Humans. *Circulation, 95*(6), 1441-1448. doi:doi:10.1161/01.CIR.95.6.1441

Palamarchuk, I., Ives, C. T., Hachinski, V., & Kimpinski, K. (2014). Baroreflex sensitivity: reliability of baroreflex components of the Valsalva maneuver. *Auton Neurosci, 185*, 138-140. doi:10.1016/j.autneu.2014.05.002

Parlow, J., Viale, J.-P., Annat, G., Hughson, R., & Quintin, L. (1995). Spontaneous cardiac baroreflex in humans. Comparison with drug-induced responses. *Hypertension, 25*, 1058-1068.

Patel, J. N., Eisenhofer, G., Coppack, S. W., & Miles, J. M. (1999). Norepinephrine spillover in forearm and subcutaneous adipose tissue before and after eating. *J Clin Endocrinol Metab, 84*(8), 2815-2819. doi:10.1210/jcem.84.8.5882

Peeters, A., Barendregt, J. J., Willekens, F., Mackenbach, J. P., Mamun, A. A., & Bonneux, L. (2003). Obesity in Adulthood and Its Consequences for Life Expectancy: A Life-Table Analysis. *Annals of internal medicine, 138*(1), 24-32. doi:10.7326/0003-4819-138-1-200301070-00008

Penev, P., Spiegel, K., Marcinkowski, T., & Van Cauter, E. (2005). Impact of Carbohydrate-Rich Meals on Plasma Epinephrine Levels: Dysregulation with Aging. *The Journal of Clinical Endocrinology & Metabolism, 90*(11), 6198-6206. doi:10.1210/jc.2005-0415

Piccirillo, G., Munizzi, M. R., Fimognari, F. L., & Marigliano, V. (1996). Heart rate variability in hypertensive subjects. *International Journal of Cardiology, 53*(3), 291-298.

Pitzalis, M. V., Mastropasqua, F., Massari, F., Forleo, C., Di Maggio, M., Passantino, A., . . . Rizzon, P. (1996). Short- and long-term reproducibility of time and frequency domain heart rate variability measurements in normal subjects. *Cardiovascular Research, 32*(2), 226-233. doi:10.1016/0008-6363(96)00086-7

Poirier, P., Giles, T. D., Bray, G. A., Hong, Y., Stern, J. S., Pi-Sunyer, F. X., . . . Metabolism. (2006). Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss: an update of the 1997 American Heart Association Scientific Statement on Obesity and Heart Disease from the Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism. *Circulation, 113*(6), 898-918. doi:10.1161/CIRCULATIONAHA.106.171016

Pomeranz, B., Macaulay, R. J. B., Caudill, M. A., Kutz, I., Adam, D., Gordon, D., . . . Benson, H. (1985). Assessment of autonomic function in humans by heart rate spectral analysis. *Am. J. Physiol, 248*, H151-H153.

Pontet, J., Contreras, P., Curbelo, A., Medina, J., Noveri, S., Bentancourt, S., & Migliaro, E. R. (2003). Heart rate variability as early marker of multiple organ dysfunction syndrome in septic patients. *Journal of Critical Care, 18*(3), 156-163. doi:https://doi.org/10.1016/j.jcrc.2003.08.005

Practice Guidelines for Preoperative Fasting and the Use of Pharmacologic Agents to Reduce the Risk of Pulmonary Aspiration: Application to Healthy Patients Undergoing Elective Procedures: An Updated Report by the American Society of Anesthesiologists Task Force on Preoperative Fasting and the Use of Pharmacologic Agents to Reduce the Risk of Pulmonary Aspiration*. (2017). *Anesthesiology, 126*(3), 376-393. doi:10.1097/aln.0000000000001452

Prentice, A. M., & Jebb, S. A. (2003). Fast foods, energy density and obesity: a possible mechanistic link. *Obesity Reviews, 4*(4), 187-194. doi:https://doi.org/10.1046/j.1467-789X.2003.00117.x

Ray, C. A., & Carter, J. R. (2010). Effects of aerobic exercise training on sympathetic and renal responses to mental stress in humans. *Am J Physiol Heart Circ Physiol, 298*(1), H229-234. doi:10.1152/ajpheart.00880.2009

Reid, C., Nelson, M. R., Shiel, L., Chew, D., Connor, G., & DeLooze, F. (2008). Australians at Risk: Management of Cardiovascular Risk Factors in the REACH Registry. *Heart, Lung and Circulation, 17*(2), 114-118. doi:https://doi.org/10.1016/j.hlc.2007.07.009

Reimers, C. D., Knapp, G., & Reimers, A. K. (2012). Does physical activity increase life expectancy? A review of the literature. *Journal of aging research, 2012*, 243958-243958. doi:10.1155/2012/243958

Robertson, D., Wade, D., & Robertson, R. M. (1981). Postprandial alterations in cardiovascular hemodynamics in autonomic dysfunction states. *Am J Cardiol, 48*(6), 1048-1052. doi:10.1016/0002-9149(81)90319-2

Rocchini, A. P., Moorehead, C. P., DeRemer, S., & Bondie, D. (1989). Pathogenesis of weight-related changes in blood pressure in dogs. *Hypertension, 13*(6_pt_2), 922-928. doi:doi:10.1161/01.HYP.13.6.922

Rodrigues, J. A. L., Yamane, A. C., Gonçalves, T. C. P., Kalva-Filho, C., Papoti, M., & Júnior, C. R. B. (2019). Fed and fasted states on heart rate variability, hemodynamic heart rate and blood pressure in adults submitted to moderate aerobic exercise. *IJC Heart & Vasculature, 23*, 100378. doi:https://doi.org/10.1016/j.ijcha.2019.100378

Rovere, M. T. L., Pinna, G. D., Hohnloser, S. H., Marcus, F. I., Mortara, A., Nohara, R., . . . Schwartz, P. J. (2001). Baroreflex Sensitivity and Heart Rate Variability in the Identification of Patients at Risk for Life-Threatening Arrhythmias. *Circulation, 103*(16), 2072-2077. doi:doi:10.1161/01.CIR.103.16.2072

Rovere, M. T. L., Pinna, G. D., Maestri, R., Mortara, A., Capomolla, S., Febo, O., . . . Cobelli, F. (2003). Short-Term Heart Rate Variability Strongly Predicts Sudden Cardiac Death in Chronic Heart Failure Patients. *Circulation, 107*(4), 565-570. doi:doi:10.1161/01.CIR.0000047275.25795.17

Rowell, L. B. (1993). *Human cardiovascular control*: Oxford University Press, USA.

Samad, F., Qazi, F., Pervaiz, M. B., Kella, D. K., Mansoor, M., Osmani, B. Z., . . . Kadir, M. M. (2015). EFFECTS OF RAMADAN FASTING ON BLOOD PRESSURE IN NORMOTENSIVE MALES. *Journal of Ayub Medical College, Abbottabad : JAMC, 27*(2), 338-342. Retrieved from http://europepmc.org/abstract/MED/26411111

Sanders, J. S., Ferguson, D. W., & Mark, A. L. (1988). Arterial baroreflex control of sympathetic nerve activity during elevation of blood pressure in normal man: dominance of aortic baroreflexes. *Circulation, 77*(2), 279-288.

Shaffer, F., McCraty, R., & Zerr, C. L. (2014). A healthy heart is not a metronome: an integrative review of the heart's anatomy and heart rate variability. *Frontiers in Psychology, 5*(1040). doi:10.3389/fpsyg.2014.01040

Shykoff, B. E., Naqvi, S. S., Menon, A. S., & Slutsky, A. S. (1991). Respiratory sinus arrhythmia in dogs. Effects of phasic afferents and chemostimulation. *The Journal of Clinical Investigation, 87*(5), 1621-1627. doi:10.1172/JCI115176

Sidery, M. B., Macdonald, I. A., Cowley, A. J., & Fullwood, L. J. (1991). Cardiovascular responses to high-fat and high-carbohydrate meals in young subjects. *Am J Physiol, 261*(5 Pt 2), H1430-1436. doi:10.1152/ajpheart.1991.261.5.H1430

Silva, L. E. V., Dias, D. P. M., da Silva, C. A. A., Salgado, H. C., & Fazan, R., Jr. (2019). Revisiting the Sequence Method for Baroreflex Analysis. *Frontiers in neuroscience, 13*, 17-17. doi:10.3389/fnins.2019.00017

Skarphedinsson, J. O., Elam, M., Jungersten, L., & Wallin, B. G. (1997). Sympathetic nerve traffic correlates with the release of nitric oxide in humans: implications for blood pressure control. *The Journal of*

Physiology, 501(3), 671-675. doi:https://doi.org/10.1111/j.1469-7793.1997.671bm.x
Solianik, R., Sujeta, A., Terentjeviene, A., & Skurvydas, A. (2016). Effect of 48 h Fasting on Autonomic Function, Brain Activity, Cognition, and Mood in Amateur Weight Lifters. *Biomed Res Int, 2016*, 1503956. doi:10.1155/2016/1503956
Someya, N., Endo, M. Y., Fukuba, Y., Hirooka, Y., & Hayashi, N. (2010). Effects of a mental task on splanchnic blood flow in fasting and postprandial conditions. *European journal of applied physiology, 108*(6), 1107-1113.
Spence, J., LeManach, Y., Chan, M. T. V., Wang, C. Y., Sigamani, A., Xavier, D., . . . Devereaux, P. J. (2019). Association between complications and death within 30 days after noncardiac surgery. *Canadian Medical Association Journal, 191*(30), E830. doi:10.1503/cmaj.190221
Stekovic, S., Hofer, S. J., Tripolt, N., Aon, M. A., Royer, P., Pein, L., . . . Url, J. (2019). Alternate day fasting improves physiological and molecular markers of aging in healthy, non-obese humans. *Cell metabolism, 30*(3), 462-476. e466.
Stimson, R. H., Mohd-Shukri, N. A., Bolton, J. L., Andrew, R., Reynolds, R. M., & Walker, B. R. (2014). The Postprandial Rise in Plasma Cortisol in Men Is Mediated by Macronutrient-Specific Stimulation of Adrenal and Extra-Adrenal Cortisol Production. *The Journal of Clinical Endocrinology & Metabolism, 99*(1), 160-168. doi:10.1210/jc.2013-2307
Sundlof, G., & Wallin, B. G. (1977). The variability of muscle nerve sympathetic activity in resting recumbent man. *J. Physiol, 272*, 383-397.
Sundlof, G., & Wallin, B. G. (1978). Human muscle nerve sympathetic activity at rest. Relationship to blood pressure and age. *J. Physiol, 274*, 621-637.
Tonacio, A., Trombetta, I., Rondon, M., Batalha, L., Kuniyoshi, F., Laterza, M., . . . Halpern, A. (2006). Effects of diet and exercise training on neurovascular control during mental stress in obese women. *Brazilian Journal of Medical and Biological Research, 39*, 53-62.
Torres, S. J., & Nowson, C. A. (2007). Effect of a weight-loss program on mental stress–induced cardiovascular responses and recovery. *Nutrition, 23*(7), 521-528. doi:https://doi.org/10.1016/j.nut.2007.04.016
Truijen, J., van Lieshout, J. J., Wesselink, W. A., & Westerhof, B. E. (2012). Noninvasive continuous hemodynamic monitoring. *Journal of clinical monitoring and computing, 26*(4), 267-278. doi:10.1007/s10877-012-9375-8
Vallbo, Å. B., Hagbarth, K.-E., & Wallin, B. G. (2004). Microneurography: how the technique developed and its role in the investigation of the

sympathetic nervous system. *Journal of Applied Physiology, 96*(4), 1262-1269. doi:10.1152/japplphysiol.00470.2003

van Baak, M. A. (2008). Meal-induced activation of the sympathetic nervous system and its cardiovascular and thermogenic effects in man. *Physiol Behav, 94*(2), 178-186. doi:10.1016/j.physbeh.2007.12.020

van Wijnen, V. K., Finucane, C., Harms, M. P. M., Nolan, H., Freeman, R. L., Westerhof, B. E., . . . Wieling, W. (2017). Noninvasive beat-to-beat finger arterial pressure monitoring during orthostasis: a comprehensive review of normal and abnormal responses at different ages. *Journal of Internal Medicine, 282*(6), 468-483. doi:https://doi.org/10.1111/joim.12636

Varady, K. A., Bhutani, S., Klempel, M. C., Kroeger, C. M., Trepanowski, J. F., Haus, J. M., . . . Calvo, Y. (2013). Alternate day fasting for weight loss in normal weight and overweight subjects: a randomized controlled trial. *Nutrition journal, 12*(1), 146.

Varady, K. A., & Hellerstein, M. K. (2007). Alternate-day fasting and chronic disease prevention: a review of human and animal trials. *The American Journal of Clinical Nutrition, 86*(1), 7-13. doi:10.1093/ajcn/86.1.7

Vaz, M., Jennings, G., Turner, A., Cox, H., Lambert, G., & Esler, M. (1997). Regional Sympathetic Nervous Activity and Oxygen Consumption in Obese Normotensive Human Subjects. *Circulation, 96*(10), 3423-3429. doi:doi:10.1161/01.CIR.96.10.3423

Wallin, B., Thompson, J., Jennings, G., & Esler, M. (1996). Renal noradrenaline spillover correlates with muscle sympathetic activity in humans. *The Journal of Physiology, 491*(3), 881-887.

