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Cardiovascular and Autonomic Nervous System Function: Impact of Glucose Ingestion, Hydration Status and Exercise in Heated Environments

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CARDIOVASCULAR AND AUTONOMIC NERVOUS SYSTEM FUNCTION: IMPACT
OF GLUCOSE INGESTION, HYDRATION STATUS AND EXERCISE IN HEATED
ENVIRONMENTS

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The School of Kinesiology

by

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ABSTRACT

Cardiovascular function is under the influence of autonomic nervous system, both of which can be assessed non-invasively. The purpose of this dissertation was to examine these non-invasive markers of cardiovascular and autonomic function and their relationships with exercise training, glucose ingestion and hydration status. A series of three studies were conducted to gain insight to various influences on cardiovascular and autonomic function.

The first study examined the influence of exercise training of brachial artery flow-mediated dilation (BAFMD) using meta-analytic techniques. Sixty-six studies included in the analysis demonstrated exercise training improves BAFMD compared to controls. Results indicated exercise training significantly alters BAFMD, a well-known factor associated with prevention of cardiovascular diseases. Exercise training interventions including greater intensity and duration may optimize increases in BAFMD.

The second study observed glucose ingestion alters autonomic nervous system function, shifting the sympathetic/parasympathetic balance to higher sympathetic activity. Higher exercise intensity decreased fasting heart rate variability 24-hrs after cessation of exercise whereas lower exercise intensity did not alter heart rate variability. Acute exercise increased heart rate variability after an oral glucose tolerance test, but was not affected by exercise intensity.

The last study determined the effect of chronic dehydration on cardiovascular and sweat responses during exercise in a heated environment. Dehydration altered blood and urine markers of hydration status, and cardiovascular response to exercise in the heat. In addition, BAFMD was related to the change in weighted skin temperature and body temperature during exercise in the heat, and increased LF/HF at rest was associated with

increased peak heat storage. Together these data suggest resting cardiovascular health may influence the ability to thermoregulate during exercise in the heat.

CHAPTER 1. INTRODUCTION

The cardiovascular system is adaptive and dynamic, constantly monitored by a number of mechanisms to provide precise alterations under various physiological conditions. The autonomic nervous system provides dual innervation to the cardiovascular system where it has short- and long-term effects. Each beat-to-beat of the heart provides a circulatory pattern, or arterial pressure, which is subject to neural control via sympathetic and parasympathetic nerves. A high beat-to-beat variability, or heart rate variability (HRV), is telling of a healthy heart. Alterations in HRV can be utilized to assess autonomic imbalance in healthy and diseased states. Kleiger et al. (1987) was a landmark study demonstrating decreased parasympathetic tone, measured by HRV, independently predicts mortality in post-myocardial infarction patients¹. Several large epidemiological studies including the Framingham Heart Study and Atherosclerosis Risk in Communities (ARIC) Study, have since supported reduced parasympathetic activity, measured by HRV, to be associated with mortality in high and low risk cardiovascular disease (CVD) populations^{2,3}. In addition to HRV, endothelial function, measured by brachial artery flow-mediated dilation (BAFMD), has been linked to CVD as an independent predictor of future CVD events^{4,5}. The endothelial lining of blood vessels is innervated via the sympathetic nervous system, physically and physiologically connecting the cardiovascular and autonomic nervous systems. Therefore, it is feasible autonomic imbalance and endothelial dysfunction are involved in the development of CVD.

Strategies of CVD management include surgical and pharmacological interventions, usually in the form of vasodilator medications⁶, leaving disparities in other treatment strategies including physical activity. The relevance of exercise to the structure and function

of the cardiovascular system to cardiovascular health has been substantiated in both healthy and diseased patients⁷. A sedentary lifestyle, one of the six major risk factors along with dyslipidemia, diabetes, hypertension, obesity and smoking, can be combated with increased physical activity, exercise and reduced sedentary time thereby reducing the likelihood of adverse cardiovascular events^{8,9}. Recent popularity in non-invasive measurements of cardiovascular and autonomic function has yielded insights into the impact exercise training on the intertwined systems. The non-invasive techniques utilized in this dissertation include brachial artery flow-mediated dilation (BAFMD), pulse wave velocity (PWV) and heart rate variability (HRV), which assess vascular function, arterial stiffness and autonomic balance, respectively.

The purpose of this dissertation was to study non-invasive markers of cardiovascular and autonomic function in response various conditions including exercise, glucose ingestion and dehydration. Chapter 2 is an extensive review of scientific literature pertaining to cardiovascular and autonomic assessment and control during exercise. In the following chapters (3-5), novel research addressed the following questions: 1) BAFMD and exercise training, 2) heart rate variability responses to glucose ingestion, and 3) cardiac and vascular function changes during exercise in the heat under hydrated and chronic, persistent dehydrated states. Finally, Chapter 6 is a conclusion devised to summarize the findings outlined in this dissertation and provide future research directions.

The first project (Chapter 3) conducted was a meta-analysis examining the effects of exercise training on BAFMD. To gain additional insight to potential modifiers of vascular function, meta-analytic techniques were applied to summarize and strengthen information on how exercise training impacts BAFMD. We hypothesized that exercise training modified

BAFMD suggesting endothelial function can be improved with regular exercise. In addition, this study also examined potential factors that moderate the impact of exercise training on BAFMD included disease status, fitness level and type, intensity and volume of exercise training.

Chapter 4 consisted of a crossover, counterbalanced study investigating the effect of glucose ingestion after acute exercise (at two different intensities) on HRV. Following glucose ingestion, components of HRV have been shown to shift toward greater sympathetic tone in healthy and obese adults^{10,11}. Although exercise intensity is recognized as a factor affecting blood glucose, few studies have examined the impact of acute exercise at different intensities on HRV in response to glucose ingestion. We hypothesized that a single bout of exercise would increase autonomic response to an OGTT and exercise intensity would mediate the response.

The final project (Chapter 5) sought to determine whether chronic hypohydration, achieved by fluid restriction over a 3-day period, alters cardiovascular function and sweat responsiveness during exercise in a heated environment compared to a euhydrated state. In addition, this study assessed whether an individual's resting cardiovascular function influences their cardiovascular, blood flow, and sweat responses to exercise in a heated environment. Exercise in a heated environment has detrimental effects on cardiovascular function, core body temperature regulation and performance because of the increased demand for adequate sweat rate and heat dissipation through the skin^{12,13}. The combination of heat stress and dehydration may have an additive negative consequence on cardiovascular function and sweat responses^{14,15}. We hypothesized that (1) cardiovascular function and sweat responsiveness will be greater in the euhydrated state compared with the dehydrated

state during exercise in a heated environment due to less cardiovascular strain and heat stress during exercise; (2) resting cardiovascular profiles evaluated by PWV, BAFMD and HRV will influence cardiovascular function and sweat responsiveness more after chronic, progressive dehydration.

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CHAPTER 2. LITERATURE REVIEW

2.1 Introduction

According to the Centers for Disease Control and Prevention 2010 update, diseases of the heart are the primary cause of mortality in the United States, accounting for 24.2% of total deaths^{1,2}. Cardiovascular disease (CVD) is an extensive public health issue with a heavy economic cost in the United States estimated at ~\$315 billion spent on associated health care³. Additionally, racial and sex differences prevail in CVD and the associated risk factors, particularly among African-Americans⁴. The inequalities and burden of CVD explains the rapid expansion of clinical and scholarly interest focused on the etiology, treatment and management of health outcomes related to CVD and associated CVD risk factors. Popular strategies of CVD management include surgical and pharmacological, usually in the form of vasodilator medications³, leaving disparities in other treatment strategies including physical activity. The relevance of exercise to the structure and function of the cardiovascular system has been substantiated in both overtly healthy and diseased patients⁵. A sedentary lifestyle, one of the five major risk factors along with dyslipidemia, hypertension, obesity and smoking, can be combated with increased physical activity and exercise and reduced sedentary time thereby reducing the likelihood of adverse cardiovascular events^{6,7}.

The cardiovascular system is adaptive and dynamic, constantly being monitored by a number of mechanisms to provide precise alterations under various physiological conditions. The heart, vast network of blood vessels, and contents of fluid (blood) that compose the cardiovascular system serve the following functions: (1) transport oxygen, nutrients and hormones to cells; (2) remove metabolic waste; and (3) regulate core temperature, pH and fluid balance⁸. Blood pumped from the heart is transported through the two closed circuits

(pulmonary and systemic) of vasculature⁹. During exercise, the vasoconstrictive and vasodilatory responses of the blood vessels direct blood flow to the active tissues through autonomic and vasoactive regulation. The autonomic nervous system provides dual innervation to the cardiovascular system where it has short- and long-term effects. Each beat-to-beat of the heart provides a circulatory pattern, or arterial pressure, which is subject to neural control via sympathetic and parasympathetic nerves. A high beat-to-beat variability, or heart rate variability, is telling of a healthy heart.

Several cardiovascular and metabolic diseases manifest in the vasculature. Therefore, examining the association between both autonomic and vascular regulation, especially as they relate to physical activity and exercise, is warranted. However, few studies have examined the relationship between autonomic and cardiovascular function in relation to demographic differences. Although African-Americans have been reported to have differences in autonomic and vascular function compared to Caucasians¹⁰⁻¹², the mechanisms behind these differences are poorly understood. Therefore, the purpose of this review is to identify and describe structural and physiological features and techniques used in the measurement of the autonomic nervous and cardiovascular systems. Furthermore, this review will describe the impact of race on the autonomic nervous and vascular systems as well as their responses to exercise.

2.2 Cardiovascular Anatomy and Physiology

The cardiovascular system is responsible for bringing low-pressure venous blood to the heart, oxygenating blood through the pulmonary circuit and ejecting high-pressure arterial blood into the systemic circuit. This closed circuit ensures the circulation and distribution of blood flow, and is necessary for achieving and maintaining homeostasis under

various conditions such as exercise. The structure and function of the vascular network changes as blood travels away from the heart down the vascular tree. In this section, I will describe the cardiovascular anatomy and the autonomic nervous system innervation of the cardiovascular system.

2.2.1 The Heart

The heart is a four-chambered organ which pumps de-oxygenated blood to the lungs (pulmonary circulation) and oxygenated blood the rest of the body (systemic circulation). The two smaller, upper chambers of the heart are the atria while the two larger, lower chambers of the heart are called ventricles. In general, the left ventricle has a thicker muscle (myocardium) and is capable of generating enough force to deliver blood to the entire systemic circuit. The tricuspid and mitral valves separate the atria from the ventricles while the pulmonary and aortic valves separate the ventricles from pulmonary and systemic circuits, respectively. The valves function to ensure unidirectional flow of blood through the heart. The myocardium is composed of cardiomyocytes and intercalated discs giving the heart its myogenic property. Meaning, the atria and ventricles act as separate syncytium in which nervous impulses spread rapidly through intercalated discs to allow the atria to contract separately from the ventricles.

The rhythmic contraction-relaxation process of the heart's chambers is called the cardiac cycle. The rest, diastole or relaxation phase of the cardiac cycle is approximately 0.5 seconds and allows for the heart to fill and receive its own blood supply via the coronary arteries. The contraction or systole phase of the cardiac cycle is approximately 0.3 seconds and will eject an amount of blood (stroke volume). In the first phase of the cardiac cycle, blood passively flows into the atria. When the atria contract and atrial pressure increases, the

atrioventricular (tricuspid and mitral valves) open causing blood to flow into the ventricles. As atrial contraction completes, atrial pressure begins to fall causing the atrioventricular valves to close. Following atrial systole, there is a period of time in which no blood flow is occurring and the ventricles are contracting (isovolumetric contraction). Once the pressure in the ventricles exceeds the pressure in the arterial branches, the semilunar valves open and blood ejected. After reaching a peak, ventricular pressure gradually declines causing semilunar valves to close and intraventricular pressure to continue to decrease as the ventricles relax (isovolumetric relaxation). Lastly, once ventricular pressure drops below the pressure in atria, atrioventricular valves open, thus beginning the cycle again. Given the heart has approximately one cardiac cycle per second, a healthy individuals heart rate is 60-80 beat per minute⁸.

Heart rhythm requires the synchronized contraction of all myocardium, which is initiated by electrical impulses originating at the sinoatrial (SA) node. The SA node located in the right atrium serves as the physiological pacemaker and is regulated through autonomic innervation and hormones. Sinoatrial (SA) node electrical impulses cause atrial depolarization (atrial contraction), and propagate to the atrioventricular (AV) node located in the interatrial septum where the signal slows briefly to allow for ventricular filling. From the AV node, electrical impulses rapidly pass through the bundle of His, left and right bundle branches and purkinje fibers to cause ventricular depolarization (ventricular contraction). The heart's electrical activity can be detected using an electrocardiogram (ECG).

2.2.2 The Autonomic Nervous System

The heart's involuntary contraction is under the control of the autonomic nervous system (ANS). The ANS is the motor division of the peripheral nervous system that is

essential to homeostatic functions (i.e. heart, lung, and digestive function, blood pressure). The ANS can be further broken into a parasympathetic and sympathetic nervous systems (PNS and SNS). Parasympathetic and sympathetic neural outflow is primarily regulated by the medulla oblongata located in the brainstem that receives input from a wide range of higher centers including the cerebral cortex and hypothalamus¹³. Within the medulla oblongata lie several integrative cardiovascular centers including the nucleus tractus solitarius (NTS), where sensory input from baroreceptors and peripheral chemoreceptors is received. The neural mechanism most known for the short-term, tight control of blood pressure is called the baroreflex, which will be discussed in future sections¹⁴.

The parasympathetic (“rest and digest”) and sympathetic (“fight or flight”) divisions are often antagonistic, but together they create a balance or tone. The preganglionic nerves of the parasympathetic (craniosacral) division arise from the brain stem, or cranial, and sacral segments of the spinal cord. The axons of preganglionic nerves that exit the central nervous system are long, with ganglia in or near the target organ. The specific preganglionic neurons with parasympathetic activity are carried through cranial nerves. The oculomotor nerve (III) innervates the pupil and levator palpebrae superioris muscle; the facial nerve (VII) innervates muscles of facial expression as well as two-thirds of the tongue; the glossopharyngeal nerve (IX) innervates the tongue and pharynx; and the vagus nerve (X) innervates the viscera of the thoracic and upper abdominal cavity. The majority of parasympathetic neural outflow is carried through the vagus nerve. Preganglionic neurons from sacral segments S2-S4 join the pelvic nerves to innervate viscera of the pelvic cavity. Within the ganglion both PNS and SNS preganglionic neurons release acetylcholine (cholinergic) at the synapse with the postganglionic neuron. Postganglionic neurons of the

PNS are short in length due to their proximity to the effector tissues and primarily release acetylcholine.

Preganglionic neurons of the sympathetic (thoracolumbar) division arise from the thoracic and lumbar (T1-L2) segments of the spinal cord, which travel through both pairs of spinal nerves. Most of these axons are short reaching and synapse at the ganglia of the paravertebral sympathetic chain. The sympathetic chain consists of 22 ganglia on either side of the spinal cord. Postganglionic neurons may synapse with a postganglionic neuron at the level they exit from the central nervous system, travel up or down in the sympathetic chain to other ganglia, or may even synapse with more than one postganglionic neuron. Other preganglionic neurons pass through the chain to sympathetic collateral ganglia located halfway between the central nervous system and the target tissue. Preganglionic neurons may also synapse within the adrenal medulla where modified postganglionic neurons secrete catecholamines (norepinephrine and epinephrine) into the bloodstream for longer lasting effects. Axons of the postganglionic neurons synapsing at the sympathetic chain are long reaching and release norepinephrine (adrenergic) at the effector site.

Catecholamines and acetylcholine released from postganglionic neurons stimulate or inhibit activity of an innervated tissue depending on the receptors located at the target. Acetylcholine will bind to nicotinic or muscarinic receptors. Muscarinic receptors are slow to alter metabolic activity because they are linked to second messenger systems¹⁵. Of the five subtypes of muscarinic receptors (M1-M5) M1, M3 and M5 activation leads to increased intracellular calcium while M2 and M4 activation inhibits the second messenger cascade. Nicotinic receptor activation rapidly increases cellular permeability of sodium and calcium, which excites and depolarizes the specific tissue. Catecholamines bind to either alpha (α) or

beta (β) classes of adrenergic receptors. Adrenergic receptors work through second messenger systems and the response depends on the subtype of the receptor. Activation of α_1 receptors causes smooth muscle contraction; α_2 inhibits insulin and induces glucagon; β_1 causes increased heart rate and contractility; β_2 causes smooth muscle relaxation; and β_3 enhances lipolysis.

The ANS innervates both the heart and blood vessels. Heart rate is controlled by the SA node and is under the “tonic” influence of both the PNS and SNS. At rest the parasympathetic (vagal) tone dominates¹⁶. Muscarinic-2 (M2) receptors on the SA and AV nodes are stimulated by release of acetylcholine from postganglionic fibers. Activation of M2 receptors causes inhibition of the second messenger cAMP, leading to less cellular permeability to calcium. This slows the speed of depolarization thereby decreasing heart rate¹⁴. Conversely, the SNS has an excitatory effect on the heart due to postganglionic fibers releasing norepinephrine at postsynaptic terminals within the heart. Norepinephrine targets β_1 receptors located on the SA and AV nodes¹⁴. Increased permeability to sodium increases the rate of depolarization leading to an increase in heart rate. Bristow et al. demonstrated failing human hearts have a reduction in β_1 receptor density and therefore increased tolerance to higher rates of sympathetic activity¹⁷. Excessive sympathetic activity associated with heart failure promotes an increased concentration of norepinephrine. Elevated norepinephrine concentrations predict heart failure survival independent of the severity of heart failure¹⁸.

Smooth muscle of blood vessels are sympathetically innervated and lack parasympathetic innervation, giving blood vessels a constant, partial vasoconstriction due to the discharge of sympathetic activity¹⁹. The vasoconstriction can be illustrated by

interruption of sympathetic tone with an α -adrenergic agent resulting in an increase of blood flow to skeletal muscle²⁰. However, the endothelium of blood vessels appears to have parasympathetic cholinergic innervation. M3 receptors located on the vascular endothelium are involved with the production of nitric oxide, a potent vasodilator. Blockage of nitric oxide production leads to M2 and M3 receptors causing vasoconstriction. The latter finding may be suggestive of vascular dysfunction where nitric oxide production is impaired. Similarly, removal of the vascular endothelium in rabbit carotid arteries increased the release of norepinephrine from sympathetic nerves, suggesting vascular function is important to ANS regulation²¹.

2.2.3 General Vascular Wall Structure

The general wall arrangements of an artery and vein are depicted in Figure 2.1. The vascular system is generally divided into five classes of vessels including arteries, arterioles, capillaries, venules and veins. The extensive vascular network has a wall structure composed of three histological layers: the internal endothelial coat or tunica intima; the middle muscular layer or tunica media; and external connective tissue coat or tunica adventitia⁹. With the exception of the capillaries, which are solely an endothelium layer, each class of vessel has a variation of the three layers forming its wall. The tunica intima is the innermost layer formed by a single celled lining of endothelium mounted on a fenestrated elastic membrane⁹. Beneath this membrane lies a sub endothelial layer of connective tissue. The relationship of the tunica intima to the tunica media is of particular importance because it not only provides a physical barrier between the tunica media and the contents of the lumen, but also facilitates actions of the tunica media. Tunica media is the middle layer comprised of smooth muscle cells in a concentric framework of elastic sheets, collagenous fibers, and

elastic fibrils²². Actions of the tunica media include acute changes in diameter in response to mechanical or neuro-hormonal mechanisms as well as strengthen the vessel wall to protect against blood pressure forces. Finally, the outermost layer, tunica adventitia (externa), is a dense fibro-elastic sheath surrounding the vessel. Chiefly comprised of collagen fibers, the tunica adventitia blends with adjacent tissues to anchor and stabilize the vessel and protect the vessel under high pressure²².

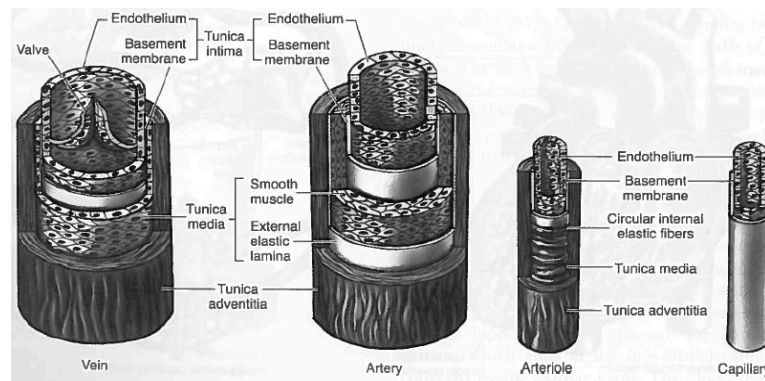


Figure 2.1. Comparison of wall structure of blood vessels. Taken with permission from²³.