Wallin, B. G., Esler, M., Dorward, P., Eisenhofer, G., Rerrier, C., Westerman, R., & Jennings, G. (1992). Simultaneous measurements of cardiac noradrenaline spillover and sympathetic outflow to skeletal muscle in humans. *J. Physiol, 453*, 45-58.

Wallin, B. G., Kunimoto, M. M., & Sellgren, J. (1993). Possible genetic influence on the strength of human muscle nerve sympathetic activity at rest. *Hypertension, 22*(3), 282-284. doi:doi:10.1161/01.HYP.22.3.282

Wallin, B. G., Sundlöf, G., & Delius, W. (1975). The effect of carotid sinus nerve stimulation on muscle and skin nerve sympathetic activity in man. *Pflugers Arch, 358*(2), 101-110. doi:10.1007/bf00583921

Waseem, T., Mogensen, K. M., Lautz, D. B., & Robinson, M. K. (2007). Pathophysiology of Obesity: Why Surgery Remains the Most Effective Treatment. *Obesity Surgery, 17*(10), 1389. doi:10.1007/s11695-007-9220-1

Watkins, E., & Serpell, L. (2016). The Psychological Effects of Short-Term Fasting in Healthy Women. *Frontiers in nutrition, 3*, 27-27. doi:10.3389/fnut.2016.00027

Webber, J., & Macdonald, I. A. (1994). The cardiovascular, metabolic and hormonal changes accompanying acute starvation in men and women. *British Journal of Nutrition, 71*(3), 437-447. doi:10.1079/BJN19940150
Webber, J., Taylor, J., Greathead, H., Dawson, J., Buttery, P., & Macdonald, I. (1995). The effects of fasting on the thermogenic, metabolic and cardiovascular responses to infused adrenaline. *British Journal of Nutrition, 74*(4), 477-490.
Wehrwein, E. A., Orer, H. S., & Barman, S. M. (2011). Overview of the anatomy, physiology, and pharmacology of the autonomic nervous system. *Comprehensive Physiology, 6*(3), 1239-1278.
Westerveld, H. (1998). Estrogens and postprandial lipid metabolism. *Atherosclerosis, 141*, S105-S107.
Whitney, R. (1953). The measurement of volume changes in human limbs. *The Journal of Physiology, 121*(1), 1-27.
Wiinberg, N., Høegholm, A., Christensen, H. R., Bang, L. E., Mikkelsen, K. L., Nielsen, P. E., . . . Bentzon, M. W. (1995). 24-h ambulatory blood pressure in 352 normal Danish subjects, related to age and gender. *American journal of hypertension, 8*(10), 978-986.
William Tank, A., & Lee Wong, D. (2011). Peripheral and central effects of circulating catecholamines. *Comprehensive Physiology, 5*(1), 1-15.
Wilson, P. W. (1997). An epidemiologic perspective of systemic hypertension, ischemic heart disease, and heart failure. *The American journal of cardiology, 80*(9), 3J-8J.
Wright, J. D., Kennedy-Stephenson, J., Wang, C.-Y., McDowell, M., & Johnson, C. L. (2004). Trends in intake of energy and macronutrients-United States, 1971-2000. *MMWR: Morbidity & Mortality Weekly Report, 53*(4), 80-80.
Yamazaki, F., Yamauchi, K., Tsutsui, Y., Endo, Y., Sagawa, S., & Shiraki, K. (2003). Whole body heating reduces the baroreflex response of sympathetic nerve activity during Valsalva straining. *Auton Neurosci, 103*(1-2), 93-99. doi:10.1016/s1566-0702(02)00140-6
Yan, L. L., Liu, K., Matthews, K. A., Daviglus, M. L., Ferguson, T. F., & Kiefe, C. I. (2003). Psychosocial factors and risk of hypertension: the Coronary Artery Risk Development in Young Adults (CARDIA) study. *JAMA, 290*(16), 2138-2148. doi:10.1001/jama.290.16.2138
Yang, H., Durocher, J. J., Larson, R. A., Dellavalla, J. P., & Carter, J. R. (2012). Total sleep deprivation alters cardiovascular reactivity to acute stressors in humans. *Journal of applied physiology (Bethesda, Md. : 1985), 113*(6), 903-908. doi:10.1152/japplphysiol.00561.2012
Yates, B. J., Bolton, P. S., & Macefield, V. G. (2014). Vestibulo-sympathetic responses. *Comprehensive Physiology, 4*(2), 851-887. doi:10.1002/cphy.c130041
Young, C. N., Deo, S. H., Chaudhary, K., Thyfault, J. P., & Fadel, P. J. (2010). Insulin enhances the gain of arterial baroreflex control of

muscle sympathetic nerve activity in humans. *J Physiol, 588*(Pt 18), 3593-3603. doi:10.1113/jphysiol.2010.191866
Young, J. B., & Landsberg, L. (1977). Suppression of sympathetic nervous system during fasting. *Science, 196*(4297), 1473-1475.
Young, J. B., Rosa, R. M., & Landsberg, L. (1984). Dissociation of sympathetic nervous system and adrenal medullary responses. *American Journal of Physiology-Endocrinology and Metabolism, 247*(1), E35-E40. doi:10.1152/ajpendo.1984.247.1.E35
Zouhal, H., Jacob, C., Delamarche, P., & Gratas-Delamarche, A. (2008). Catecholamines and the effects of exercise, training and gender. *Sports medicine, 38*(5), 401-423.

A APPENDICES

A.1 Example Subject Information Sheet

ID code: _____Sex: _____ Age: _____

Weight History
Most recent weight: _____
Height: _____

Medical History:
Have you been diagnosed with any nutrition related conditions (ie: eating disorder, celiac disease, etc)?

_____

Do you take any vitamin, mineral, or dietary supplements? If so, please list

_____

_____

Physical Activity: Circle your current physical activity level
Sedentary **Mild** (15-30 minutes 2-3x per week) **Moderate** (30-60 min 3-4x per week)
Heavy (>60 min 5-7x per week) **Extreme** (Very demanding/difficult to achieve, >2 hours daily)

Diet History:
1. List any food allergies:_____
2. Foods you avoid for religious, personal, cultural reasons:_____
3. Circle the types of foods you eat and how many times in a typical week:
   - Home-cooked meals _____
   - Fast foods/ Take-out _____
   - Restaurants_____

4. Do you skip meals? Yes or No If yes, how many meals per week?

_____

5. Do you snack? Yes or No If yes, how many times per day do you snack?

_____

6. Please provide an example of what you typically eat in a 24 hour period:
Breakfast: _____
Lunch: _____
Dinner:_____
Snacks: _____

7. Is there anything else that you would like us to know about your eating habits?

_____

A.2 Calories to Points Calculation Example

Activity Factor:

1.2	*Sedentary*	
1.375	*Mild*	15-30 minutes, 2-3x per week
1.55	*Moderate*	30-60 minutes, 3-4x per week
1.725	*Heavy*	>60 minutes, 5-7x per week
1.9	*Extreme*	very demanding/difficult to achieve, >2 hours daily

Choosen Factor: 1.2

Height:	170	cm		inches
Weights:	80	kg		
BMI:	27.7	kg/m^2		
Age	20			

IF BMI IS ABOVE 30, USE BELOW EQUATION!!

Ideal Body Weight

Men	-88	kg
Women	-92.5	kg

Adjusted Weight

Men	-24.2	kg
Women	-37.3	kg

Resting Metabolic Rate

Men	727.7	calories
Women	430.5	calories

Calorie needs

Men	873.3	calories
Women	516.6	calories

Points Alloted

9

IF BMI IS BELOW 30, USE BELOW EQUATI

Resting Metabolic Rate

Men	1768.3	calories
Women	1602.3	calories

Calorie needs

Men	2122.0	calories
Women	1922.8	calories

Cal Need	Cal Lunch	Points allowed
1500	500	7
1700	565	8
1900	630	9
2100	700	10
2300	765	11
2500	830	12
2700	900	13
2900	965	13
3100	1030	14
3300	1100	15
3500	1165	16

A.3 Subway Lunch

Lunch Options:

Guidelines: Must choose 1 main entree. If you have points remaining, you may choose between sides or sauces to complete the meal plan.

For example if your meal plan consists of 8 points, an acceptable option would be:

1) 6" Turkey Sub (Cheese + Veggies + Light Mayo **(6)** + Baked Lays chips **(2)**

Your Meal Plan: _____

Please circle food choices from each group:

Main Entree	**Sides**	**Salad Dressing/Sauce**
Sub Sandwiches: Bread/Veggies of choice 6" Turkey Breast Sub **(4)** Wrap **(5)** 12" Sub **(8)** 6" Black Forest Ham Sub **(4)** Wrap **(5)** 12" Sub **(8)** 6" Veggie Delight Sub **(4)** Wrap **(5)** 12 " Sub **(8)** **Do you want cheese? Add additional points: 6" sub/wrap = **1** 12" sub = **2**	Baked Lays Chips **(2)** Sun Chips **(3)** Lays Classic Chips **(4)** Doritos Nacho Cheese **(4)** Cookie -Chocolate Chip **(3)** -Oatmeal Raisin **(3)** -Peanut butter **(3)**	Vinegar **(0)** Light Mayonnaise **(1)** Honey Mustard **(1)** Oil **(1)** Sweet Onion **(1)** Mustard **(1)** Guacamole **(1)** Mayonnaise **(2)** Ranch **(2)** Italian **(2)** Chipotle Southwest **(2)**

A.4 LBNP Normalization Procedure

	-30 mmHg Presyncope By Condition (1 fed 2 fasted)	-45 mmHg Presyncope By Condition (1 fed 1 fasted)	-60 mmHg Presyncope By Condition (7 Fed 7 Fasted)	-70 mmHg Presyncope By Condition (6 Fasted 4 Fed)	-80 mmHg Presyncope By Condition (5 Fed 2 Fasted)
0% Data Points Fed = 18 Fasted = 18	Base	Base	Base	Base	Base
20% Fed = 9 Fasted = 8				-15	-15
40% Fed = 18 Fasted= 18	-15	-15	-15	-30	-30
60% Fed= 16 Fasted = 15			-30	-45	-60
80% Fed=17 Fasted = 16		-30	-45	-60	-70
100% Fed = 18 Fasted= 18	-30	-45	-60	-70	-80

B BLOOD BIOMARKER STATISTICS

Paired t-test: Wednesday, May 12, 2021 9:38:59 AM

Data source: Data 2 in Notebook1

Dependent Variable: Weight

Normality Test (Shapiro-Wilk): Passed (P = 0.114)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	25	0	76.340	15.385	3.077
2.000	25	0	75.620	15.179	3.036
Difference	25	0	0.720	3.192	0.638

t = 1.128 with 24 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -0.598 to 2.038

Two-tailed P-value = 0.271

The change that occurred with the treatment is not great enough to exclude the possibility that the difference is due to chance (P = 0.271)

One-tailed P-value = 0.135

Paired t-test: Wednesday, May 12, 2021 9:39:25 AM

Data source: Data 2 in Notebook1

Dependent Variable: Urine SG

Normality Test (Shapiro-Wilk): Passed (P = 0.548)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	25	2	1.015	0.00857	0.00179
2.000	25	2	1.011	0.00933	0.00194
Difference	25	3	0.00360	0.0102	0.00218

t = 1.654 with 21 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -0.000927 to 0.00814

Two-tailed P-value = 0.113

The change that occurred with the treatment is not great enough to exclude the possibility that the difference is due to chance (P = 0.113)

One-tailed P-value = 0.0565

Paired t-test: Wednesday, May 12, 2021 9:39:39 AM

Data source: Data 2 in Notebook1

Dependent Variable: TC

Normality Test (Shapiro-Wilk): Passed (P = 0.271)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	25	0	172.400	35.210	7.042
2.000	25	0	172.720	29.145	5.829
Difference	25	0	-0.320	20.083	4.017

t = -0.0797 with 24 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -8.610 to 7.970

Two-tailed P-value = 0.937

The change that occurred with the treatment is not great enough to exclude the possibility that the difference is due to chance (P = 0.937)

One-tailed P-value = 0.469

Paired t-test: Wednesday, May 12, 2021 9:39:56 AM

Data source: Data 2 in Notebook1

Dependent Variable: LDL

Normality Test (Shapiro-Wilk): Passed (P = 0.933)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	25	3	95.636	29.904	6.376
2.000	25	5	105.450	21.466	4.800
Difference	25	8	-5.882	20.448	4.959

t = -1.186 with 16 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -16.396 to 4.631

Two-tailed P-value = 0.253

The change that occurred with the treatment is not great enough to exclude the possibility that the difference is due to chance (P = 0.253)

One-tailed P-value = 0.126

Paired t-test: Wednesday, May 12, 2021 9:39:45 AM

Data source: Data 2 in Notebook1

Dependent Variable: HDL

Normality Test (Shapiro-Wilk): Passed (P = 0.444)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	25	0	53.840	18.991	3.798
2.000	25	0	56.240	20.268	4.054
Difference	25	0	-2.400	8.098	1.620

t = -1.482 with 24 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -5.743 to 0.943

Two-tailed P-value = 0.151

The change that occurred with the treatment is not great enough to exclude the possibility that the difference is due to chance (P = 0.151)

One-tailed P-value = 0.0757

Paired t-test: Wednesday, May 12, 2021 9:40:03 AM

Data source: Data 2 in Notebook1

Dependent Variable: TRG

Normality Test (Shapiro-Wilk): Failed (P < 0.050)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	25	0	120.800	61.553	12.311
2.000	25	0	77.160	37.888	7.578
Difference	25	0	43.640	55.323	11.065

t = 3.944 with 24 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: 20.804 to 66.476

Two-tailed P-value = 0.000607

The change that occurred with the treatment is greater than would be expected by chance; there is a statistically significant change (P = <0.001)

One-tailed P-value = 0.000303

Paired t-test: Wednesday, May 12, 2021 9:40:17 AM

Data source: Data 2 in Notebook1

Dependent Variable: GLU

Normality Test (Shapiro-Wilk): Passed (P = 0.271)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	25	0	98.760	11.699	2.340
2.000	25	0	80.880	7.960	1.592
Difference	25	0	17.880	13.163	2.633

t = 6.792 with 24 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: 12.446 to 23.314

Two-tailed P-value = 0.000000504

The change that occurred with the treatment is greater than would be expected by chance; there is a statistically significant change (P = <0.001)

One-tailed P-value = 0.000000252

Paired t-test: Wednesday, May 12, 2021 9:40:23 AM

Data source: Data 2 in Notebook1

Dependent Variable: Blood Ketone

Normality Test (Shapiro-Wilk): Failed (P < 0.050)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	25	0	0.128	0.131	0.0262
2.000	25	0	0.536	0.488	0.0976
Difference	25	0	-0.408	0.415	0.0831

t = -4.913 with 24 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -0.579 to -0.237

Two-tailed P-value = 0.0000519

The change that occurred with the treatment is greater than would be expected by chance; there is a statistically significant change (P = <0.001)

One-tailed P-value = 0.0000259

Wilcoxon test	
Table Analyzed	TRG
Column B	Fasted
vs.	vs.
Column A	Fed
Wilcoxon matched-pairs signed rank test	
P value	0.0033
Exact or approximate P value?	Exact
P value summary	**
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of positive, negative ranks	57.00 , -268.0
Sum of signed ranks (W)	-211.0
Number of pairs	25
Number of ties (ignored)	0
Median of differences	
Median	-36.00
How effective was the pairing?	
rs (Spearman)	0.09613
P value (one tailed)	0.3238
P value summary	ns
Was the pairing significantly effective?	No

Wilcoxon test	
Table Analyzed	Ketones
Column B	Fasted
vs.	vs.
Column A	Fed
Wilcoxon matched-pairs signed rank test	
P value	<0.0001
Exact or approximate P value?	Exact
P value summary	****
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of positive, negative ranks	253.0 , 0.000
Sum of signed ranks (W)	253.0
Number of pairs	25
Number of ties (ignored)	3
Median of differences	
Median	0.3000
How effective was the pairing?	
rs (Spearman)	0.2687
P value (one tailed)	0.0970
P value summary	ns
Was the pairing significantly effective?	No

C ACTIGRAPHY STATISTICS

Paired t-test: Wednesday, May 12, 2021 9:48:11 AM

Data source: Data 1 in Notebook1

Dependent Variable: Time in Bed

Normality Test (Shapiro-Wilk): Passed (P = 0.380)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	21	0	7.666	1.655	0.361
2.000	21	0	8.935	1.905	0.416
Difference	21	0	-1.270	1.870	0.408

t = -3.111 with 20 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -2.121 to -0.418

Two-tailed P-value = 0.00551

The change that occurred with the treatment is greater than would be expected by chance; there is a statistically significant change (P = 0.006)

One-tailed P-value = 0.00275

Paired t-test: Wednesday, May 12, 2021 9:48:25 AM

Data source: Data 1 in Notebook1

Dependent Variable: Total Sleep

Normality Test (Shapiro-Wilk): Passed (P = 0.492)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	21	0	6.560	1.553	0.339
2.000	21	0	7.611	1.602	0.350
Difference	21	0	-1.051	1.725	0.376

t = -2.791 with 20 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -1.836 to -0.266

Two-tailed P-value = 0.0113

The change that occurred with the treatment is greater than would be expected by chance; there is a statistically significant change (P = 0.011)

One-tailed P-value = 0.00563

Paired t-test: Wednesday, May 12, 2021 9:48:38 AM

Data source: Data 1 in Notebook1

Dependent Variable: Effciency %

Normality Test (Shapiro-Wilk): Passed (P = 0.212)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	21	0	84.732	6.706	1.463
2.000	21	0	85.390	4.380	0.956
Difference	21	0	-0.658	5.827	1.272

t = -0.518 with 20 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -3.310 to 1.994

Two-tailed P-value = 0.610

The change that occurred with the treatment is not great enough to exclude the possibility that the difference is due to chance (P = 0.610)

One-tailed P-value = 0.305

Paired t-test: Wednesday, May 12, 2021 9:48:33 AM

Data source: Data 1 in Notebook1

Dependent Variable: Latency

Normality Test (Shapiro-Wilk): Passed (P = 0.209)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	21	1	13.475	16.460	3.681
2.000	21	0	22.000	17.813	3.887
Difference	21	1	-8.550	24.807	5.547

t = -1.541 with 19 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -20.160 to 3.060

Two-tailed P-value = 0.140

The change that occurred with the treatment is not great enough to exclude the possibility that the difference is due to chance (P = 0.140)

One-tailed P-value = 0.0699

Paired t-test: Wednesday, May 12, 2021 9:48:55 AM

Data source: Data 1 in Notebook1

Dependent Variable: #Awak

Normality Test (Shapiro-Wilk): Passed (P = 0.901)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	21	0	30.286	12.042	2.628
2.000	21	0	35.810	14.261	3.112
Difference	21	0	-5.524	11.343	2.475

t = -2.232 with 20 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -10.687 to -0.361

Two-tailed P-value = 0.0372

The change that occurred with the treatment is greater than would be expected by chance; there is a statistically significant change (P = 0.037)

One-tailed P-value = 0.0186

Paired t-test: Wednesday, May 12, 2021 9:48:48 AM

Data source: Data 1 in Notebook1

Dependent Variable: WASO

Normality Test (Shapiro-Wilk): Passed (P = 0.721)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	21	0	36.214	15.491	3.380
2.000	21	0	37.929	17.924	3.911
Difference	21	0	-1.714	16.758	3.657

t = -0.469 with 20 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -9.343 to 5.914

Two-tailed P-value = 0.644

The change that occurred with the treatment is not great enough to exclude the possibility that the difference is due to chance (P = 0.644)

One-tailed P-value = 0.322

Paired t-test: Wednesday, May 12, 2021 9:49:20 AM

Data source: Data 1 in Notebook1

Dependent Variable: Active

Normality Test (Shapiro-Wilk): Failed ($P < 0.050$)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	21	1	612.250	234.587	52.455
2.000	21	0	588.190	361.692	78.928
Difference	21	1	19.600	271.519	60.713

$t = 0.323$ with 19 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -107.475 to 146.675

Two-tailed P-value = 0.750

The change that occurred with the treatment is not great enough to exclude the possibility that the difference is due to chance ($P = 0.750$)

One-tailed P-value = 0.375

Wilcoxon test	
Table Analyzed	Active Minutes
Column B	Fasted
vs.	vs.
Column A	Fed
Wilcoxon matched-pairs signed rank test	
P value	0.1738
Exact or approximate P value?	Exact
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
Sum of positive, negative ranks	68.00 , -142.0
Sum of signed ranks (W)	-74.00
Number of pairs	20
Number of ties (ignored)	0
Median of differences	
Median	-36.00
How effective was the pairing?	
rs (Spearman)	0.7063
P value (one tailed)	0.0003
P value summary	***
Was the pairing significantly effective?	Yes

D AMBULATORY BLOOD PRESSURE STATISTICS

Paired t-test: Wednesday, May 12, 2021 9:36:00 AM

Data source: Data 1 in Notebook1

Dependent Variable: All SAP

Normality Test (Shapiro-Wilk): Passed (P = 0.652)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	20	0	109.970	7.542	1.686
2.000	20	0	107.750	6.584	1.472
Difference	20	0	2.220	4.632	1.036

t = 2.144 with 19 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: 0.0523 to 4.388

Two-tailed P-value = 0.0452

The change that occurred with the treatment is greater than would be expected by chance; there is a statistically significant change (P = 0.045)

One-tailed P-value = 0.0226

Paired t-test: Wednesday, May 12, 2021 9:36:20 AM

Data source: Data 1 in Notebook1

Dependent Variable: ALL DAP

Normality Test (Shapiro-Wilk): Passed (P = 0.960)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	20	0	64.365	5.610	1.254
2.000	20	0	62.750	5.919	1.324
Difference	20	0	1.615	3.818	0.854

t = 1.892 with 19 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -0.172 to 3.402

Two-tailed P-value = 0.0739

The change that occurred with the treatment is not great enough to exclude the possibility that the difference is due to chance (P = 0.074)

One-tailed P-value = 0.0369

Paired t-test: Wednesday, May 12, 2021 9:36:29 AM

Data source: Data 1 in Notebook1

Dependent Variable: All Map

Normality Test (Shapiro-Wilk): Passed (P = 0.702)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	20	0	80.550	5.266	1.178
2.000	20	0	78.500	5.434	1.215
Difference	20	0	2.050	4.136	0.925

t = 2.217 with 19 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: 0.115 to 3.985

Two-tailed P-value = 0.0390

The change that occurred with the treatment is greater than would be expected by chance; there is a statistically significant change (P = 0.039)

One-tailed P-value = 0.0195

Paired t-test: Wednesday, June 23, 2021 8:00:36 PM

Data source: Data 1 in Notebook1

Dependent Variable: ALL PP

Normality Test (Shapiro-Wilk): Passed (P = 0.553)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	20	0	46.600	6.386	1.428
2.000	20	0	45.600	5.698	1.274
Difference	20	0	1.000	2.636	0.589

t = 1.697 with 19 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -0.234 to 2.234

Two-tailed P-value = 0.106

The change that occurred with the treatment is not great enough to exclude the possibility that the difference is due to chance (P = 0.106)

One-tailed P-value = 0.0530

Paired t-test: Wednesday, May 12, 2021 9:36:36 AM

Data source: Data 1 in Notebook1

Dependent Variable: All HR

Normality Test (Shapiro-Wilk): Passed (P = 0.407)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	20	0	68.750	8.397	1.878
2.000	20	0	64.850	9.986	2.233
Difference	20	0	3.900	5.241	1.172

t = 3.328 with 19 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: 1.447 to 6.353

Two-tailed P-value = 0.00353

The change that occurred with the treatment is greater than would be expected by chance; there is a statistically significant change (P = 0.004)

One-tailed P-value = 0.00177

The sample mean of treatment 1.000 exceeds the sample mean of treatment 2.000 by an amount that is greater than would be expected by chance, rejecting the hypothesis that the population mean of treatment 2.000 is greater than or equal to the population mean of treatment 1.000. (P = 0.004)

Paired t-test: Wednesday, May 12, 2021 9:36:43 AM

Data source: Data 1 in Notebook1

Dependent Variable: Wake SAP

Normality Test (Shapiro-Wilk): Failed (P < 0.050)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	20	0	115.050	8.841	1.977
2.000	20	0	112.500	8.332	1.863
Difference	20	0	2.550	5.256	1.175

t = 2.170 with 19 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: 0.0900 to 5.010

Two-tailed P-value = 0.0429

The change that occurred with the treatment is greater than would be expected by chance; there is a statistically significant change (P = 0.043)

One-tailed P-value = 0.0215

Paired t-test: Wednesday, May 12, 2021 9:36:49 AM

Data source: Data 1 in Notebook1

Dependent Variable: Wake DAP

Normality Test (Shapiro-Wilk): Passed (P = 0.169)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	20	0	69.200	6.598	1.475
2.000	20	0	67.750	7.312	1.635
Difference	20	0	1.450	5.708	1.276

t = 1.136 with 19 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -1.221 to 4.121

Two-tailed P-value = 0.270

The change that occurred with the treatment is not great enough to exclude the possibility that the difference is due to chance (P = 0.270)

One-tailed P-value = 0.135

Paired t-test: Wednesday, May 12, 2021 9:36:55 AM

Data source: Data 1 in Notebook1

Dependent Variable: Wake MAP

Normality Test (Shapiro-Wilk): Passed (P = 0.144)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	20	0	84.800	6.709	1.500
2.000	20	0	83.100	6.866	1.535
Difference	20	0	1.700	4.996	1.117

t = 1.522 with 19 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -0.638 to 4.038

Two-tailed P-value = 0.145

The change that occurred with the treatment is not great enough to exclude the possibility that the difference is due to chance (P = 0.145)

One-tailed P-value = 0.0723

Paired t-test: Wednesday, June 23, 2021 8:00:56 PM

Data source: Data 1 in Notebook1

Dependent Variable: Wake PP

Normality Test (Shapiro-Wilk): Failed (P < 0.050)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	20	0	45.900	5.581	1.248
2.000	20	0	43.041	9.287	2.077
Difference	20	0	2.859	6.866	1.535

t = 1.862 with 19 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -0.355 to 6.072

Two-tailed P-value = 0.0782

The change that occurred with the treatment is not great enough to exclude the possibility that the difference is due to chance (P = 0.078)

One-tailed P-value = 0.0391

Paired t-test: Wednesday, May 12, 2021 9:37:33 AM

Data source: Data 1 in Notebook1

Dependent Variable: Wake HR

Normality Test (Shapiro-Wilk): Passed (P = 0.961)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	20	0	73.800	9.094	2.033
2.000	20	0	68.450	10.495	2.347
Difference	20	0	5.350	6.081	1.360

t = 3.935 with 19 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: 2.504 to 8.196