Extending into the lumen from the endothelium is a hair-like layer termed endothelial glycocalyx. Endothelial glycocalyx, also known as the endothelial surface layer (ESL), is connected to the endothelium through several negatively charged “backbone” molecules, mainly proteoglycans and also glycoproteins²⁴. The thickness of this layer increases as vascular diameter increases²⁵ and is subject to a balance of biosynthesis and shedding²⁶. Endothelial glycocalyx functions as a permeable “gatekeeper” to the endothelium by mediating the access of certain molecules and interacting in leukocyte and thrombocyte adhesion. The vasoprotective properties of the endothelial glycocalyx make it an anatomical structure of interest in health and diseased states²⁷.

2.2.4 Arterial System

Arteries, or efferent vessels, distribute blood throughout the body and decrease in size as they move away from the heart. The arterial system (arteries and arterioles) is subjected to high pressure, which is why arteries are classified as elastic, or conducting vessels¹⁶. Arteries are the largest in diameter and allow for stretch with each cardiac cycle without collapsing due to a high ratio of elastic fibers to smooth muscle fibers and thick intima layer²². The aorta (~2.5cm) is a major elastic artery of the body that receives the volume and pressure load from the left ventricle and supplies all of the systemic circulation.

Arteries subdivide into arterioles, such as the brachial or popliteal arteries, and are classified as muscular arteries. These muscular, or conduit arteries, are responsible for carrying blood to major regional vascular beds. Though arterioles have poorly delineated layers within the tunica adventitia and a multi-layer, concentric tunica media^{16,22}, they have the greatest influence on peripheral pressure and blood volume distribution. Due to the relatively thicker layer of smooth muscle fibers, the ability to regulate vessel diameter allows arterioles to control blood flow. Arterioles progressively divide in to smaller branches considered terminal arterioles where the internal elastic lamina disappears and smooth muscle fibers become continuous, leaving solely the tunica interna¹⁶.

Capillaries are the smallest diameter blood vessels that arise from arterioles, but have the largest cross-sectional area in the vascular network. The capillary wall is composed of only the intima layer of endothelium and a thin basement membrane with occasional gaps, and terminate in networks of vessels that pervade every tissue in the body⁹. The capillary diameter (~8µm) is typically wide enough for the passage of a single column of erythrocytes, which allows the exchange of gases and nutrients to occur quickly. Capillaries are classified

according to their structure as continuous, fenestrated or sinusoidal (discontinuous).

Continuous capillaries are abundant in skin and muscle and have a complete lining of endothelium with tight junctions¹⁶. Fenestrated capillaries have pores due to an incomplete lining of endothelium to allow greater permeability in glandular, intestinal and kidney tissue. Lastly, sinusoidal or discontinuous capillaries located in organs including the heart, liver and spleen and are in direct contact with the cells of the organ⁹.

Two categories of capillary beds exist. First, several arterioles directly form thoroughfare channels, which are an intermediate between arterioles and venules¹⁶. The meta-arteriole contains smooth muscle cells and is responsible for the delivery of blood into capillary beds. Secondly, true capillaries stem from the meta-arteriole and provide gas and nutrient exchange. Typically, a band of smooth muscle (pre-capillary sphincter) surrounds the root of each true capillary at the thoroughfare channel to regulate blood flow into the capillary bed. Depending on the need for blood supply at the local level, pre-capillary sphincters can open (sensing oxygen, carbon dioxide, blood pH levels) for blood to flow to the tissues or close for blood to be shunted through the channel.

2.2.5 Venous System

Venules and veins collect the blood from the capillary beds and serve as and serve as a low-pressure reservoir under rested conditions eventually returning blood to the heart. The venous system differs from the arterial system in that the tunica media is much less developed allowing for veins to collapse⁹. The collapsibility of the vessel is advantageous when muscle tissue contracting around venous walls is able to compress the veins and aid in return of blood to the heart. This mechanism called the ‘muscle pump’ will be discussed in further detail in latter sections. Connecting the arterial and venous side are post-capillary

venules. Venules have a small number of smooth muscle cells and eventually pour into collecting venules and in turn small collecting veins¹⁶. Veins carry blood from venules to the large veins and ultimately the heart. Due to their large lumen that can collapse and expand as well as the low pressure of venous system, veins can be considered capacitance vessels where 60-80% of the total blood volume lies at rest⁸. Finally, large veins specifically return blood to the heart (i.e. superior and inferior vena cava).

A second marked difference between arteries and veins is the presence of one-way valves. Formed by a invagination of tunica intima and reinforced with connective tissue and elastic fibers⁹, one-way valves are located in the lumen to prevent the back-flow of blood away from the heart. These valves play a role in postural transitions, particularly when moving from supine to upright, blood pooling in the periphery is prevented²⁸.

2.3 Autonomic and Vascular Assessments

With an appreciation of cardiovascular structure and function, it is appropriate to methodologically quantify cardiovascular physiology. Although there are several techniques applicable to the field of autonomic and cardiovascular assessment, the non-invasive tools related to this specific review will be discussed.

2.3.1 Autonomic Function Assessments

Historically, the variation in the beat-to-beat time length during the respiratory cycle was first observed in horses by Stephen Hales in 1733; however, Carl Ludwig was the first to be accredited for the report of cardiac variability in humans²⁹. Heart rate variability (HRV) is defined as the degree of fluctuation in the length of intervals between heart beats³⁰. A number of techniques for the assessment of cardiovascular autonomic control have been developed to quantify the heart rate variability³¹. The assessment of HRV requires acquiring

either a long-term (24-hour) or short-term (5-minute) ECG whose signal is converted to a tachogram. This simple method of HRV detection identifies variations in time intervals between QRS complexes of an ECG, which can be called R-R or N-N (normal-to-normal) intervals. During inspiration, parasympathetic activity is inhibited by the inflation of the lungs, causing an increase in heart rate. During expiration, the parasympathetic activity is stimulated to slow heart rate. Thus, N-N intervals have different lengths depending on the point in the respiration cycle. The two most common techniques for measuring HRV are time and frequency domain measurements. Table 2.1 describes the commonly used parameters used to measure HRV.

Table 2.1. Frequently used HRV parameters for autonomic assessment.

Variable	Units	Description
<i>Time Domain Measurements</i>		
Mean RR	ms	Average of all RR (NN) intervals
SDNN	ms	Standard deviation of RR (NN) intervals
RMSSD	ms	Square root of the mean of the sum of squares of differences between adjacent RR (NN) intervals
NN50	count	Number of RR (NN) intervals >50ms apart
pNN50	%	NN50/Total RR (NN) intervals x100; Percent of adjacent RR (NN) intervals >50ms apart
<i>Frequency Domain Measurements</i>		
VLF	ms ²	Very Low Frequency (0.0-0.04Hz)
LF	ms ²	Low Frequency (0.04-0.15Hz); represents mixed sympathetic and parasympathetic activity
HF	ms ²	High frequency (0.15-0.40Hz); represents parasympathetic activity
LF/HF Ratio	-	Ratio of sympathetic and parasympathetic activity; represents autonomic balance
Total power	ms ²	Total variance of RR (NN) intervals; represents total HRV

ms=milliseconds; %=percent

Time domain analysis is used to evaluate heart rate fluctuation as a function of time. The standard deviation of the N-N interval (SDNN) is one of the most widely used time domain indices of HRV³². The SDNN (milliseconds) value depends on the length of the recording (i.e. the longer the recording, the lower SDNN value)³³. SDNN reflects all factors that contribute to HRV. A lower SDNN would indicate lower variability and greater

sympathetic activity, and conversely a higher SDNN would indicate greater variability and greater parasympathetic activity. The square root of the mean squared differences of successive N-N intervals called the RMSSD (milliseconds) can be calculated. The RMSSD is an estimate of short-term variation and provides information regarding vagal activity of the heart. The percent of adjacent N-N intervals less than 50ms apart (pNN50 (%)) is a function is virtually independent of circadian rhythms and reflects vagal-mediated alterations in autonomic function. Time domain analyses may be simple to calculate, but do not quantify autonomic balance between the SNS and PNS because they are of first order statistics³⁰. SDNN is a function of heart rate behavior over time and gives long-term information that is sensitive circadian rhythms. RMSSD and pNN50 are parameters based on interval differences that are not sensitive to diurnal variation and reflect short-term beat-to-beat variation that is caused by vagal-mediated parasympathetic activity³¹.

Frequency domain analysis, also called power spectral analysis, reflects the amplitude of the heart fluctuations at different frequencies³¹. The NN intervals can be decomposed into different frequency bands depending on the autonomic activity. Lower frequency bands are associated with sympathetic activity and higher frequency bands are corresponding to parasympathetic activity³⁴. Very low frequency (VLF) domains are frequency bands between 0.003-0.04Hz, which generally indicate the activity of slow mechanisms controlling sympathetic function. Low frequency (LF) domains are also indicators of sympathetic activity and range from 0.04-0.15Hz. However, LF domains can reflect parasympathetic activity as well, especially when an individual's respiration is extremely low, for instance, during sleep. High frequency (HF) bands range from 0.15-0.40Hz. HF domains are also called the "respiratory" band because they correspond with NN variations caused by

respiration. HF domains represent a measure of parasympathetic activity and are generally associated with HRV (increased HF, increased HRV). The LF/HF Ratio is a measure of the sympathovagal balance. A high ratio would reflect sympathetic dominance and conversely a low ratio would suggest parasympathetic dominance.

Testing baroreflex sensitivity has also been used as a simple method to examine autonomic cardiovascular control. As stated previously, the baroreflex is the neural mechanism required for short-term control of blood pressure. Baroreceptors, also known as mechanoreceptors, are stretch receptors located in the carotid sinus and aortic arch that are sensitive to changes in intravascular pressure. Presumed inactive at pressures lower than 60mmHg, baroreceptors increase the rate of firing when pressures reach 60-180mmHg and 90-210mmHg in the carotid arteries and aorta, respectively¹⁴. For example, the baroreceptors sensing an increase in pressure would stimulate nerve propagations to the NTS, inhibiting the vasoconstrictor center and exciting the parasympathetic center. In turn, this would reduce the heart rate and myocardial contractility leading to a drop in blood pressure.