Two-tailed P-value = 0.000890

The change that occurred with the treatment is greater than would be expected by chance; there is a statistically significant change (P = <0.001)

One-tailed P-value = 0.000445

Paired t-test: Wednesday, May 12, 2021 9:37:38 AM

Data source: Data 1 in Notebook1

Dependent Variable: Sleep SAP

Normality Test (Shapiro-Wilk): Passed (P = 0.911)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	20	0	99.750	6.774	1.515
2.000	20	0	98.900	5.438	1.216
Difference	20	0	0.850	6.243	1.396

t = 0.609 with 19 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -2.072 to 3.772

Two-tailed P-value = 0.550

The change that occurred with the treatment is not great enough to exclude the possibility that the difference is due to chance (P = 0.550)

One-tailed P-value = 0.275

Paired t-test: Wednesday, May 12, 2021 9:37:44 AM

Data source: Data 1 in Notebook1

Dependent Variable: Sleep DAP

Normality Test (Shapiro-Wilk): Passed (P = 0.119)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	20	0	54.250	5.180	1.158
2.000	20	0	53.150	4.626	1.034
Difference	20	0	1.100	5.088	1.138

t = 0.967 with 19 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -1.281 to 3.481

Two-tailed P-value = 0.346

The change that occurred with the treatment is not great enough to exclude the possibility that the difference is due to chance (P = 0.346)

One-tailed P-value = 0.173

Paired t-test: Wednesday, May 12, 2021 9:37:49 AM

Data source: Data 1 in Notebook1

Dependent Variable: Sleep MAP

Normality Test (Shapiro-Wilk): Passed (P = 0.678)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	20	0	71.650	4.870	1.089
2.000	20	0	69.950	4.536	1.014
Difference	20	0	1.700	5.232	1.170

t = 1.453 with 19 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -0.749 to 4.149

Two-tailed P-value = 0.163

The change that occurred with the treatment is not great enough to exclude the possibility that the difference is due to chance (P = 0.163)

One-tailed P-value = 0.0813

Paired t-test: Wednesday, May 12, 2021 9:37:55 AM

Data source: Data 1 in Notebook1

Dependent Variable: Sleep HR

Normality Test (Shapiro-Wilk): Passed (P = 0.994)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	20	0	58.850	8.810	1.970
2.000	20	0	57.300	10.443	2.335
Difference	20	0	1.550	6.117	1.368

t = 1.133 with 19 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -1.313 to 4.413

Two-tailed P-value = 0.271

The change that occurred with the treatment is not great enough to exclude the possibility that the difference is due to chance (P = 0.271)

One-tailed P-value = 0.136

Paired t-test: Wednesday, June 23, 2021 8:01:12 PM

Data source: Data 1 in Notebook1

Dependent Variable: Sleep PP

Normality Test (Shapiro-Wilk): Passed (P = 0.735)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	20	0	45.450	5.326	1.191
2.000	20	0	45.200	4.021	0.899
Difference	20	0	0.250	4.908	1.098

t = 0.228 with 19 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -2.047 to 2.547

Two-tailed P-value = 0.822

The change that occurred with the treatment is not great enough to exclude the possibility that the difference is due to chance (P = 0.822)

One-tailed P-value = 0.411

Wilcoxon test	
Table Analyzed	Wake PP
Column B	Data Set-B
vs.	vs.
Column A	Data Set-A
Wilcoxon matched-pairs signed rank test	
P value	0.3705
Exact or approximate P value?	Exact
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
Sum of positive, negative ranks	98.00 , -155.0
Sum of signed ranks (W)	-57.00
Number of pairs	22
Number of ties (ignored)	0
Median of differences	
Median	0.08000
How effective was the pairing?	
rs (Spearman)	0.1910
P value (one tailed)	0.1973
P value summary	ns
Was the pairing significantly effective?	No

Wilcoxon test	
Table Analyzed	Wake SAP
Column B	Fasted
vs.	vs.
Column A	Fed
Wilcoxon matched-pairs signed rank test	
P value	0.0181
Exact or approximate P value?	Exact
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of positive, negative ranks	43.00 , -167.0
Sum of signed ranks (W)	-124.0
Number of pairs	20
Number of ties (ignored)	0
Median of differences	
Median	-3.000
How effective was the pairing?	
rs (Spearman)	0.7177
P value (one tailed)	0.0002
P value summary	***
Was the pairing significantly effective?	Yes

E CONTROLLED BREATHING STATISTICS

Paired t-test: Wednesday, May 12, 2021 9:52:14 AM

Data source: Data 2 in Notebook1

Dependent Variable: RRI

Normality Test (Shapiro-Wilk): Passed (P = 0.101)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	25	0	992.400	151.383	30.277
2.000	25	0	1059.440	186.041	37.208
Difference	25	0	-67.040	160.837	32.167

t = -2.084 with 24 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -133.430 to -0.650

Two-tailed P-value = 0.0480

The change that occurred with the treatment is greater than would be expected by chance; there is a statistically significant change (P = 0.048)

One-tailed P-value = 0.0240

Paired t-test: Wednesday, May 12, 2021 2:13:51 PM

Data source: Data 2 in Actigraphy & 10 min Controlled Breathing.JNB

Dependent Variable: SAP

Normality Test (Shapiro-Wilk): Passed (P = 0.254)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	25	0	107.360	12.305	2.461
2.000	25	0	107.240	11.092	2.218
Difference	25	0	0.120	12.380	2.476

t = 0.0485 with 24 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -4.990 to 5.230

Two-tailed P-value = 0.962

The change that occurred with the treatment is not great enough to exclude the possibility that the difference is due to chance (P = 0.962)

One-tailed P-value = 0.481

Paired t-test: Wednesday, May 12, 2021 2:14:03 PM

Data source: Data 2 in Actigraphy & 10 min Controlled Breathing.JNB

Dependent Variable: DAP

Normality Test (Shapiro-Wilk): Passed (P = 0.892)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	25	0	57.400	9.381	1.876
2.000	25	0	57.720	9.745	1.949
Difference	25	0	-0.320	9.137	1.827

t = -0.175 with 24 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -4.091 to 3.451

Two-tailed P-value = 0.862

The change that occurred with the treatment is not great enough to exclude the possibility that the difference is due to chance (P = 0.862)

One-tailed P-value = 0.431

Paired t-test: Wednesday, May 12, 2021 9:53:19 AM

Data source: Data 2 in Notebook1

Dependent Variable: MSNA b/min

Normality Test (Shapiro-Wilk): Passed (P = 0.649)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	25	12	15.231	11.307	3.136
2.000	25	12	15.538	7.954	2.206
Difference	25	13	1.583	5.744	1.658

t = 0.955 with 11 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -2.066 to 5.233

Two-tailed P-value = 0.360

The change that occurred with the treatment is not great enough to exclude the possibility that the difference is due to chance (P = 0.360)

One-tailed P-value = 0.180

Paired t-test: Wednesday, May 12, 2021 9:53:26 AM

Data source: Data 2 in Notebook1

Dependent Variable: MSNA b/100hb

Normality Test (Shapiro-Wilk): Passed (P = 0.104)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	25	12	23.308	15.829	4.390
2.000	25	12	26.231	13.596	3.771
Difference	25	13	-0.417	7.549	2.179

t = -0.191 with 11 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -5.213 to 4.380

Two-tailed P-value = 0.852

The change that occurred with the treatment is not great enough to exclude the possibility that the difference is due to chance (P = 0.852)

One-tailed P-value = 0.426

Paired t-test: Wednesday, May 12, 2021 9:55:56 AM

Data source: Data 2 in Notebook1

Dependent Variable: RRI nu

Normality Test (Shapiro-Wilk): Passed (P = 0.104)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	25	0	0.545	0.129	0.0257
2.000	25	0	0.618	0.159	0.0318
Difference	25	0	-0.0734	0.173	0.0346

t = -2.126 with 24 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -0.145 to -0.00213

Two-tailed P-value = 0.0440

The change that occurred with the treatment is greater than would be expected by chance; there is a statistically significant change (P = 0.044)

One-tailed P-value = 0.0220

Paired t-test: Wednesday, May 12, 2021 9:56:01 AM

Data source: Data 2 in Notebook1

Dependent Variable: RRI Lfnu

Normality Test (Shapiro-Wilk): Passed (P = 0.104)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	25	0	0.455	0.129	0.0257
2.000	25	0	0.382	0.159	0.0318
Difference	25	0	0.0734	0.173	0.0346

t = 2.126 with 24 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: 0.00213 to 0.145

Two-tailed P-value = 0.0440

The change that occurred with the treatment is greater than would be expected by chance; there is a statistically significant change (P = 0.044)

One-tailed P-value = 0.0220

Paired t-test: Wednesday, May 12, 2021 9:55:37 AM

Data source: Data 2 in Notebook1

Dependent Variable: SV

Normality Test (Shapiro-Wilk): Failed (P < 0.050)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	25	0	87.952	19.765	3.953
2.000	25	1	99.958	26.407	5.390
Difference	25	1	-13.675	29.515	6.025

t = -2.270 with 23 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -26.138 to -1.212

Two-tailed P-value = 0.0329

The change that occurred with the treatment is greater than would be expected by chance; there is a statistically significant change (P = 0.033)

One-tailed P-value = 0.0165

Paired t-test: Wednesday, May 12, 2021 6:38:41 PM

Data source: Data 1 in Notebook1

Dependent Variable: FBF

Normality Test (Shapiro-Wilk): Passed (P = 0.599)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	23	0	2.407	0.892	0.186
2.000	23	0	2.192	1.065	0.222
Difference	23	0	0.215	0.939	0.196

t = 1.096 with 22 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -0.192 to 0.621

Two-tailed P-value = 0.285

The change that occurred with the treatment is not great enough to exclude the possibility that the difference is due to chance (P = 0.285)

One-tailed P-value = 0.143

Paired t-test: Wednesday, May 12, 2021 6:38:59 PM

Data source: Data 1 in Notebook1

Dependent Variable: FVR

Normality Test (Shapiro-Wilk): Passed (P = 0.433)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	23	0	36.890	17.445	3.638
2.000	23	0	46.239	28.052	5.849
Difference	23	0	-9.350	20.370	4.248

t = -2.201 with 22 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -18.159 to -0.541

Two-tailed P-value = 0.0385

The change that occurred with the treatment is greater than would be expected by chance; there is a statistically significant change (P = 0.039)

One-tailed P-value = 0.0193

F CARDIOVAGAL BAROREFLEX STATISTICS

Paired t-test: Wednesday, May 12, 2021 9:52:51 AM

Data source: Data 2 in Notebook1

Dependent Variable: BRS-Up

Normality Test (Shapiro-Wilk): Failed (P < 0.050)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	25	0	19.960	11.708	2.342
2.000	25	2	26.196	23.119	4.821
Difference	25	2	-7.874	20.610	4.298

t = -1.832 with 22 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -16.786 to 1.039

Two-tailed P-value = 0.0805

The change that occurred with the treatment is not great enough to exclude the possibility that the difference is due to chance (P = 0.080)

One-tailed P-value = 0.0402

Paired t-test: Wednesday, May 12, 2021 9:53:09 AM

Data source: Data 2 in Notebook1

Dependent Variable: BRS-Down

Normality Test (Shapiro-Wilk): Failed (P < 0.050)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	25	0	17.096	7.994	1.599
2.000	25	0	21.364	12.025	2.405
Difference	25	0	-4.268	14.134	2.827

t = -1.510 with 24 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -10.102 to 1.566

Two-tailed P-value = 0.144

The change that occurred with the treatment is not great enough to exclude the possibility that the difference is due to chance (P = 0.144)

One-tailed P-value = 0.0721

Paired t-test: Wednesday, May 12, 2021 10:04:54 AM

Data source: Data 1 in Notebook1

Dependent Variable: SAP-RRI Slope

Normality Test (Shapiro-Wilk): Failed (P < 0.050)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	25	3	10.768	4.284	0.913
2.000	25	3	11.778	6.497	1.385
Difference	25	3	-1.010	6.864	1.463

t = -0.690 with 21 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -4.053 to 2.034

Two-tailed P-value = 0.498

The change that occurred with the treatment is not great enough to exclude the possibility that the difference is due to chance (P = 0.498)

One-tailed P-value = 0.249

Paired t-test: Wednesday, May 12, 2021 10:06:36 AM

Data source: Data 2 in Notebook1

Dependent Variable: SAP-RRI Slope

Normality Test (Shapiro-Wilk): Passed (P = 0.115)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	25	2	9.381	4.975	1.037
2.000	25	2	11.542	5.754	1.200
Difference	25	2	-2.161	4.158	0.867

t = -2.493 with 22 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -3.959 to -0.363

Two-tailed P-value = 0.0207

The change that occurred with the treatment is greater than would be expected by chance; there is a statistically significant change (P = 0.021)

One-tailed P-value = 0.0104

Paired t-test: Wednesday, May 12, 2021 10:06:11 AM

Data source: Data 2 in Notebook1

Dependent Variable: RRI delta

Normality Test (Shapiro-Wilk): Passed (P = 0.186)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	25	2	414.580	202.453	42.214
2.000	25	2	484.261	194.908	40.641
Difference	25	2	-69.681	162.742	33.934

t = -2.053 with 22 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -140.056 to 0.694

Two-tailed P-value = 0.0521

The change that occurred with the treatment is not great enough to exclude the possibility that the difference is due to chance (P = 0.052)