Assessing the baroreflex can be done invasively with intravenous vasoactive drugs, but also non-invasively through the Valsalva maneuver. Baroreflex sensitivity can be quantified as the time lag between systolic pressure and NN intervals (ms/mmHg)³⁵. To perform the maneuver, forced expiration forces the glottis closed which transiently elevates the intrathoracic pressure. Initially, the increase in pressure deactivates baroreceptors that then trigger vasoconstriction and increases heart rate. Immediately following the maneuver, baroreceptors are activated which leads to increased blood pressure and a decreased heart rate.

The response time of the PNS and SNS differ greatly due to their efferent pathways. Typically, the parasympathetic activation is an immediate response while sympathetic activation can take 2-3 seconds. Therefore, the ability of the baroreflex to control beat-to-beat regulation is done through vagal activity³⁵. Cardiovascular diseases such as hypertension, myocardial infarction and heart failure are associated with increased sympathetic activity and impaired baroreflex mechanisms^{36,37}.

2.3.2 Conduit Vessel Assessments

A widely accepted non-invasive method to assess macro-vascular function is Doppler Ultrasonography. In the 1990's, Doppler Ultrasonography was employed to provide structural and functional information for large conduit arteries (i.e. brachial or popliteal artery) as well as blood flow and velocity. A pulsed ultrasound wave is emitted through a target tissue and a returning 'echo' is briefly detected by a crystalized probe, called a time gate. Depending on the length of time between signal emissions and returning activation, the depth of the targeted structure can be estimated³⁸. This gives a three dimensional map of the vessel. The frequency of reflected ultrasound waves corresponds the blood flow velocity³⁹. This can be visualized in real time (duplex mode), which allows for blood flow velocity and direction determination.

Doppler ultrasound had been employed to examine responses to a physical stimulus. Initially, Celermajer and colleagues (1992)⁴⁰ developed a protocol using the brachial artery since it could easily be imaged by ultrasound. After acquiring a resting image of the brachial artery a period of forearm occlusion was used to create a shear stress. The percent change in vessel diameter after forearm occlusion (termed flow-mediated dilation; FMD) is used as a marker of vascular function and is also thought to be a potential marker for coronary artery

disease⁴¹. Several factors have been known to influence vaso-reactivity including subject preparation, time of day, meal composition, smoking and exercise. Therefore, technical guidelines have provided a more universal approach to minimize biological and environmental variability⁴¹⁻⁴³. The measurement of flow-mediated dilation (FMD) has been shown reproducible and accurate under these standardized conditions^{44,45}. Furthermore, FMD is predictive of future cardiovascular events in healthy⁴⁶ and diseased⁴⁷ populations.

2.3.3 Resistance Vessel Assessments

The systemic vasculature has mechanical properties important to the cardiac work of the heart. Hence arterial distensibility and compliance have been targeted as potential indicators of cardiovascular health. The non-invasive method used to evaluate these characteristics in resistance arteries is applanation tonometry. Using a hand-held strain gauge pressure sensor over the radial artery, the arterial pulse is digitally recorded. From this recording, a series of algorithms generates pressure waveforms and provides central blood pressure, augmentation pressure and index. The augmentation index is defined as the ratio of height of the peak above the shoulder of the wave to the pulse pressure, and indicates the pressure rise resulting from the peak flow input into the vasculature prior to wave reflection⁴⁸. An increase in the pulse pressure and augmentation index represents arterial waves reaching the heart during systole, which leads to an increase in cardiac afterload (forces opposing ejection of blood from the heart)⁴⁹.

Another noninvasive measure that can be obtained from applanation tonometry is pulse wave velocity. Pulse wave velocity (PWV) is based on the pulse pressure generated by the ventricular ejection that travels through the vasculature at a certain speed. Using the same strain gauge pressure sensor, arterial pulse waves are recorded at two different sites

along the vascular tree (i.e. carotid and radial arteries). The PWV is derived from foot-to-foot transit time and the distance between the two waves. The distance from the carotid pulse to the suprasternal notch and from the suprasternal notch down to the radial artery are measured by gulick tape. The speed at which this pulse travels is altered by the mechanical properties of the vessel giving a gauge of distensibility and compliance. Higher PWV demonstrates a greater vascular resistance and rigidity of the vascular wall⁵⁰. The ease of this measurement and high reproducibility (0.14 ± 0.829 m/s within-observer and -0.44 ± 1.09 m/s between-observer)⁵¹ has allowed for applanation tonometry to be applied in clinical settings. PWV is strongly associated with cardiovascular events and all-cause mortality^{52,53} in hypertensive patients⁵³.

2.4 Neural Cardiovascular Control during Dynamic Exercise

Exercise is defined as a structured form of physical activity aimed at improving or maintaining physical fitness⁶. Cardiovascular responses to aerobic exercise are a result of central neural command and skeletal muscle mechanical stimuli. Depending on the physical task presented to the body, the acute responses and chronic adaptation of the cardiovascular and nervous system vary in order to provide the most efficient response. In this section, I will address cardiovascular and autonomic regulation at the onset of aerobic exercise and after chronic aerobic training.

2.4.1. Cardiac Performance

The heart functions to maintain blood pressure, which is accomplished through alterations in cardiac output. Cardiac output (Q), the product of heart rate and stroke volume, is the amount of blood pumped by the heart per minute (L/min). Adjustments in either heart rate or stroke volume will influence cardiac output (Q). Stroke volume during exercise is

determined by the following factors: preload, afterload, myocardial contractility and heart rate⁸. At the onset of exercise demand for oxygen to active tissues promoting a shift of oxygen offloading into the tissue. In humans, the Fick equation explains the rate of oxygen uptake (VO_2) from inspired air (Equation 4.1). The amount of oxygen taken up (VO_2) into the blood is the difference in the rate (Q) of the amount of oxygen leaving (oxygenated blood) and entering (deoxygenated blood) the lungs. Therefore, oxygen uptake (VO_2) is the product of Q and arterial-venous oxygen concentration difference (a-v O_2 diff).

$$\text{Equation 4.1 } \text{VO}_2 = Q * \text{a-vO}_2 \text{ diff or } \text{VO}_2 = \text{HR} * \text{SV} * \text{a-vO}_2 \text{ diff}$$

Each component (HR, SV and a-v O_2 diff) can increase VO_2 during exercise. The heart is under the influence of the autonomic nervous system that has the ability to increase HR, SA node firing rate and force of contraction. At rest the parasympathetic nervous system dominates typically keeping HR at 60-80beats/min⁸. The body is capable to overriding the PNS activity to the heart as seen with the anticipatory response prior to exercise. The anticipatory response causes a rise in HR prior to or leading up to the start of exercise due to a release in epinephrine is not uncommon when individuals are put in a stressful environment such as competitions or exercise testing. At the onset of exercise, HR increases due to the withdrawal of parasympathetic (vagal) tone and the increase in sympathetic activity⁵⁴. During submaximal aerobic exercise (~100-150beats/min), HR increases to raise cardiac output to meet the oxygen demand of the active muscle mass. At a low-to-moderate intensity, the rise in HR is predominately mediated by the withdrawal of parasympathetic activity⁸. At approximately 50-60% of $\text{VO}_{2\text{max}}$ the increase in HR is a result of sympathetic activity⁵⁵. Steady state HR for a given workload can be achieved typically within 2 minutes, but will increase linearly as exercise intensity increases. The point at

which heart rate begins to level off with an increase in intensity is indicative of maximal exercise capacity (~180-200bpm). Immediately after the cessation of exercise, heart rate drops quickly (heart rate recovery) due to the rapid parasympathetic reactivation. Individuals with higher levels of fitness have higher resting levels of parasympathetic activity leading to bradycardia, and therefore have lower resting HR and more rapid HR recovery compared to unfit individuals⁵⁶. Several pharmacological agents also influence heart rate. For example, atropine is commonly used to block parasympathetic activity and increase heart rate, while propranolol selectively blocks sympathetic activity resulting in a decreased heart rate⁵⁴.

Stroke volume (SV) (~70mL) is the difference between left ventricular end-diastolic volume (~130mL) and left ventricular systolic volume (~60mL). In an upright position, SV increases curvilinear with work rate during exercise to ~90mL, or about 50% of its maximum where reaches a plateau. Preload, afterload, myocardial contractility and heart rate⁸ are each factors that influence SV. The relationship between SV and preload (myocardial stretch) is described by the Frank-Starling mechanism. Briefly, an increase in end-diastolic volume increases the preload, which stretches the ventricular muscle fibers. This stretch leads to an increased force of contraction causing the heart to eject the larger volume of blood. The extent of left ventricular preload is subject to several factors including the ventricular cavity size, total blood volume, venous tone, intrathoracic pressure, body position and the blood return to the heart due to the muscle pump. During exercise in the upright position, preload is increased via an increase in venous return; venous constriction, the muscle pump and respiratory activity increasing and decreasing intrathoracic pressure to rhythmically move more blood to the heart (respiratory pump) to achieve a greater preload. Conversely, venous return can be decreased by vena cava compression as seen during a Valsalva maneuver. The

Valsalva maneuver raises intrathoracic pressure leading to a decreased transmural pressure (pressure across the wall of a blood vessel). This pressure differential results in venous compression, which impedes venous return into the thorax and reduces preload. Venous return is also sensitive to alterations in body posture in the absence of compensatory mechanisms. In particular, standing body posture results in gravity driven pooling of blood in the lower extremities that reduces blood to the heart and preload. Orthostatic intolerance encompasses several conditions where when standing the skeletal muscle pump mechanism is not activated to maintain venous return, sympathetic nerve activity becomes less effective in venous constriction and blood pools⁵⁷.

The last component that influences VO_2 is the a-vO_2 diff, or the amount of oxygen extracted at the tissue level. During exercise a-vO_2 difference increases with an increase in exercise intensity from $\sim 5\text{mL O}_2/100\text{mL blood}$ to $\sim 15\text{mL}/100\text{mL blood}$ at maximal exercise. The a-vO_2 difference is dependent on the arterial oxygen carrying capacity, the ability of the respiratory system to load oxygen and the ability to unload oxygen in the active tissues. Trained individuals tend to have greater oxygen carrying capacity and a-vO_2 difference than sedentary counterparts during maximal exercise. No matter the exercise intensity, some oxygenated blood will supply other tissues that are not fully active⁸.

2.4.2 Circulation

2.4.2.1 Principles of Blood Flow

The peripheral vascular network is responsible for the circulation of ~ 5 liters of blood. Circulation through the different vascular compartments changes as each has different properties that manipulate hemodynamics, and therefore alters rate of blood flow to a specific region of the body. Control of blood flow, or Q can be described in terms of principles of

fluid mechanics (flow, pressure and resistance) and the assumption that fluids are continuous. The driving force of blood flow is the pressure differences at two ends of a vessel, which is almost always along a gradient of high to low areas of pressures⁸. The relationship of flow to pressure and resistance is described below, where Q is directly proportional to the change in pressure (ΔP) between two ends of a blood vessel and inversely related to the total peripheral resistance (TPR) (Equation 4.2). Total blood flow, expressed Q in liters per minute (L/min), is ~5L/min at rest and is capable of increasing five to six fold during exercise (25-30L/min)⁸.

$$\text{Equation 4.2 } Q = \Delta P / \text{TPR}$$

The movement of blood at a steady rate through a vessel in concentric, streamline layers is called laminar flow⁵⁸. During laminar flow, the velocity in the center of the lumen is greater than that at the edges due to contact with the intima. The drag of the outermost layer of blood causes the next layer slip by and so on forming a parabolic velocity profile where blood layers in the middle are able to move by rapidly. In contrast to laminar flow is turbulent flow, which can occur as blood passes obstructions such as curvatures or bifurcations. Turbulent flow is unparallelled, disorganized and creates detectable sounds (known as Korotkoff sounds)⁵⁸. The probability or tendency of turbulent blood flow can be predicted by Reynold's number (Re) which is directly proportional to the mean velocity of blood flow (v), vessel diameter (d), density of blood (ρ), and inversely proportional to the viscosity of the blood (η) (Equation 4.3). When the value of Re is greater than 2000 (unitless), turbulence is almost always present⁵⁸. For instance, an atherosclerotic plaque narrowing the artery would create high blood flow velocity through the point of constriction and loud, turbulent blood flow beyond that point.

$$\text{Equation 4.3 } Re = vd\rho/\eta$$

Furthermore, this principle can be applied when the measuring blood pressure; the cuff narrows the artery creating turbulent flow identified as systolic pressure, and as flow becomes laminar the diastolic pressure can be determined. In situations where the vessel is small and essentially no rapidly flowing center of blood exists, Poiseuille's Law can be applied (Equation 4.4) where the rate of blood flow (Q) is directly proportional to the pressure change (ΔP) and radius (r^4) and inversely related to viscosity (η) and length of the vessel (l). Note that the radius of the blood vessel markedly affects blood flow (to the fourth power). Hence arterioles, which are capable of precise diameter changes, have pronounced effects on the regulation of peripheral resistance and blood flow. In equation 4.4, viscosity of blood is taken into account, but does not have a large effect on blood flow unless there is a considerable change in the hematocrit⁵⁸ (i.e. blood doping or dehydration).

$$\text{Equation 4.4 } Q = \pi \Delta P r^4 / 8 \eta l$$

2.4.2.2 Blood Flow Regulation

Local control of blood flow is mediated through multiple mechanisms to adjust blood flow to match metabolic demands while maintaining systemic and cerebral blood pressures. Transitioning from rest to exercise increases demand for skeletal muscle and skin blood flow and decreases demand for visceral blood flow. This rapid shunting of blood from one system to another involves autoregulation and neural control via the sympathetic nervous system. At rest, the sympathetic nerves that innervate arterioles exhibit a tonic discharge giving vessels a resting, constricted tone¹⁹. During exercise, sympathetic outflow is increased causing vasoconstriction at inactive tissue mediated through α_1 or α_2 receptors and vasodilation mediated through β_1 , β_2 or M3 receptors at active tissue. However, sympathetic discharge

balances parasympathetic activity in exercising muscle even during high intensity exercise in order to maintain blood pressure^{59,60}. Buckwalter et al. (2001) found alpha-adrenergic receptor sensitivity of arterial vasculature in exercising muscle is attenuated from rest to exercise in an intensity-dependent manner, suggesting a decrease in the magnitude of vasoconstriction allowing for the active skeletal muscle to receive more blood flow⁶¹. Factors that affect sympathetic constriction of the arteries and arterioles include, but are not limited to, density of sympathetic nerves and subtype of adrenergic receptors, differences in norepinephrine kinetics, degree of resting tone, and concentration of metabolites from muscle activity¹⁹. Although sympathetic nerves are active during exercise, NO released from the endothelium may blunt vascular response to sympathetic activity, a phenomenon called functional sympatholysis⁶². Jendzjowsky et al. (2013) demonstrated NO inhibition augmented sympathetic vasoconstriction while NO blockade did not, suggesting a NO-mediated mechanism is behind functional sympatholysis during rest and exercise⁶³.

Blood flow is coupled with metabolic regulation of peripheral tissues. The formation of vasodilatory metabolites (potassium, magnesium, carbon dioxide, adenosine, hydrogen ions) and hormones (bradykinin, histamine) occurs when the metabolic rate of a specific tissue increases or oxygen delivery decreases¹⁴. Under exercising conditions, metabolic factors are released from active muscle, diffuse through the interstitial space, and act on smooth muscle cells to increase blood flow. Conversely, vasoconstricting metabolites (calcium, endothelin-A) and hormones (angiotensin II, vasopressin) may decrease blood flow. Occluding a vessel can also interfere with blood flow to produce an increase in vasodilatory substances, called reactive hyperemia or hypoxia-induced vasodilation. Reduced oxygen supply (oxygen tension) and increased oxygen demand to active tissues

creates a hypoxic environment. Upon release of clamping a vessel blood flow increases above resting level and remains elevated until metabolic conditions return to homeostasis¹³. Local vasodilation in response to tissue hypoxia ensures oxygen delivery to metabolically stressed areas under normal conditions⁶⁴.

One of the most important local dilating factors is endothelial derived NO. Furchgott and Zawadzki (1980) were the first to recognize the importance of the endothelium in controlling vascular smoother muscle tone⁶⁵. In rabbit arteries, disruption of the endothelium abolishes NO-mediating response of acetylcholine, suggesting the intact endothelium is necessary for vasodilation in response to acetylcholine. Physical stimuli such as reactive hyperemia can lead to endothelium-dependent vasodilation. For example, contracting skeletal muscles during exercise produce shear stress on a vessel, increasing calcium flux into the intracellular space through specific stretch activated calcium channels. The rise in intracellular calcium triggers vascular smooth muscle relaxation through three pathways; 1) production of nitric oxide (NO) via nitric oxide synthase (eNOS), 2) production of prostacyclin (PGI-2) and 3) endothelium-derived hyperpolarizing factor (EDHF)⁶⁶. Figure 2.2 is a model of receptor-signal transduction in endothelium cell which leads to vasodilation⁶⁷. Nitric oxide (NO) is produced from the metabolic conversion of L-arginine to L-citrulline via eNOS. The NO formed in the endothelium diffuses to the smooth muscle cells, where it interacts with guanylate cyclase to produce cyclic guanosine monophosphate (cGMP)⁵⁸. The cGMP activates additional protein kinases, increasing calcium release and uptake, and subsequently relaxation in the smooth muscle cells⁶⁶.

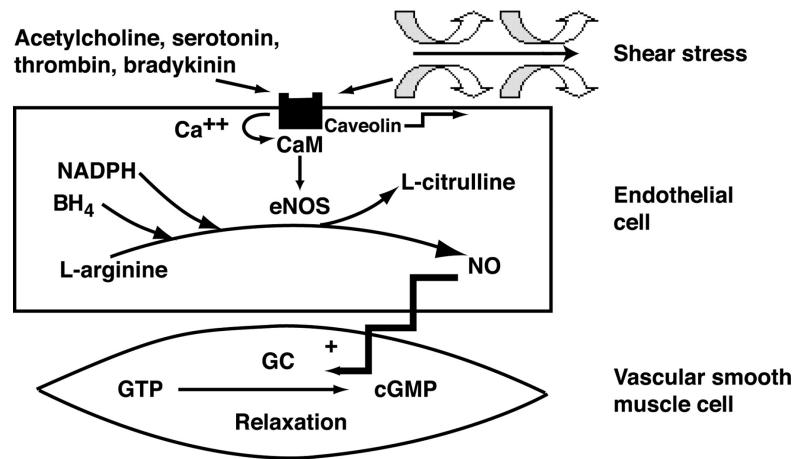


Figure 2.2. Production of nitric oxide (NO) by endothelial cells. NO is produced by the action of endothelial nitric oxide synthase (eNOS) on l-arginine. This reaction requires a number of cofactors, including tetrahydrobiopterin (BH_4) and nicotinamide adenine dinucleotide phosphate (NADPH). Increased intercellular Ca^{++} in response to vasodilator agonists or shear stress displaces the inhibitor caveolin from calmodulin (CaM), activating eNOS. NO diffuses to vascular smooth muscle and causes relaxation by activating guanylate cyclase (GC), thereby increasing intracellular cyclic guanosine monophosphate (cGMP). Reprinted with permission from⁶⁷.

Myogenic regulation occurs in direct response to a rise or fall in blood pressure to maintain a vascular tone. Under increasing transmural pressure vascular smooth muscle contracts, thus allowing for blood flow through a vessel to remain constant. This inherent behavior is most pronounced in arterioles to protect against over-perfusion of capillaries. The stimulus for the myogenic action, or Bayliss effect, postulated to be triggered by an increase in wall tension from stretch in the blood vessel. The distended vessel then opens stretch-activated ion channels to release calcium. The influx of calcium leads to smooth muscle contraction and in turn reduces the radius of the vessel¹⁴.

2.4.3 Adaptations to Exercise Training

Considerable data demonstrates long-term (weeks to months) exercise training increases cardiorespiratory fitness, which is associated with lower heart rate, increased vagal tone and improved endothelial function. Typically with 6-months of exercise training, a 15-

20% increase in $\text{VO}_{2\text{max}}$ can be expected⁶⁸. Previous cross-sectional studies suggest both young and older physically active participants have superior vascular and autonomic health compared to sedentary, inactive individuals⁶⁹⁻⁷³. Furthermore, clinical patients with the highest CVD risk and lowest cardiorespiratory fitness who increase their levels of physical activity may have the greatest health benefits⁷⁴. Since exercise capacity has been strongly associated with cardiovascular events and all-cause mortality in patients with known CVD^{6,75}, exercise training is imperative to developing positive cardiovascular adaptations that combat disease in both healthy and sick populations.