One-tailed P-value = 0.0261

Wilcoxon test	
Table Analyzed	SP cvBRS up-up
Column B	Fasted
vs.	vs.
Column A	Fed
Wilcoxon matched-pairs signed rank test	
P value	0.0286
Exact or approximate P value?	Exact
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	One-tailed
Sum of positive, negative ranks	200.5 , -75.50
Sum of signed ranks (W)	125.0
Number of pairs	23
Number of ties (ignored)	0
Median of differences	
Median	2.400
How effective was the pairing?	
rs (Spearman)	0.4251
P value (one tailed)	0.0216
P value summary	*
Was the pairing significantly effective?	Yes

Wilcoxon test	
Table Analyzed	SP cvBRS down-down
Column B	Fasted
vs.	vs.
Column A	Fed
Wilcoxon matched-pairs signed rank test	
P value	0.0758
Exact or approximate P value?	Exact
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	One-tailed
Sum of positive, negative ranks	201.0 , -99.00
Sum of signed ranks (W)	102.0
Number of pairs	25
Number of ties (ignored)	1
Median of differences	
Median	3.200
How effective was the pairing?	
rs (Spearman)	0.2347
P value (one tailed)	0.1294
P value summary	ns
Was the pairing significantly effective?	No

Wilcoxon test	
Table Analyzed	VM cvBRS Phase II
Column B	Fasted
vs.	vs.
Column A	Fed
Wilcoxon matched-pairs signed rank test	
P value	0.4746
Exact or approximate P value?	Exact
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	One-tailed
Sum of positive, negative ranks	124.0 , -129.0
Sum of signed ranks (W)	-5.000
Number of pairs	22
Number of ties (ignored)	0
Median of differences	
Median	-0.8408
How effective was the pairing?	
rs (Spearman)	0.3168
P value (one tailed)	0.0755
P value summary	ns
Was the pairing significantly effective?	No

G MENTAL STRESS STATISTICS

Two Way Repeated Measures ANOVA (Two Factor Repetition) Tuesday, June 22, 2021 11:18:58 AM

Data source: Data 1 in Notebook1

Balanced Design

Dependent Variable: ΔHR

Normality Test (Shapiro-Wilk): Failed (P < 0.050)

Equal Variance Test (Brown-Forsythe): Failed (P < 0.050)

Source of Variation	DF	SS	MS	F	P
Subject	24	7278.513	303.271		
Cond	1	2.430	2.430	0.0308	0.862
Cond x Subject	24	1896.153	79.006		
Time	5	4172.057	834.411	44.314	<0.001
Time x Subject	120	2259.527	18.829		
Cond x Time	5	50.910	10.182	1.403	0.228
Residual	120	871.007	7.258		
Total	299	16530.597	55.286		

The difference in the mean values among the different levels of Cond is not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in Time. There is not a statistically significant difference (P = 0.862).

The difference in the mean values among the different levels of Time is greater than would be expected by chance after allowing for effects of differences in Cond. There is a statistically significant difference (P = <0.001). To isolate which group(s) differ from the others use a multiple comparison procedure.

The effect of different levels of Cond does not depend on what level of Time is present. There is not a statistically significant interaction between Cond and Time. (P = 0.228)

Power of performed test with alpha = 0.0500: for Cond : 0.0500
Power of performed test with alpha = 0.0500: for Time : 1.000
Power of performed test with alpha = 0.0500: for Cond x Time : 0.152

Least square means for Cond :

Group	Mean
1.000	7.947
2.000	8.127

Std Err of LS Mean = 0.726

Least square means for Time :

Group	Mean
0.000	0.000
1.000	11.680
2.000	9.220
3.000	8.500
4.000	9.100
5.000	9.720

Std Err of LS Mean = 0.614

Least square means for Cond x Time :

Group	Mean
1.000 x 0.000	0.000
1.000 x 1.000	10.680
1.000 x 2.000	9.400
1.000 x 3.000	8.520
1.000 x 4.000	9.240
1.000 x 5.000	9.840
2.000 x 0.000	0.000
2.000 x 1.000	12.680
2.000 x 2.000	9.040
2.000 x 3.000	8.480
2.000 x 4.000	8.960
2.000 x 5.000	9.600

Std Err of LS Mean = 0.539

All Pairwise Multiple Comparison Procedures (Fisher LSD Method):

Comparisons for factor: **Cond**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
2.000 vs. 1.000	0.180	2.118	0.862	No

Comparisons for factor: **Time**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
1.000 vs. 0.000	11.680	1.718	<0.001	Yes
1.000 vs. 3.000	3.180	1.718	<0.001	Yes
1.000 vs. 4.000	2.580	1.718	0.004	Yes
1.000 vs. 2.000	2.460	1.718	0.005	Yes
1.000 vs. 5.000	1.960	1.718	0.026	Yes
5.000 vs. 0.000	9.720	1.718	<0.001	Yes
5.000 vs. 3.000	1.220	1.718	0.162	No
5.000 vs. 4.000	0.620	1.718	0.476	Do Not Test
5.000 vs. 2.000	0.500	1.718	0.566	Do Not Test
2.000 vs. 0.000	9.220	1.718	<0.001	Yes
2.000 vs. 3.000	0.720	1.718	0.408	Do Not Test
2.000 vs. 4.000	0.120	1.718	0.890	Do Not Test
4.000 vs. 0.000	9.100	1.718	<0.001	Yes
4.000 vs. 3.000	0.600	1.718	0.491	Do Not Test
3.000 vs. 0.000	8.500	1.718	<0.001	Yes

Comparisons for factor: **Time within 1**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
1.000 vs. 0.000	10.680	2.014	<0.001	Yes
1.000 vs. 3.000	2.160	2.014	0.036	Yes
1.000 vs. 4.000	1.440	2.014	0.160	No
1.000 vs. 2.000	1.280	2.014	0.212	Do Not Test
1.000 vs. 5.000	0.840	2.014	0.412	Do Not Test
5.000 vs. 0.000	9.840	2.014	<0.001	Yes
5.000 vs. 3.000	1.320	2.014	0.198	No
5.000 vs. 4.000	0.600	2.014	0.558	Do Not Test
5.000 vs. 2.000	0.440	2.014	0.667	Do Not Test
2.000 vs. 0.000	9.400	2.014	<0.001	Yes
2.000 vs. 3.000	0.880	2.014	0.390	Do Not Test
2.000 vs. 4.000	0.160	2.014	0.876	Do Not Test

4.000 vs. 0.000	9.240	2.014	<0.001	Yes
4.000 vs. 3.000	0.720	2.014	0.482	Do Not Test
3.000 vs. 0.000	8.520	2.014	<0.001	Yes

Comparisons for factor: **Time within 2**

Comparison	**Diff of Means**	**LSD(alpha=0.050)**	**P**	**Diff >= LSD**
1.000 vs. 0.000	12.680	2.014	<0.001	Yes
1.000 vs. 3.000	4.200	2.014	<0.001	Yes
1.000 vs. 4.000	3.720	2.014	<0.001	Yes
1.000 vs. 2.000	3.640	2.014	<0.001	Yes
1.000 vs. 5.000	3.080	2.014	0.003	Yes
5.000 vs. 0.000	9.600	2.014	<0.001	Yes
5.000 vs. 3.000	1.120	2.014	0.274	No
5.000 vs. 4.000	0.640	2.014	0.532	Do Not Test
5.000 vs. 2.000	0.560	2.014	0.584	Do Not Test
2.000 vs. 0.000	9.040	2.014	<0.001	Yes
2.000 vs. 3.000	0.560	2.014	0.584	Do Not Test
2.000 vs. 4.000	0.0800	2.014	0.938	Do Not Test
4.000 vs. 0.000	8.960	2.014	<0.001	Yes
4.000 vs. 3.000	0.480	2.014	0.639	Do Not Test
3.000 vs. 0.000	8.480	2.014	<0.001	Yes

Comparisons for factor: **Cond within 0**

Comparison	**Diff of Means**	**LSD(alpha=0.050)**	**P**	**Diff >= LSD**
1.000 vs. 2.000	0.000	2.492	1.000	No

Comparisons for factor: **Cond within 1**

Comparison	**Diff of Means**	**LSD(alpha=0.050)**	**P**	**Diff >= LSD**
2.000 vs. 1.000	2.000	2.492	0.113	No

Comparisons for factor: **Cond within 2**

Comparison	**Diff of Means**	**LSD(alpha=0.050)**	**P**	**Diff >= LSD**
1.000 vs. 2.000	0.360	2.492	0.773	No

Comparisons for factor: **Cond within 3**

Comparison	**Diff of Means**	**LSD(alpha=0.050)**	**P**	**Diff >= LSD**
1.000 vs. 2.000	0.0400	2.492	0.974	No

Comparisons for factor: **Cond within 4**

Comparison	**Diff of Means**	**LSD(alpha=0.050)**	**P**	**Diff >= LSD**
1.000 vs. 2.000	0.280	2.492	0.822	No

Comparisons for factor: **Cond within 5**

Comparison	**Diff of Means**	**LSD(alpha=0.050)**	**P**	**Diff >= LSD**
1.000 vs. 2.000	0.240	2.492	0.847	No

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no

Two Way Repeated Measures ANOVA (Two Factor Repetition) Tuesday, June 22, 2021 11:22:58 AM

Data source: Data 1 in Notebook1

Balanced Design

Dependent Variable: ΔMAP

Normality Test (Shapiro-Wilk): Failed (P < 0.050)

Equal Variance Test (Brown-Forsythe): Failed (P < 0.050)

Source of Variation	DF	SS	MS	F	P
Subject	24	4716.347	196.514		
Cond	1	115.320	115.320	0.711	0.407
Cond x Subject	24	3892.347	162.181		
Time	5	4734.080	946.816	54.696	<0.001
Time x Subject	120	2077.253	17.310		
Cond x Time	5	26.320	5.264	0.395	0.851
Residual	120	1599.013	13.325		
Total	299	17160.680	57.394		

The difference in the mean values among the different levels of Cond is not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in Time. There is not a statistically significant difference (P = 0.407).

The difference in the mean values among the different levels of Time is greater than would be expected by chance after allowing for effects of differences in Cond. There is a statistically significant difference (P = <0.001). To isolate which group(s) differ from the others use a multiple comparison procedure.

The effect of different levels of Cond does not depend on what level of Time is present. There is not a statistically significant interaction between Cond and Time. (P = 0.851)

Power of performed test with alpha = 0.0500: for Cond : 0.0500
Power of performed test with alpha = 0.0500: for Time : 1.000
Power of performed test with alpha = 0.0500: for Cond x Time : 0.0500

Least square means for Cond :

Group	Mean
1.000	8.240
2.000	7.000

Std Err of LS Mean = 1.040

Least square means for Time :

Group	Mean
0.000	0.000
1.000	4.800
2.000	9.200
3.000	10.600
4.000	10.540
5.000	10.580

Std Err of LS Mean = 0.588

Least square means for Cond x Time :

Group	Mean
1.000 x 0.000	0.000
1.000 x 1.000	5.400
1.000 x 2.000	10.120
1.000 x 3.000	11.240
1.000 x 4.000	11.320
1.000 x 5.000	11.360
2.000 x 0.000	0.000
2.000 x 1.000	4.200
2.000 x 2.000	8.280
2.000 x 3.000	9.960
2.000 x 4.000	9.760
2.000 x 5.000	9.800

Std Err of LS Mean = 0.730

All Pairwise Multiple Comparison Procedures (Fisher LSD Method):

Comparisons for factor: **Cond**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
1.000 vs. 2.000	1.240	3.035	0.407	No

Comparisons for factor: **Time**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
3.000 vs. 0.000	10.600	1.648	<0.001	Yes
3.000 vs. 1.000	5.800	1.648	<0.001	Yes
3.000 vs. 2.000	1.400	1.648	0.095	No
3.000 vs. 4.000	0.0600	1.648	0.943	Do Not Test
3.000 vs. 5.000	0.0200	1.648	0.981	Do Not Test
5.000 vs. 0.000	10.580	1.648	<0.001	Yes
5.000 vs. 1.000	5.780	1.648	<0.001	Yes
5.000 vs. 2.000	1.380	1.648	0.100	Do Not Test
5.000 vs. 4.000	0.0400	1.648	0.962	Do Not Test
4.000 vs. 0.000	10.540	1.648	<0.001	Yes
4.000 vs. 1.000	5.740	1.648	<0.001	Yes
4.000 vs. 2.000	1.340	1.648	0.110	Do Not Test
2.000 vs. 0.000	9.200	1.648	<0.001	Yes
2.000 vs. 1.000	4.400	1.648	<0.001	Yes
1.000 vs. 0.000	4.800	1.648	<0.001	Yes

Comparisons for factor: **Time within 1**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
5.000 vs. 0.000	11.360	2.181	<0.001	Yes
5.000 vs. 1.000	5.960	2.181	<0.001	Yes
5.000 vs. 2.000	1.240	2.181	0.264	No
5.000 vs. 3.000	0.120	2.181	0.914	Do Not Test
5.000 vs. 4.000	0.0400	2.181	0.971	Do Not Test
4.000 vs. 0.000	11.320	2.181	<0.001	Yes
4.000 vs. 1.000	5.920	2.181	<0.001	Yes
4.000 vs. 2.000	1.200	2.181	0.279	Do Not Test
4.000 vs. 3.000	0.0800	2.181	0.942	Do Not Test
3.000 vs. 0.000	11.240	2.181	<0.001	Yes
3.000 vs. 1.000	5.840	2.181	<0.001	Yes
3.000 vs. 2.000	1.120	2.181	0.313	Do Not Test