From the Fick equation, $\text{VO}_{2\text{max}}$ following exercise training is increased due to adaptations in HR, SV and a- vO_2 diff. At rest a trained individual has a lower resting and submaximal exercise HR compared to an untrained individual; however, at maximal exercise HR does not differ greatly. Exercise training enhances parasympathetic modulation and decreases sympathetic activity at rest in healthy populations^{54,76}, which may contribute to bradycardia. Several cross-sectional studies comparing athletes to sedentary controls reflect an increase in HRV, particularly an increase in the high frequency domain (parasympathetic activity)⁷⁷⁻⁷⁹. For example, long distance runners have demonstrated a lower resting HR, greater high frequency (parasympathetic activity) and reduced low frequency (sympathetic activity) compared to sedentary controls⁷⁹. Longitudinal exercise training studies in sedentary participants have also demonstrated an improvement in autonomic balance^{70,80}. For example, Melanson et al. (2001) showed a 14% increase in $\text{VO}_{2\text{max}}$ and increase in pNN50, RMSSD and HF after 16 weeks of moderate exercise training⁷⁰.

Stroke volume (SV) increases approximately 20% with exercise training, with the increases occurring at rest, during submaximal and maximal exercise⁸. A trained individual's

stroke volume may approach ~150-200 mL, compared to 110-130 mL in untrained people, which can potentially be attributed to increased blood volume and contractility. A longer adaptive response includes hypertrophy of cardiac muscle fibers. Higher Q values in trained individuals at maximal exercise are related to the increases in SV as opposed to changes in maximal a-vO₂ diff. The rate of blood flow and oxygen delivery to the tissue is higher, which may lead to the faster unloading of oxygen. The slight adaptation in a-vO₂ diff at rest and during submaximal and maximal exercise may have to do with oxygen's affinity to hemoglobin. With exercise training the blood may loosen the binding of oxygen to hemoglobin to unload oxygen more easily to the tissues⁸. Other adaptations include increased capillary density and blood volume and decreased blood pressure and total peripheral resistance which contribute to reduced blood flow demand of the heart⁸. Therefore, during exercise a more trained individual has less strain on the heart.

In the peripheral vasculature functional and structural changes also occur with exercise training. Repetitive skeletal muscle contraction on the arterial walls during exercise causes an induced shear stress^{81,82}. The response to the intermittent shear stress from several bouts of exercise leads to the release of NO from the endothelium therefore dilating the vessel. The NO machinery may lead to vascular remodeling in order to cope with the shear stress imposed during exercise⁸¹. As previously discussed, flow-mediated dilation (conduit vessel assessment) and pulse wave analysis (resistance vessel assessment) are barometers of vascular health. Although literature conflicts, the general consensus is that FMD and PWV measures improve in response to exercise training^{83,84}. In a recent meta-analysis, FMD was found to improve with exercise training, regardless of exercise modality^{85,86}. The magnitude of change did appear to be related to the exercise intensity and duration. Tanaka et al. (1998)

found pulse wave velocity to be 30% lower in aerobically trained women compared to sedentary women⁸⁷. Resistance vessel adaptation may be due to neuro-humoral mechanisms improving vasomotor function⁸².

2.5 Clinical Relevance of Autonomic and Vascular Function

The structural components of the autonomic nervous and cardiovascular system and their mechanisms of control are altered by several controllable (exercise, diet, smoking) and uncontrollable (age, gender, race, family history) factors. Both controllable and uncontrollable factors have an important role in the development of cardiovascular diseases. Age, gender and race predispose an individual to autonomic imbalance and vascular dysfunction associated with cardiovascular disease, which may be accelerated by controllable factors. In this section, I will discuss predisposed factors (aging, gender and race) that affect autonomic and vascular function, and examine the impact of cardiovascular disease on autonomic and vascular properties.

2.5.1 Impact of Age and Gender

Aging is associated with a decline in cardiovascular adaptations, neural control and aerobic capacity, which accelerates with advancing age^{8,88}. Deteriorations in both the cardiovascular and nervous system with aging contributes to endothelial dysfunction, arterial stiffness, and increased sympathetic activity⁸. All of these factors are apparent during exercise where blood flow is not as tightly regulated and maximal heart rate and cardiac output are reduced.

Structural and functional changes occur in the heart and blood vessels during the aging process leading to “stiffer” vasculature. As previously discussed, surrounding the tunica media is an extracellular matrix composed of collagen, elastin and a sheath of

microfibrils to maintain the vessel's integrity. During aging, microfibrils disappear causing the vessel to slowly lose elasticity and impede the control of blood flow⁸⁹. The smooth muscle of the tunica media can also alter vascular stiffness by displacing force on the vessel walls. Carroll et al. (1991) infused nitroprusside, a vasodilator, in patients with dilated cardiomyopathy across young, middle and older aged groups⁹⁰. The oldest group had the greatest reduction in aortic stiffness, suggesting augmented smooth muscle tone contributes to increased stiffening with age. Due to these structural changes, PWV is greater⁹¹ and FMD is lower⁹² in older individuals. Age-related changes in PWV and FMD suggest arterial thickening and stiffness and endothelial dysfunction are due to the increased presence of atherosclerosis, which leads to the development of cardiovascular diseases⁹³.

The loss of baroreflex sensitivity may be in part due to vascular stiffening in the regions where baroreceptors are found. With aging, there is less stretch in the arterial wall and therefore a reduced ability of baroreceptors to transmit signals⁹⁴. Older individuals also appear to have diminished response to β -adrenergic receptors and elevated plasma levels of norepinephrine⁹⁵. The excess plasma norepinephrine often, called 'spillover' in the elderly, is believed to be due to an increase in sympathetic discharge and reduced uptake at nerve junctions. The prolonged exposure to norepinephrine spillover seen with aging desensitizes β -adrenergic receptors causing smooth muscle to remain vasoconstricted¹⁹.

Heart rate modulation reflects the aging process through a decreased maximal heart rate and variability. Increased sympathetic activity is associated with aging and can be detected as a decreased HRV⁹⁶⁻⁹⁸. Interestingly, the effect of aging on HRV is not linear throughout the entire life span. Through childhood, HRV steadily increases up to 10 years old⁹⁹, after which, it linearly increases until 60 years old followed by a decline in later

decades. Developmental changes in the cardiovascular system account for a younger individuals heart to respond differently than an adult heart¹⁰⁰. Older age is associated with decreased HF and increased LF/HF, suggesting that the sympathovagal balance progressively increases and parasympathetic tone decreases with age¹¹.

Generally females have been found to have significantly lower HRV parameters than men suggesting an enhanced parasympathetic tone^{97,101,102}. In addition, during periods of cardiac stress, women have been found to have greater indices of vagal activity compared with men¹⁰³. The mechanisms responsible for the observed gender differences have not been completely elucidated; however, some researchers have suggested that female hormones are involved. Estrogen, for example, has been found to alter calcium and potassium flux in cardiomyocytes, which would affect catecholamine uptake and change ANS function¹⁰⁴. Women also have a lower cardiovascular risk compared to men, suggesting that the lower HRV found in women may provide cardio-protection against arrhythmia and atherosclerosis development¹⁰⁵. Gender influences seem to disappear between 40-60 years old^{101,105} and may be associated with menopause or a general decline in parasympathetic activity.

2.5.2 Impact of Race

In a recent publication in the *New England Journal of Medicine*, researchers found African-Americans had much higher incidences of heart failure that develops at a younger age compared to other races¹⁰⁶. The incidence of CVD affects African-Americans (AA) disproportionally, believed to be largely attributable to the higher prevalence of CVD risk factors. African-Americans have the higher rates of obesity¹⁰⁷, hypertension¹⁰⁸ and a higher prevalence of type-2-diabetes than Caucasian counterparts¹⁰⁹. In addition, AA have a significantly lower exercise capacity¹⁰⁷. Racial differences in CVD risk factors are complex

and multifactorial and may relate to vascular and autonomic tone. However, the extent of racial influence on vascular and autonomic measures is not yet fully understood.

Arterial wall function may vary between AA and Caucasians leading to alterations in vascular homeostasis. Lang et al.¹¹⁰ found vasodilation in response to brachial artery infusion of isoproterenol (a vasodilator) was significantly attenuated in AA. Other research has also found FMD to be notably blunted in AA¹¹¹⁻¹¹³, suggesting that the predisposition AA have to development of CVD risk factors may begin with endothelial dysfunction. The loss of endothelial nitric-oxide synthase activity and bioavailability of nitric oxide to the smooth muscle in the arterial wall may bring about flawed cell signaling and impairing vasodilation; in turn, contributing to the pathogenesis of CVD and explain the increased risk in AA. In addition to FMD, pulse wave analysis in AA has suggested they have a higher prevalence of arterial stiffness than that of Caucasians at rest¹¹⁴⁻¹¹⁸. Reasons for this difference are not clear, but may relate to racial differences in vessel-wall structure¹¹⁷. AA may have a smooth muscle phenotype and activity that affects the proportion of collagen, thereby influencing vascular stiffness⁴⁹.

Interestingly, few studies have linked conduit (FMD) and resistance (PWV) vessel function with autonomic (HRV) function, especially in relation to race. Reduced arterial stiffness (PWV) has been correlated with parasympathetic activity^{119,120} in healthy, Caucasian individuals and sympathetic activity¹²¹ in healthy, Asian individuals, suggesting race may influence autonomic activity. Pinter et al.¹²² found FMD positively correlated with parasympathetic HRV parameters, suggesting endothelial NO may mediate both functions. The autonomic nervous system and endothelial factors work together to maintain vascular tone, and NO may augment vagal tone in healthy individuals¹²³. However, this study was

limited to a younger, male sample with no race differences reported. Presently, research investigating the effects of race on FMD, PWV and HRV function, and the relationship of these variables to each other, is limited.