2.000 vs. 0.000	10.120	2.181	<0.001	Yes
2.000 vs. 1.000	4.720	2.181	<0.001	Yes
1.000 vs. 0.000	5.400	2.181	<0.001	Yes

Comparisons for factor: **Time within 2**

Comparison	**Diff of Means**	**LSD(alpha=0.050)**	**P**	**Diff >= LSD**
3.000 vs. 0.000	9.960	2.181	<0.001	Yes
3.000 vs. 1.000	5.760	2.181	<0.001	Yes
3.000 vs. 2.000	1.680	2.181	0.130	No
3.000 vs. 4.000	0.200	2.181	0.857	Do Not Test
3.000 vs. 5.000	0.160	2.181	0.885	Do Not Test
5.000 vs. 0.000	9.800	2.181	<0.001	Yes
5.000 vs. 1.000	5.600	2.181	<0.001	Yes
5.000 vs. 2.000	1.520	2.181	0.171	Do Not Test
5.000 vs. 4.000	0.0400	2.181	0.971	Do Not Test
4.000 vs. 0.000	9.760	2.181	<0.001	Yes
4.000 vs. 1.000	5.560	2.181	<0.001	Yes
4.000 vs. 2.000	1.480	2.181	0.183	Do Not Test
2.000 vs. 0.000	8.280	2.181	<0.001	Yes
2.000 vs. 1.000	4.080	2.181	<0.001	Yes
1.000 vs. 0.000	4.200	2.181	<0.001	Yes

Comparisons for factor: **Cond within 0**

Comparison	**Diff of Means**	**LSD(alpha=0.050)**	**P**	**Diff >= LSD**
1.000 vs. 2.000	0.000	3.515	1.000	No

Comparisons for factor: **Cond within 1**

Comparison	**Diff of Means**	**LSD(alpha=0.050)**	**P**	**Diff >= LSD**
1.000 vs. 2.000	1.200	3.515	0.495	No

Comparisons for factor: **Cond within 2**

Comparison	**Diff of Means**	**LSD(alpha=0.050)**	**P**	**Diff >= LSD**
1.000 vs. 2.000	1.840	3.515	0.298	No

Comparisons for factor: **Cond within 3**

Comparison	**Diff of Means**	**LSD(alpha=0.050)**	**P**	**Diff >= LSD**
1.000 vs. 2.000	1.280	3.515	0.467	No

Comparisons for factor: **Cond within 4**

Comparison	**Diff of Means**	**LSD(alpha=0.050)**	**P**	**Diff >= LSD**
1.000 vs. 2.000	1.560	3.515	0.376	No

Comparisons for factor: **Cond within 5**

Comparison	**Diff of Means**	**LSD(alpha=0.050)**	**P**	**Diff >= LSD**
1.000 vs. 2.000	1.560	3.515	0.376	No

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no

Paired t-test: Tuesday, June 22, 2021 11:29:19 AM

Data source: Data 2 in Notebook1

Dependent Variable: Perceived Stress

Normality Test (Shapiro-Wilk): Passed (P = 0.327)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	25	0	2.180	0.776	0.155
2.000	25	0	2.240	1.001	0.200
Difference	25	0	-0.0600	0.882	0.176

t = -0.340 with 24 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -0.424 to 0.304

Two-tailed P-value = 0.737

The change that occurred with the treatment is not great enough to exclude the possibility that the difference is due to chance (P = 0.737)

One-tailed P-value = 0.368

Paired t test Tabular results	
Table Analyzed	FBF
Column B	Fasted
vs.	vs.
Column A	Fed
Paired t test	
P value	0.2318
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=1.254, df=13
Number of pairs	14
How big is the difference?	
Mean of differences (B - A)	12.39
SD of differences	36.95
SEM of differences	9.875
95% confidence interval	-8.948 to 33.72
R squared (partial eta squared)	0.1079
How effective was the pairing?	
Correlation coefficient (r)	0.5258
P value (one tailed)	0.0267
P value summary	*
Was the pairing significantly effective?	Yes

Paired t test Tabular results	
Table Analyzed	MS FVR
Column B	Fasted
vs.	vs.
Column A	Fed
Paired t test	
P value	0.1866
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=1.394, df=13
Number of pairs	14
How big is the difference?	
Mean of differences (B - A)	-10.32
SD of differences	27.70
SEM of differences	7.404
95% confidence interval	-26.32 to 5.673
R squared (partial eta squared)	0.1301
How effective was the pairing?	
Correlation coefficient (r)	0.4206
P value (one tailed)	0.0671
P value summary	ns
Was the pairing significantly effective?	No

Paired t test
Tabular results

Table Analyzed	MS delta HR
Column B	Fasted
vs.	vs.
Column A	Fed
Paired t test	
P value	0.8623
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.1754, df=24
Number of pairs	25
How big is the difference?	
Mean of differences (B - A)	0.2160
SD of differences	6.158
SEM of differences	1.232
95% confidence interval	-2.326 to 2.758
R squared (partial eta squared)	0.001280
How effective was the pairing?	
Correlation coefficient (r)	0.5869
P value (one tailed)	0.0010
P value summary	**
Was the pairing significantly effective?	Yes

Paired t test
Tabular results

Table Analyzed	MS delta MAP
Column B	Fasted
vs.	vs.
Column A	Fed
Paired t test	
P value	0.4808
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.7162, df=24
Number of pairs	25
How big is the difference?	
Mean of differences (B - A)	-1.288
SD of differences	8.992
SEM of differences	1.798
95% confidence interval	-5.000 to 2.424
R squared (partial eta squared)	0.02093
How effective was the pairing?	
Correlation coefficient (r)	0.05397
P value (one tailed)	0.3989
P value summary	ns
Was the pairing significantly effective?	No

Paired t test
Tabular results

Table Analyzed	MS delta Burst frequency
Column B	Fasted
vs.	vs.
Column A	Fed
Paired t test	
P value	0.2283
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=1.276, df=11
Number of pairs	12
How big is the difference?	
Mean of differences (B - A)	-3.700
SD of differences	10.05
SEM of differences	2.900
95% confidence interval	-10.08 to 2.682
R squared (partial eta squared)	0.1289
How effective was the pairing?	
Correlation coefficient (r)	-0.2924
P value (one tailed)	0.1782
P value summary	ns
Was the pairing significantly effective?	No

Paired t test
Tabular results

Table Analyzed	MS delta Total activity
Column B	Fasted
vs.	vs.
Column A	Fed
Paired t test	
P value	0.2638
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=1.178, df=11
Number of pairs	12
How big is the difference?	
Mean of differences (B - A)	107.9
SD of differences	317.3
SEM of differences	91.59
95% confidence interval	-93.72 to 309.5
R squared (partial eta squared)	0.1120
How effective was the pairing?	
Correlation coefficient (r)	0.4384
P value (one tailed)	0.0770
P value summary	ns
Was the pairing significantly effective?	No

H LOWER BODY NEGATIVE PRESSURE STATISTICS

Paired t-test: Tuesday, June 22, 2021 5:50:57 PM

Data source: Data 5 in Notebook1

Dependent Variable: DNP

Normality Test (Shapiro-Wilk): Passed (P = 0.187)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	18	0	1370.778	381.350	89.885
2.000	18	0	1229.667	403.011	94.991
Difference	18	0	141.111	328.865	77.514

t = 1.820 with 17 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -22.429 to 304.652

Two-tailed P-value = 0.0863

The change that occurred with the treatment is not great enough to exclude the possibility that the difference is due to chance (P = 0.086)

One-tailed P-value = 0.0432

Paired t-test: Tuesday, June 22, 2021 5:49:57 PM

Data source: Data 5 in Notebook1

Dependent Variable: CSI

Normality Test (Shapiro-Wilk): Passed (P = 0.298)

Treatment Name	N	Missing	Mean	Std Dev	SEM
1.000	18	0	832.672	367.248	86.561
2.000	18	0	725.189	376.597	88.765
Difference	18	0	107.483	309.216	72.883

t = 1.475 with 17 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -46.286 to 261.253

Two-tailed P-value = 0.159

The change that occurred with the treatment is not great enough to exclude the possibility that the difference is due to chance (P = 0.159)

One-tailed P-value = 0.0793

Two Way Repeated Measures ANOVA (Two Factor Repetition) Monday, June 28, 2021 12:09:23 PM

Data source: Data 6 in Notebook1

General Linear Model

Dependent Variable: FVR

Normality Test (Shapiro-Wilk): Passed (P = 0.999)

Equal Variance Test (Brown-Forsythe): Passed (P = 0.801)

Source of Variation	DF	SS	MS	F	P
Subject	13	30584.398	2352.646	0.986	0.506
Condition	1	990.455	990.455	0.583	0.458
Condition x Subject	13	28807.048	2215.927		
%	3	13839.548	4613.183	16.135	<0.001
% x Subject	39	11648.005	298.667		
Condition x %	3	2292.096	764.032	6.362	0.002
Residual	33	3963.134	120.095		
Total	105	97575.144	929.287		

Main effects cannot be properly interpreted if significant interaction is determined. This is because the size of a factor's effect depends upon the level of the other factor.

The effect of different levels of Condition depends on what level of % is present. There is a statistically significant interaction between Condition and %. (P = 0.002)

Power of performed test with alpha = 0.0500: for Condition : 0.0500
Power of performed test with alpha = 0.0500: for % : 1.000
Power of performed test with alpha = 0.0500: for Condition x % : 0.902

Expected Mean Squares:
Approximate DF Residual for Condition = 13.463
Approximate DF Residual for % = 41.403
Approximate DF Residual for Subject = 14.986

Expected MS(Condition) = var(res) + 2.667 var(Condition x Subject) + var(Condition)
Expected MS(%) = var(res) + 1.714 var(% x Subject) + var(%)
Expected MS(Subject) = var(res) + 3.538 var(Condition x Subject) +1.769 var(% x Subject) +7.077 var(Subject)
Expected MS(Condition x Subject) = var(res) + 3.538 var(Condition x Subject)
Expected MS(Condition x %) = var(res) + var(Condition x %)
Expected MS(% x Subject) = var(res) + 1.846 var(% x Subject)
Expected MS(Residual) = var(res)

Least square means for Condition :

Group	Mean	SEM
1.000	64.709	6.290
2.000	57.425	8.896

Least square means for % :

Group	Mean	SEM

0.000	42.677	3.266
40.000	58.737	3.771
80.000	68.396	3.771
100.000	74.458	3.771

Least square means for Condition x % :

Group	Mean	SEM
1.000 x 0.000	39.610	2.929
1.000 x 40.000	61.086	2.929
1.000 x 80.000	73.138	2.929
1.000 x 100.000	85.000	2.929
2.000 x 0.000	45.743	2.929
2.000 x 40.000	56.387	3.781
2.000 x 80.000	63.653	3.781
2.000 x 100.000	63.915	3.781

All Pairwise Multiple Comparison Procedures (Fisher LSD Method):

Comparisons for factor: **Condition**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
1.000 vs. 2.000	7.284	23.538	0.515	No

Comparisons for factor: **%**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
100.000 vs. 0.000	31.781	10.091	<0.001	Yes
100.000 vs. 40.000	15.721	10.788	0.005	Yes
100.000 vs. 80.000	6.062	10.788	0.263	No
80.000 vs. 0.000	25.719	10.091	<0.001	Yes
80.000 vs. 40.000	9.659	10.788	0.078	No
40.000 vs. 0.000	16.060	10.091	0.003	Yes

Comparisons for factor: **% within 1**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
100.000 vs. 0.000	45.390	11.127	<0.001	Yes
100.000 vs. 40.000	23.914	11.127	<0.001	Yes
100.000 vs. 80.000	11.862	11.127	0.037	Yes
80.000 vs. 0.000	33.528	11.127	<0.001	Yes
80.000 vs. 40.000	12.052	11.127	0.034	Yes
40.000 vs. 0.000	21.476	11.127	<0.001	Yes

Comparisons for factor: **% within 2**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
100.000 vs. 0.000	18.172	12.848	0.006	Yes
100.000 vs. 40.000	7.528	14.364	0.299	No
100.000 vs. 80.000	0.262	14.364	0.971	Do Not Test
80.000 vs. 0.000	17.910	12.848	0.007	Yes
80.000 vs. 40.000	7.266	14.364	0.316	Do Not Test
40.000 vs. 0.000	10.644	12.848	0.103	No

Comparisons for factor: **Condition within 0**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
2.000 vs. 1.000	6.133	21.313	0.551	No

Comparisons for factor: **Condition within 40**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
1.000 vs. 2.000	4.699	24.611	0.692	No

Comparisons for factor: **Condition within 80**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
1.000 vs. 2.000	9.485	24.611	0.427	No

Comparisons for factor: **Condition within 100**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
1.000 vs. 2.000	21.086	24.611	0.088	No

Paired t test Tabular results	
Table Analyzed	%LBNP FVR
Column B	Fasted
vs.	vs.
Column A	Fed
Paired t test	
P value	0.0349
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	One-tailed
t, df	t=2.008, df=11
Number of pairs	12
How big is the difference?	
Mean of differences (B - A)	-93.64
SD of differences	161.6
SEM of differences	46.64
95% confidence interval	-196.3 to 9.015
R squared (partial eta squared)	0.2682
How effective was the pairing?	
Correlation coefficient (r)	-0.06520
P value (one tailed)	0.4202
P value summary	ns
Was the pairing significantly effective?	No

Two Way Repeated Measures ANOVA (Two Factor Repetition) Monday, June 28, 2021 10:08:55 AM

Data source: Data 1 in Notebook1

General Linear Model

Dependent Variable: HR

The following subject was deleted from the calculations because of the pattern of missing data:
FAS-038

Normality Test (Shapiro-Wilk): Passed (P = 0.996)

Equal Variance Test (Brown-Forsythe): Passed (P = 0.695)

Source of Variation	DF	SS	MS	F	P
Subject	16	11232.956	702.060	3.311	0.009
Cond	1	475.666	475.666	2.387	0.142
Cond x Subject	16	3201.191	200.074		
Percent to Presyncope	3	28011.069	9337.023	241.063	<0.001
Percent to Pr x Subject	48	1864.544	38.845		
Cond x Percent to Presyncope	3	20.356	6.785	0.253	0.859
Residual	47	1259.103	26.789		
Total	134	46323.733	345.700		

The difference in the mean values among the different levels of Cond is not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in Percent to Presyncope. There is not a statistically significant difference (P = 0.142).

The difference in the mean values among the different levels of Percent to Presyncope is greater than would be expected by chance after allowing for effects of differences in Cond. There is a statistically significant difference (P = <0.001). To isolate which group(s) differ from the others use a multiple comparison procedure.

The effect of different levels of Cond does not depend on what level of Percent to Presyncope is present. There is not a statistically significant interaction between Cond and Percent to Presyncope. (P = 0.859)

Power of performed test with alpha = 0.0500: for Cond : 0.198
Power of performed test with alpha = 0.0500: for Percent to Presyncope : 1.000
Power of performed test with alpha = 0.0500: for Cond x Percent to Presyncope : 0.0500

Expected Mean Squares:
Approximate DF Residual for Cond = 16.021
Approximate DF Residual for Percent to Presyncope = 48.621
Approximate DF Residual for Subject = 17.649

Expected MS(Cond) = var(res) + 3.918 var(Cond x Subject) + var(Cond)
Expected MS(Percent to Presyncope) = var(res) + 1.961 var(Percent to Pr x Subject) + var(Percent to Presyncope)
Expected MS(Subject) = var(res) + 3.937 var(Cond x Subject) +1.969 var(Percent to Pr x Subject) +7.875 var(Subject)
Expected MS(Cond x Subject) = var(res) + 3.937 var(Cond x Subject)
Expected MS(Cond x Percent to Presyncope) = var(res) + var(Cond x Percent to Presyncope)

Expected MS(Percent to Pr x Subject) = var(res) + 1.979 var(Percent to Pr x Subject)
Expected MS(Residual) = var(res)

Least square means for Cond :

Group	Mean	SEM
1.000	77.456	1.715
2.000	73.677	1.751

Least square means for Percent to Presyncope :

Group	Mean	SEM
0.000	60.676	1.069
40.000	63.176	1.069
80.000	82.854	1.113
100.000	95.559	1.069

Least square means for Cond x Percent to Presyncope :

Group	Mean	SEM
1.000 x 0.000	62.765	1.255
1.000 x 40.000	64.941	1.255
1.000 x 80.000	85.235	1.255
1.000 x 100.000	96.882	1.255
2.000 x 0.000	58.588	1.255
2.000 x 40.000	61.412	1.255
2.000 x 80.000	80.472	1.356
2.000 x 100.000	94.235	1.255

All Pairwise Multiple Comparison Procedures (Fisher LSD Method):

Comparisons for factor: **Cond**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
1.000 vs. 2.000	3.779	5.196	0.143	No

Comparisons for factor: **Percent to Presyncope**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
100.000 vs. 0.000	34.882	3.039	<0.001	Yes
100.000 vs. 40.000	32.382	3.039	<0.001	Yes
100.000 vs. 80.000	12.705	3.102	<0.001	Yes
80.000 vs. 0.000	22.177	3.102	<0.001	Yes
80.000 vs. 40.000	19.677	3.102	<0.001	Yes
40.000 vs. 0.000	2.500	3.039	0.105	No

Comparisons for factor: **Percent to Presyncope within 1**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
100.000 vs. 0.000	34.118	3.906	<0.001	Yes
100.000 vs. 40.000	31.941	3.906	<0.001	Yes
100.000 vs. 80.000	11.647	3.906	<0.001	Yes
80.000 vs. 0.000	22.471	3.906	<0.001	Yes
80.000 vs. 40.000	20.294	3.906	<0.001	Yes
40.000 vs. 0.000	2.176	3.906	0.271	No

Comparisons for factor: **Percent to Presyncope within 2**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
100.000 vs. 0.000	35.647	3.906	<0.001	Yes
100.000 vs. 40.000	32.824	3.906	<0.001	Yes
100.000 vs. 80.000	13.763	4.066	<0.001	Yes
80.000 vs. 0.000	21.884	4.066	<0.001	Yes
80.000 vs. 40.000	19.060	4.066	<0.001	Yes
40.000 vs. 0.000	2.824	3.906	0.155	No

Comparisons for factor: **Cond within 0**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
1.000 vs. 2.000	4.176	5.898	0.158	No

Comparisons for factor: **Cond within 40**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
1.000 vs. 2.000	3.529	5.898	0.231	No

Comparisons for factor: **Cond within 80**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
1.000 vs. 2.000	4.763	6.139	0.123	No

Comparisons for factor: **Cond within 100**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
1.000 vs. 2.000	2.647	5.898	0.366	No

Two Way Repeated Measures ANOVA (Two Factor Repetition) Monday, June 28, 2021 10:09:19 AM

Data source: Data 1 in Notebook1

General Linear Model

Dependent Variable: SAP

The following subject was deleted from the calculations because of the pattern of missing data:
FAS-038

Normality Test (Shapiro-Wilk): Passed (P = 0.855)

Equal Variance Test (Brown-Forsythe): Passed (P = 0.152)

Source of Variation	DF	SS	MS	F	P
Subject	16	6338.573	396.161	1.366	0.269
Cond	1	181.858	181.858	0.636	0.437
Cond x Subject	16	4594.335	287.146		
Percent to Presyncope	3	1101.742	367.247	9.983	<0.001
Percent to Pr x Subject	48	1767.076	36.814		
Cond x Percent to Presyncope	3	61.996	20.665	0.608	0.613
Residual	47	1597.129	33.981		
Total	134	15675.081	116.978		

The difference in the mean values among the different levels of Cond is not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in Percent to Presyncope. There is not a statistically significant difference (P = 0.437).

The difference in the mean values among the different levels of Percent to Presyncope is greater than would be expected by chance after allowing for effects of differences in Cond. There is a statistically significant difference (P = <0.001). To isolate which group(s) differ from the others use a multiple comparison procedure.

The effect of different levels of Cond does not depend on what level of Percent to Presyncope is present. There is not a statistically significant interaction between Cond and Percent to Presyncope. (P = 0.613)

Power of performed test with alpha = 0.0500: for Cond : 0.0500
Power of performed test with alpha = 0.0500: for Percent to Presyncope : 0.993
Power of performed test with alpha = 0.0500: for Cond x Percent to Presyncope : 0.0500

Expected Mean Squares:
Approximate DF Residual for Cond = 16.018
Approximate DF Residual for Percent to Presyncope = 48.831
Approximate DF Residual for Subject = 16.152

Expected MS(Cond) = var(res) + 3.918 var(Cond x Subject) + var(Cond)
Expected MS(Percent to Presyncope) = var(res) + 1.961 var(Percent to Pr x Subject) + var(Percent to Presyncope)
Expected MS(Subject) = var(res) + 3.937 var(Cond x Subject) +1.969 var(Percent to Pr x Subject) +7.875 var(Subject)
Expected MS(Cond x Subject) = var(res) + 3.937 var(Cond x Subject)
Expected MS(Cond x Percent to Presyncope) = var(res) + var(Cond x Percent to Presyncope)

Expected MS(Percent to Pr x Subject) = var(res) + 1.979 var(Percent to Pr x Subject)
Expected MS(Residual) = var(res)

Least square means for Cond :

Group	Mean	SEM
1.000	113.735	2.055
2.000	111.399	2.097

Least square means for Percent to Presyncope :

Group	Mean	SEM
0.000	113.882	1.041
40.000	114.853	1.041
80.000	113.856	1.083
100.000	107.676	1.041

Least square means for Cond x Percent to Presyncope :

Group	Mean	SEM
1.000 x 0.000	114.882	1.414
1.000 x 40.000	115.882	1.414
1.000 x 80.000	114.235	1.414
1.000 x 100.000	109.941	1.414
2.000 x 0.000	112.882	1.414
2.000 x 40.000	113.824	1.414
2.000 x 80.000	113.477	1.527
2.000 x 100.000	105.412	1.414

All Pairwise Multiple Comparison Procedures (Fisher LSD Method):

Comparisons for factor: **Cond**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
1.000 vs. 2.000	2.337	6.225	0.438	No

Comparisons for factor: **Percent to Presyncope**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
40.000 vs. 100.000	7.176	2.959	<0.001	Yes
40.000 vs. 80.000	0.997	3.020	0.510	No
40.000 vs. 0.000	0.971	2.959	0.513	Do Not Test
0.000 vs. 100.000	6.206	2.959	<0.001	Yes
0.000 vs. 80.000	0.0263	3.020	0.986	Do Not Test
80.000 vs. 100.000	6.180	3.020	<0.001	Yes

Comparisons for factor: **Percent to Presyncope within 1**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
40.000 vs. 100.000	5.941	4.052	0.004	Yes
40.000 vs. 80.000	1.647	4.052	0.422	No
40.000 vs. 0.000	1.000	4.052	0.625	Do Not Test
0.000 vs. 100.000	4.941	4.052	0.017	Yes
0.000 vs. 80.000	0.647	4.052	0.752	Do Not Test
80.000 vs. 100.000	4.294	4.052	0.038	Yes

Comparisons for factor: **Percent to Presyncope within 2**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
40.000 vs. 100.000	8.412	4.052	<0.001	Yes
40.000 vs. 0.000	0.941	4.052	0.646	No
40.000 vs. 80.000	0.347	4.218	0.871	Do Not Test
80.000 vs. 100.000	8.065	4.218	<0.001	Yes
80.000 vs. 0.000	0.594	4.218	0.780	Do Not Test
0.000 vs. 100.000	7.471	4.052	<0.001	Yes

Comparisons for factor: **Cond within 0**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
1.000 vs. 2.000	2.000	6.966	0.561	No

Comparisons for factor: **Cond within 40**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
1.000 vs. 2.000	2.059	6.966	0.550	No

Comparisons for factor: **Cond within 80**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
1.000 vs. 2.000	0.759	7.251	0.832	No

Comparisons for factor: **Cond within 100**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
1.000 vs. 2.000	4.529	6.966	0.194	No

Two Way Repeated Measures ANOVA (Two Factor Repetition) Monday, June 28, 2021 10:09:55 AM

Data source: Data 1 in Notebook1

General Linear Model

Dependent Variable: MSNA b/min

The following subjects were deleted from the calculations because of the pattern of missing data:
FAS-011
FAS-015
FAS-016
FAS-020
FAS-023
FAS-024
FAS-026
FAS-031
FAS-033
FAS-038
FAS-045

Normality Test (Shapiro-Wilk): Passed (P = 0.924)

Equal Variance Test (Brown-Forsythe): Passed (P = 0.384)

Source of Variation	DF	SS	MS	F	P
Subject	6	6504.352	1084.059	4.291	0.021
Cond	1	27.977	27.977	0.149	0.713
Cond x Subject	6	1141.639	190.273		
Percent to Presyncope	3	7096.211	2365.404	32.985	<0.001
Percent to Pr x Subject	18	1314.031	73.002		
Cond x Percent to Presyncope	3	77.682	25.894	2.658	0.081
Residual	17	165.591	9.741		
Total	54	17172.147	318.003		

The difference in the mean values among the different levels of Cond is not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in Percent to Presyncope. There is not a statistically significant difference (P = 0.713).

The difference in the mean values among the different levels of Percent to Presyncope is greater than would be expected by chance after allowing for effects of differences in Cond. There is a statistically significant difference (P = <0.001). To isolate which group(s) differ from the others use a multiple comparison procedure.