African-Americans show signs of premature deterioration of the autonomic nervous system. Table 2.2 presents studies that have examined racial differences in HRV measurements, many of which are conflicting. Initially, Liao and colleagues (1995) found AA to have lower LF (sympathetic activity) and greater HF (parasympathetic activity) in the ARIC (Atherosclerosis Risk in Communities) longitudinal study conducted in 27% AA adults¹². On the other hand, Zion et al. (2003) and Faulkner et al. (2003) reported lower HF (parasympathetic modulation) in AA adolescents and young adults^{124,125}. Choi et al. (2006) has also demonstrated that AA subjects had lower HRV (decreased HF) than their Caucasian aged-matched counterparts¹¹. In particular, this study showed younger AA subjects to have HRV indices that were comparable to older Caucasian participants. This is interesting because it suggests that young AA may show the beginnings of vascular and autonomic dysfunction, again, predisposing them to CVD risk factors earlier in life. Autonomic dysfunction may be even more hindering during exercise, where switching from parasympathetic to sympathetic activity is blunted. Inability of the heart to meet oxygen demands by increasing rate may make exercise feel more difficult and reduce aerobic capacity. From these studies it remains unclear what effect, if any, race has on autonomic balance during exercise. Further research is necessary at rest and during exercise to further determine why racial disparities exist in the prevalence of diseases such as hypertension and obesity are higher in AA.

Table 2.2. Studies examining the impact of race on HRV parameters.

Study	Population	n (%AA)	Age (yr)	Spectral Analysis	Comments
Liao et al. 1995	Atherosclerosis	1984 (27%)	45-64	↑HF, LF/HF ↓LF	AA had higher HRV than CA; Supine, 2-min data
Urbina et al. 1998	Healthy adolescents	39 (50%)	13-17	↓ LF/HF	AA had higher HRV than CA; Supine Holter monitoring
Guzzetti et al. 2000	Hypertension	52 (50%)	45±2 (AA) 46±2 (CA)	↓LF, LF/HF	AA had higher HRV than CA; 24hr Holter monitoring
Faulkner et al. 2003	Healthy adolescents	75 (19%)	15±1.6	↓HF, LF	AA had lower HRV than CA; 24hr Holter monitoring
Zion et al. 2003	Healthy adults	61 (52%)	21.6±2.6 (AA) 23.9±3.6 (NA)	↑LF/HF ↓HF	AA had lower HRV than NA; Seated, 5-min data
Gutin et al. 2005	Healthy adolescents	304 (52%)	14-18	↑RMSSD ↓LF/HF	AA had higher HRV than CA; Supine, 1-min data
Lampert et al. 2005	Healthy adults	256 (19%)	45±3 (AA) 50±1 (CA)	↓SDNN, LF, HF	AA had lower HRV than CA; 24hr Holter monitoring
Wang et al. 2005	Healthy adolescents	166 (61%)	16±2	↑RMSSD, HF ↓LF/HF	AA had higher HRV than CA; Supine, 4-min data
Choi et al. 2006	Healthy adults	135 (43%)	37.7±8.5 (AA) 36.1±7.4 (CA)	↑LF/HF ↓HF	AA had lower HRV than older CA; Seated, 3-min data
Sloan et al. 2008	Healthy adults	757 (56%)	40±3.7	↓LF, LF/HF	AA had lower HRV than CA; Seated, 5-min data
Li et al. 2009	Healthy adults	399 (53%)	15-31	↑RMSSD, HF	AA had higher HRV than CA; Supine, 30-second data
Fuller-Rowell et al. 2013	Healthy adults	1037 (20%)	50.9±10.6 (AA) 54.5±11.6 (CA)	--	No race difference in RMSSD; Seated, 5-min data

AA= African American; CA= Caucasian; NA= Non-African American; HRV= Heart rate variability. Spectral analysis described in relation to AA study groups.

2.5.3 Impact of Cardiovascular Diseases

The Bogalusa Heart Study is one of the largest longitudinal studies in the United States that investigates the development of CVD among a population with a significant proportion of AA. The Bogalusa Heart Study has shown the pathological evidence of atherosclerosis begins in early childhood and is predictive of adulthood risk for CVD¹²⁶. AA have demonstrated greater atherosclerotic lesions than Caucasian counterparts across several age groups, suggesting AA begin the early stages of atherosclerosis earlier in life¹²⁷.

Decreased FMD and increased PWV are observed with the progression of cardiovascular disease. The vascular endothelium is functionally involved in regulating vascular tone, thrombosis and platelet adhesion. The development of atherosclerosis

interferes with these functions as well as the NO) that causes vasodilation¹²⁸. Endothelial dysfunction is believed to be one of the earliest stages of atherosclerosis, measured by FMD, and precedes hypercholesterolemia, hypertension, diabetes mellitus and obesity^{67,92}. The loss of endothelial cell integrity reduces NO bioavailability, which contributes to arterial stiffness, measured by PWV.

Hijmering et al. (2002) demonstrated that sympathetic stimulation significantly impairs FMD response in healthy individuals¹²⁹. In addition, Tsuji et al. (1996) demonstrated in apparently healthy individuals a reduced HRV (high sympathetic activity) was associated with greater risk for cardiac events, suggesting autonomic and endothelial (dys)function work concomitantly¹³⁰. A study by Huikrui and colleagues (1995) examined long-term HRV in coronary artery disease patients. SDNN independently predicted the progression of coronary artery disease¹³¹. Kleiger et al. (1987), observed patients recovering from myocardial infarctions and found those with the lowest HRV (lowest SDNN) had the greatest risk of a sudden death³². These studies point to the involvement of autonomic regulation in disease suggesting sympathetic tone directly impacts the vascular endothelium at rest. The mechanism of autonomic dysfunction may be related to an increase in arterial stiffness and altered endothelial properties.

2.6 Conclusion and Future Directions

In summary of this review, the heart and vast network of blood vessels share the common purpose to circulate blood to the necessary tissue in order to maintain homeostasis. The components of the cardiovascular system are subject to autonomic neural control, both of which are challenged under various physiological conditions including physical activity. Assessing the neural and vascular function requires a number of tools in order to fully

understand the relationships between structural and functional responses at rest and during exercise.

A large number of experimental and clinical studies involve the measurement of HRV, FMD and PWV, and have widespread acceptance as non-invasive tools for identifying potential abnormalities in cardiac autonomic function. Though standard methodologies have been established^{31,42}, there is substantial inter-individual variation including age, gender and race that should be controlled for. Future research will utilize non-invasive assessments to examine differences in apparently healthy adults and association between autonomic and cardiovascular function. The combination of PWV, FMD and HRV in assessments of cardiovascular disease and autonomic imbalance may increase overall predictive accuracy of these tools and provide a more holistic approach to the condition of cardiovascular health.

Determinants of HRV, FMD and PWV include age, gender and race. AA are at greater risk for developing CVD and CVD risk factors suggesting structural and functional changes may be occurring in the vasculature as well as autonomic imbalance at younger age compared to Caucasian counterparts. The importance of the autonomic system on the cardiovascular system is often overlooked in research despite autonomic modulation of the cardiovascular system being associated with adverse prognosis. The measurement of HRV is noninvasive and inexpensive, so it is important to relate HRV to the physiological counterparts to understand how changes in HRV parameters may be associated with CVD development. Presently, research on the relationship of HRV and race has yielded inconsistent results. In addition, few studies have examined the link between of neural *and* cardiovascular control in relationship to race. However, research has highlighted that AA are at greater risk for CVD. Future research on AA is important to identify mechanisms behind

the development of CVD and how exercise mediates changes in autonomic function.

Exercise training in AA can lead to significant gains in fitness, improved vascular function and autonomic balance, thus having the potential to delay onset of CVD, and major CVD related risk factors. Further research is necessary to identify an exercise intervention (program length, duration, intensity, mode) that optimizes HRV improvements. The use of FMD, PWV and HRV in future research including exercise interventions may allow for better risk stratification in healthy and clinical populations and provide valuable non-invasive information on autonomic and cardiovascular function as participants go through exercise training.

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CHAPTER 3. THE EFFECTS OF EXERCISE TRAINING ON BRACHIAL ARTERY FLOW-MEDIATED DILATION: A META-ANALYSIS

3.1 Introduction

In the last decade, brachial artery flow mediated dilation (BAFMD) utilized to assess endothelium-dependent vasodilatory function has become a popular barometer of vascular health, highly dependent on intact nitric oxide (NO)-machinery^{1,2}. Cardiovascular (CV) risk and future CV events in both asymptomatic^{3,4} and diseased populations⁵⁻⁷ have been associated with the BAFMD assessment. Endothelial dysfunction is considered an important early event in the development of atherosclerosis, preceding gross morphological signs and clinical symptoms of the inflammatory process and future CV disease⁸.

Growing evidence suggests exercise training (ET) improves vascular structure, NO bioavailability and reduces CV disease risk factors⁹, therefore making BAFMD assessment a common outcome measure to determine the efficacy of physical activity and ET¹⁰. Resistance and aerobic ET have demonstrated improved BAFMD in short and long term ET programs^{11,12}. The mechanical effects of muscular contractions during ET contribute to an acute oscillatory shear stress thought to serve as major stimulus for adaptations and release of NO, particularly in ‘flow-sensitive’ arteries¹³.

To date, few statistical reviews have examined the effects of ET on BAFMD^{1,14-16}. These reviews were limited by a small special population including type 2 diabetics¹⁴ or children¹⁶ and lacked comprehension of potential inclusive studies and moderating factors (modality, length of program, exercise volume, fitness status) of ET¹⁵. Thus, this systematic review will explore the effect of ET on BAFMD and potential ET and participant characteristic moderating factors.

3.2 Methods

3.2.1 Search Strategy and Selection Criteria

In a systematic search of Pubmed (from 1999 to 2013), 913 citations were obtained using the search terms “brachial artery flow-mediated dilation”, “vasodilation” or “endothelial function” or “vascular reactivity” and “exercise” or “training”. Due to technological advances and standardization of methods, articles prior to 1999 were excluded. In addition, reference lists of original and review articles were analyzed manually for full-text retrieval. The search of studies was not restricted by language, age or publication status. After an initial screening of articles based on title and abstract, potentially relevant studies investigating BAFMD and ET were assessed for inclusion and quality in the current meta-analysis (Figure 3.1). This review is in accordance with PRISMA statement guidelines¹⁷.