The effect of different levels of Cond does not depend on what level of Percent to Presyncope is present. There is not a statistically significant interaction between Cond and Percent to Presyncope. (P = 0.081)

Power of performed test with alpha = 0.0500: for Cond : 0.0500
Power of performed test with alpha = 0.0500: for Percent to Presyncope : 1.000
Power of performed test with alpha = 0.0500: for Cond x Percent to Presyncope : 0.356

Expected Mean Squares:
Approximate DF Residual for Cond = 6.007

Approximate DF Residual for Percent to Presyncope = 18.100
Approximate DF Residual for Subject = 10.087

Expected MS(Cond) = var(res) + 3.789 var(Cond x Subject) + var(Cond)
Expected MS(Percent to Presyncope) = var(res) + 1.905 var(Percent to Pr x Subject) + var(Percent to Presyncope)
Expected MS(Subject) = var(res) + 3.833 var(Cond x Subject) +1.917 var(Percent to Pr x Subject) +7.667 var(Subject)
Expected MS(Cond x Subject) = var(res) + 3.833 var(Cond x Subject)
Expected MS(Cond x Percent to Presyncope) = var(res) + var(Cond x Percent to Presyncope)
Expected MS(Percent to Pr x Subject) = var(res) + 1.944 var(Percent to Pr x Subject)
Expected MS(Residual) = var(res)

Least square means for Cond :

Group	Mean	SEM
1.000	31.381	2.748
2.000	29.929	2.607

Least square means for Percent to Presyncope :

Group	Mean	SEM
0.000	15.571	2.284
40.000	23.381	2.284
80.000	39.405	2.284
100.000	44.262	2.525

Least square means for Cond x Percent to Presyncope :

Group	Mean	SEM
1.000 x 0.000	15.714	1.180
1.000 x 40.000	25.333	1.180
1.000 x 80.000	41.239	1.180
1.000 x 100.000	43.238	1.418
2.000 x 0.000	15.429	1.180
2.000 x 40.000	21.429	1.180
2.000 x 80.000	37.571	1.180
2.000 x 100.000	45.286	1.180

All Pairwise Multiple Comparison Procedures (Fisher LSD Method):

Comparisons for factor: **Cond**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
1.000 vs. 2.000	1.452	9.268	0.715	No

Comparisons for factor: **Percent to Presyncope**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
100.000 vs. 0.000	28.690	7.152	<0.001	Yes
100.000 vs. 40.000	20.881	7.152	<0.001	Yes
100.000 vs. 80.000	4.857	7.152	0.171	No
80.000 vs. 0.000	23.834	6.785	<0.001	Yes
80.000 vs. 40.000	16.024	6.785	<0.001	Yes
40.000 vs. 0.000	7.809	6.785	0.026	Yes

Comparisons for factor: **Percent to Presyncope within 1**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
100.000 vs. 0.000	27.524	7.959	<0.001	Yes
100.000 vs. 40.000	17.905	7.959	<0.001	Yes
100.000 vs. 80.000	2.000	7.959	0.608	No
80.000 vs. 0.000	25.524	7.199	<0.001	Yes
80.000 vs. 40.000	15.906	7.199	<0.001	Yes
40.000 vs. 0.000	9.619	7.199	0.011	Yes

Comparisons for factor: **Percent to Presyncope within 2**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
100.000 vs. 0.000	29.857	7.199	<0.001	Yes
100.000 vs. 40.000	23.857	7.199	<0.001	Yes
100.000 vs. 80.000	7.714	7.199	0.037	Yes
80.000 vs. 0.000	22.143	7.199	<0.001	Yes
80.000 vs. 40.000	16.143	7.199	<0.001	Yes
40.000 vs. 0.000	6.000	7.199	0.098	No

Comparisons for factor: **Cond within 0**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
1.000 vs. 2.000	0.286	9.332	0.945	No

Comparisons for factor: **Cond within 40**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
1.000 vs. 2.000	3.904	9.332	0.362	No

Comparisons for factor: **Cond within 80**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
1.000 vs. 2.000	3.667	9.332	0.390	No

Comparisons for factor: **Cond within 100**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
2.000 vs. 1.000	2.048	10.317	0.658	No

Two Way Repeated Measures ANOVA (Two Factor Repetition) Monday, June 28, 2021 10:12:50 AM

Data source: Data 1 in Notebook1

General Linear Model

Dependent Variable: TPR

The following subjects were deleted from the calculations because of the pattern of missing data:
FAS-037
FAS-038

Normality Test (Shapiro-Wilk): Failed (P < 0.050)

Equal Variance Test (Brown-Forsythe): Passed (P = 0.461)

Source of Variation	DF	SS	MS	F	P
Subject	15	1403.623	93.575	2.538	0.085
Cond	1	35.285	35.285	0.758	0.398
Cond x Subject	15	700.084	46.672		
Percent to Presyncope	3	169.188	56.396	5.255	0.003
Percent to Pr x Subject	45	478.554	10.635		
Cond x Percent to Presyncope	3	60.544	20.181	0.985	0.409
Residual	44	901.575	20.490		
Total	126	3790.512	30.083		

The difference in the mean values among the different levels of Cond is not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in Percent to Presyncope. There is not a statistically significant difference (P = 0.398).

The difference in the mean values among the different levels of Percent to Presyncope is greater than would be expected by chance after allowing for effects of differences in Cond. There is a statistically significant difference (P = 0.003). To isolate which group(s) differ from the others use a multiple comparison procedure.

The effect of different levels of Cond does not depend on what level of Percent to Presyncope is present. There is not a statistically significant interaction between Cond and Percent to Presyncope. (P = 0.409)

Power of performed test with alpha = 0.0500: for Cond : 0.0500
Power of performed test with alpha = 0.0500: for Percent to Presyncope : 0.831
Power of performed test with alpha = 0.0500: for Cond x Percent to Presyncope : 0.0500

Expected Mean Squares:
Approximate DF Residual for Cond = 15.068
Approximate DF Residual for Percent to Presyncope = 46.721
Approximate DF Residual for Subject = 8.652

Expected MS(Cond) = var(res) + 3.913 var(Cond x Subject) + var(Cond)
Expected MS(Percent to Presyncope) = var(res) + 1.958 var(Percent to Pr x Subject) + var(Percent to Presyncope)
Expected MS(Subject) = var(res) + 3.933 var(Cond x Subject) +1.967 var(Percent to Pr x Subject) +7.867 var(Subject)
Expected MS(Cond x Subject) = var(res) + 3.933 var(Cond x Subject)

Expected MS(Cond x Percent to Presyncope) = var(res) + var(Cond x Percent to Presyncope)
Expected MS(Percent to Pr x Subject) = var(res) + 1.978 var(Percent to Pr x Subject)
Expected MS(Residual) = var(res)

Least square means for Cond :

Group	Mean	SEM
1.000	19.186	0.854
2.000	18.125	0.873

Least square means for Percent to Presyncope :

Group	Mean	SEM
0.000	16.854	0.576
40.000	18.596	0.576
80.000	20.073	0.602
100.000	19.100	0.576

Least square means for Cond x Percent to Presyncope :

Group	Mean	SEM
1.000 x 0.000	16.562	1.132
1.000 x 40.000	19.107	1.132
1.000 x 80.000	20.368	1.132
1.000 x 100.000	20.709	1.132
2.000 x 0.000	17.146	1.132
2.000 x 40.000	18.084	1.132
2.000 x 80.000	19.778	1.228
2.000 x 100.000	17.491	1.132

All Pairwise Multiple Comparison Procedures (Fisher LSD Method):

Comparisons for factor: **Cond**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
1.000 vs. 2.000	1.062	2.603	0.398	No

Comparisons for factor: **Percent to Presyncope**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
80.000 vs. 0.000	3.219	1.678	<0.001	Yes
80.000 vs. 40.000	1.477	1.678	0.083	No
80.000 vs. 100.000	0.973	1.678	0.249	Do Not Test
100.000 vs. 0.000	2.246	1.642	0.008	Yes
100.000 vs. 40.000	0.504	1.642	0.539	Do Not Test
40.000 vs. 0.000	1.742	1.642	0.038	Yes

Comparisons for factor: **Percent to Presyncope within 1**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
100.000 vs. 0.000	4.148	2.770	0.004	Yes
100.000 vs. 40.000	1.602	2.770	0.253	No
100.000 vs. 80.000	0.342	2.770	0.807	Do Not Test
80.000 vs. 0.000	3.806	2.770	0.008	Yes
80.000 vs. 40.000	1.261	2.770	0.368	Do Not Test
40.000 vs. 0.000	2.545	2.770	0.071	No

Comparisons for factor: **Percent to Presyncope within 2**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
80.000 vs. 0.000	2.631	2.891	0.074	No
80.000 vs. 100.000	2.287	2.891	0.119	Do Not Test
80.000 vs. 40.000	1.693	2.891	0.247	Do Not Test
40.000 vs. 0.000	0.938	2.770	0.502	Do Not Test
40.000 vs. 100.000	0.594	2.770	0.671	Do Not Test
100.000 vs. 0.000	0.344	2.770	0.805	Do Not Test

Comparisons for factor: **Cond within 0**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
2.000 vs. 1.000	0.584	3.700	0.752	No

Comparisons for factor: **Cond within 40**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
1.000 vs. 2.000	1.023	3.700	0.581	No

Comparisons for factor: **Cond within 80**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
1.000 vs. 2.000	0.590	3.861	0.760	No

Comparisons for factor: **Cond within 100**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
1.000 vs. 2.000	3.219	3.700	0.087	No

Two Way Repeated Measures ANOVA (Two Factor Repetition) Monday, June 28, 2021 11:59:22 AM

Data source: Data 6 in Notebook1

General Linear Model

Dependent Variable: FBF

Normality Test (Shapiro-Wilk): Failed (P < 0.050)

Equal Variance Test (Brown-Forsythe): Passed (P = 0.301)

Source of Variation	DF	SS	MS	F	P
Subject	13	16.244	1.250	1.134	0.405
Condition	1	0.000677	0.000677	0.000850	0.977
Condition x Subject	13	13.431	1.033		
%	3	10.932	3.644	25.931	<0.001
% x Subject	39	5.680	0.146		
Condition x %	3	1.026	0.342	4.626	0.008
Residual	33	2.440	0.0740		
Total	105	55.523	0.529		

Main effects cannot be properly interpreted if significant interaction is determined. This is because the size of a factor's effect depends upon the level of the other factor.

The effect of different levels of Condition depends on what level of % is present. There is a statistically significant interaction between Condition and %. (P = 0.008)

Power of performed test with alpha = 0.0500: for Condition : 0.0500
Power of performed test with alpha = 0.0500: for % : 1.000
Power of performed test with alpha = 0.0500: for Condition x % : 0.745

Expected Mean Squares:
Approximate DF Residual for Condition = 13.613
Approximate DF Residual for % = 42.030
Approximate DF Residual for Subject = 14.670

Expected MS(Condition) = var(res) + 2.667 var(Condition x Subject) + var(Condition)
Expected MS(%) = var(res) + 1.714 var(% x Subject) + var(%)
Expected MS(Subject) = var(res) + 3.538 var(Condition x Subject) +1.769 var(% x Subject) +7.077 var(Subject)
Expected MS(Condition x Subject) = var(res) + 3.538 var(Condition x Subject)
Expected MS(Condition x %) = var(res) + var(Condition x %)
Expected MS(% x Subject) = var(res) + 1.846 var(% x Subject)
Expected MS(Residual) = var(res)

Least square means for Condition :

Group	Mean	SEM
1.000	1.701	0.136
2.000	1.707	0.192

Least square means for % :

Group	Mean	SEM

0.000	2.244	0.0721
40.000	1.713	0.0833
80.000	1.510	0.0833
100.000	1.349	0.0833

Least square means for Condition x % :

Group	Mean	SEM
1.000 x 0.000	2.406	0.0727
1.000 x 40.000	1.708	0.0727
1.000 x 80.000	1.457	0.0727
1.000 x 100.000	1.232	0.0727
2.000 x 0.000	2.082	0.0727
2.000 x 40.000	1.717	0.0938
2.000 x 80.000	1.563	0.0938
2.000 x 100.000	1.466	0.0938

All Pairwise Multiple Comparison Procedures (Fisher LSD Method):

Comparisons for factor: **Condition**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
2.000 vs. 1.000	0.00602	0.508	0.980	No

Comparisons for factor: **%**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
0.000 vs. 100.000	0.895	0.223	<0.001	Yes
0.000 vs. 80.000	0.734	0.223	<0.001	Yes
0.000 vs. 40.000	0.531	0.223	<0.001	Yes
40.000 vs. 100.000	0.364	0.238	0.004	Yes
40.000 vs. 80.000	0.203	0.238	0.093	No
80.000 vs. 100.000	0.161	0.238	0.179	No

Comparisons for factor: **% within 1**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
0.000 vs. 100.000	1.174	0.253	<0.001	Yes
0.000 vs. 80.000	0.949	0.253	<0.001	Yes
0.000 vs. 40.000	0.698	0.253	<0.001	Yes
40.000 vs. 100.000	0.476	0.253	<0.001	Yes
40.000 vs. 80.000	0.251	0.253	0.052	No
80.000 vs. 100.000	0.225	0.253	0.081	No

Comparisons for factor: **% within 2**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
0.000 vs. 100.000	0.616	0.293	<0.001	Yes
0.000 vs. 80.000	0.519	0.293	<0.001	Yes
0.000 vs. 40.000	0.364	0.293	0.015	Yes
40.000 vs. 100.000	0.252	0.327	0.129	No
40.000 vs. 80.000	0.155	0.327	0.349	Do Not Test
80.000 vs. 100.000	0.0972	0.327	0.555	Do Not Test

Comparisons for factor: **Condition within 0**

Comparison	**Diff of Means**	**LSD(alpha=0.050)**	**P**	**Diff >= LSD**
1.000 vs. 2.000	0.325	0.467	0.161	No

Comparisons for factor: **Condition within 40**

Comparison	**Diff of Means**	**LSD(alpha=0.050)**	**P**	**Diff >= LSD**
2.000 vs. 1.000	0.00936	0.539	0.971	No

Comparisons for factor: **Condition within 80**

Comparison	**Diff of Means**	**LSD(alpha=0.050)**	**P**	**Diff >= LSD**
2.000 vs. 1.000	0.106	0.539	0.684	No

Comparisons for factor: **Condition within 100**

Comparison	**Diff of Means**	**LSD(alpha=0.050)**	**P**	**Diff >= LSD**
2.000 vs. 1.000	0.233	0.539	0.374	No