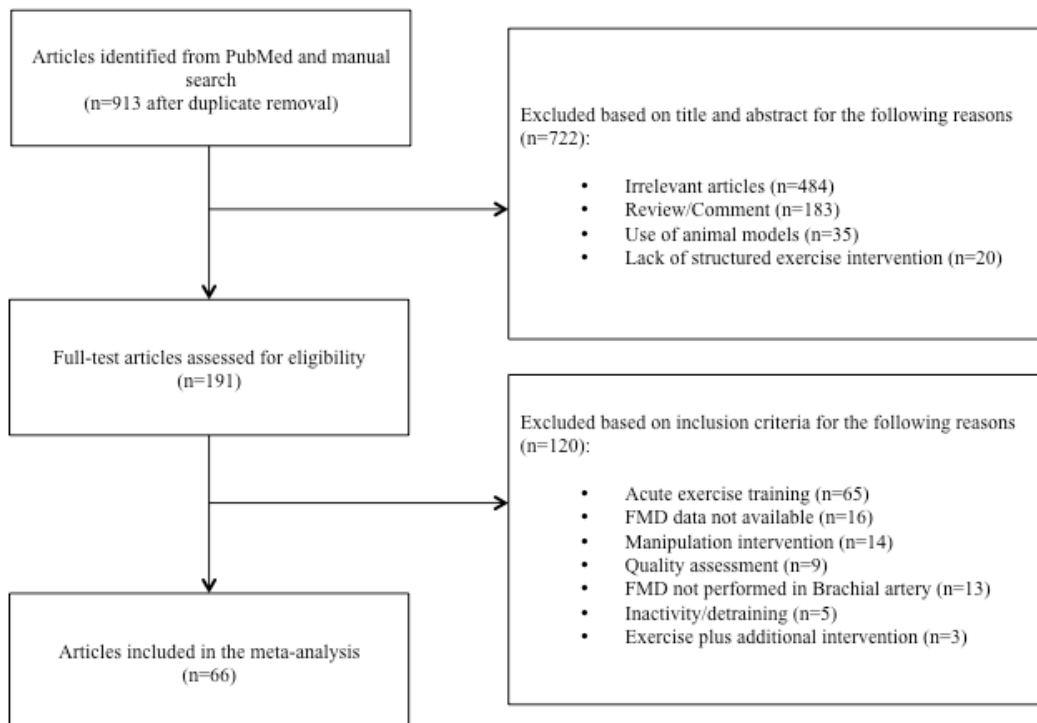


Figure 3.1. Study search and selection process. *FMD* flow-mediated dilation.

Original studies were included if they met the following criteria: 1) the use of human subjects; 2) duration of ET intervention was ≥ 1 week; 3) vascular function was evaluated by BAFMD with forearm occlusion; 4) data concerning pre- and post- ET intervention BAFMD [mean and standard deviations (SDs)] were reported. Studies involving manipulation (i.e. blood flow restriction, functional electrical stimulation, etc.), detraining or ET with additional interventions such as diet or pharmacological agents were excluded.

3.2.2 Quality Assessment and Data Extraction

Two reviewers (K.A. and A.S.) independently selected studies, extracted data and assessed the publications according to inclusion criteria. Discrepancies in inclusion/exclusion were resolved by a third party reviewer (N.J.). Reviewers judged the quality of studies by the Jadad et al. (1996)¹⁸ 3-item scoring instrument, which evaluates the study quality in terms of randomization quality, blinding, and reporting of withdrawals/dropouts. Studies scoring a 0 represented the lowest level of quality, with a possible score between 0 and 5. However, since the criterion item regarding blinding requirements was not applicable to ET interventions, the scoring was modified to assess the blinding of the outcome assessment (BAFMD); therefore, the highest possible Jadad score was 4.

The primary outcome of the present study was to investigate how ET impacts BAFMD. For each study, participant characteristics [age, sex, number of participants]; health status of participants [healthy, CV disease, metabolic diseases, hypertension, obesity, other]; ET characteristics [length of ET, duration of ET bout, frequency of sessions, ET intensity, pre- and post- fitness level]; BAFMD characteristics [baseline and peak artery diameter, absolute change of FMD, FMD mean and standard deviation (SD)]; and

methodological considerations [probe strength, cuff placement] were retrieved. ET intensity (1=very light-light, 2=moderate, 3=hard-near maximal), duration (<150min/week, \geq 150min/week) and baseline fitness level (percentile category) were rated according to ACSM's Guidelines for Exercise Testing and Prescription¹⁹. When necessary, corresponding authors were contacted for unpublished data that was not reported or displayed numerically in the content of the paper.

3.2.3 Statistical Analysis

Statistical analysis was performed using SPSS 20.0.0 statistical software for Mac OS X (SPSS Inc., Chicago, IL). Descriptive data are reported as mean \pm standard deviation (SD). BAFMD is determined as the percent change in brachial artery diameter after a 5-minute period of forearm occlusion, and is displayed as absolute (mm) and relative (%) change. To facilitate comparison of results across studies, treatment and control effect sizes were calculated by the differences in the means pre- and post-intervention divided by the pre-intervention standard deviation²⁰. Each mean effect size (ES) was calculated as a weight mean difference with 95% confidence intervals²⁰. Data were screened for normality using skewness, kurtosis and normal quantile plots. Publication bias was examined by funnel plot asymmetry as well as two formal tests: Egger's weighted regression test²¹ and Begg and Mazumdar's rank correlation test²². In the case of publication bias, the number of unpublished trials containing ES of zero is defined as $[K(d_k - d_c)]/d_c$, where K =number of obtained studies, d_k =mean ES of obtained studies, and d_c =trivial ES to which the obtained ES would be reduced²⁰.

Differences in effect of ET on BAFMD were assessed in the following subgroups: age of participants at baseline (\leq 30 versus 31-59 versus \geq 60 years), disease status (diseased

versus asymptomatic), fitness change, training type (aerobic versus resistance ET versus combined aerobic and resistance ET versus control), duration of ET program (≥ 12 weeks versus < 12 weeks), and ET intensity (very light-light, moderate, high-near maximal), volume (< 150 min/wk versus ≥ 150 min/wk), trial quality (≤ 2 versus > 2). Age and duration of training subgroups were determined in order to equally distribute ES. Correlation between fitness and BAFMD ES was evaluated using the Spearman-rank correlation analysis. To counteract multiple comparisons on the same data set, a Bonferroni correction was utilized to determine the critical P-value for each comparison.

3.3 Results

3.3.1 Literature Search and Publication Bias

A total of 191 related studies were identified from 1999 to 2013 for eligibility screening into the present study (Figure 3.1). Of these, 120 studies were excluded because they were acute ET (n=65) or detraining (n=5) regimens, a manipulation or therapy was applied (i.e. functional electrical stimulation) (n=14), ET plus additional interventions were applied (n=3), and flow mediated dilatation was not performed with forearm occlusion imaging the brachial artery (n=13), or BAFMD data was not available after author contact (n=16). Finally, of the 66 studies included in the final analysis, the sample size ranged between 7 and 146 participants with a total of 1865 ET intervention and 635 control subjects (Table 3.1). The mean and modal quality of studies (Jadad score) were 2 and 1 (range, 1-4), respectively.

Asymmetry of funnel plots suggests a potential publication bias. To confirm, the intercept of the regression relation effect between standard normal deviate and the inverse standard error was significantly different from zero (intercept=0.22, $P < 0.001$), and the

Kendall-tau correlation coefficient between the ES and its variance was also significant ($r=0.60$, $P<0.001$). The wide spread of sample size among the present studies and absence of ‘negative’ studies potentially contributed to the bias. It is estimated that the number of unpublished studies needed to bring the mean effect to a non-significant level is 411 studies²⁰.

3.3.2 Study Characteristics

The main demographic characteristics of the studies are described in Table 3.1. The mean age was 47 ± 19 (range, 9-81) years and percentage of men was 50%. Seven of the studies included children ranging from 8-14 (median, 11)²³⁻²⁹. The populations of the studies primarily included CV-related diseases ($n=19$), asymptomatic/healthy subjects ($n=18$), metabolic related diseases ($n=10$), hypertension ($n=10$), overweight and obesity ($n=7$) and thyroid related issues ($n=2$).

Prior to ET intervention, the mean baseline artery diameter and BAFMD were 3.92 ± 0.50 mm (range, 2.61-5.33mm) and $5.5\pm 2.4\%$ (range, 0.9-13.6%), respectively. Post-ET intervention groups mean BAFMD improved to $8.1\pm 3.9\%$ ($P<0.0001$) while there was no change in control group ($5.4\pm 1.9\%$, $P=0.72$). The absolute change of BAFMD in trained groups ($n=22$) was significant from pre- to post-intervention (0.26 ± 0.11 to 0.31 ± 0.13 mm, $P=0.01$), and the control group ($n=11$) absolute change in diameter decreased (0.24 ± 0.07 to 0.22 ± 0.07 mm, $P=0.23$). However, baseline artery diameter remained the same post-intervention in exercise training (3.92 ± 0.50 mm) and control groups (4.04 ± 0.56 mm). The mean ET intervention lasted 12 ± 8 (range, 1-52) weeks (Table 3.1). ET modalities included aerobic ($n=58$), resistance ($n=17$), combined aerobic and resistance ET ($n=13$), and control ($n=39$) groups. Regardless of ET modality, the average ET intervention consisted of a mean

3.5±1.5 (median, 3) sessions per week (n=87) and 46.9±13.8 (median, 45) minutes per session (n=76). The median intensity was moderate (n=84) and mean ET volume was 167±93 (median, 159) minutes per week.

3.3.3 Effect of ET on BAFMD

One hundred twenty-three mean, weighted ES were derived from 66 studies. Overall, ET induced a significant increase of BAFMD (trained ES, 8.38, 95% CI 6.16-10.59, $P<0.0001$). Eighty of the 88 training ES (91%) were larger than zero. The control group mean ES was -0.61 (95%CI, -2.15-0.94) and suggests control groups on average did not change during the interventional period (CI encompasses 0). No differences were observed between training modalities when compared to the control; aerobic ET tended to have the greatest ES (9.30; 95%CI, 6.29-12.31), followed by combined aerobic and resistance ET (ES, 7.62; 95%CI, 2.32-12.92) and resistance ET (ES, 5.80; 95%CI, 1.29-10.30) (Table 3.2). Of the 66 studies (71 ES) that reported a pre- and post-ET intervention fitness measure, 100% showed an improvement in fitness (i.e. VO_{2peak} , one-repetition maximum, maximal voluntary contraction). The mean percent change in fitness was 16±12% (median, 12%). A modest relationship was found between the change in fitness and BAFMD ES ($r=0.51$, $P<0.0001$).

Table 3.1. Participant and exercise intervention characteristics of studies included in the meta-analysis.

Study Characteristics						Exercise Intervention Characteristics				
Author	Journal	Population	Age (years)	Males (%)	N (I/C)	Length (wks)	Type	Frequency (sess/wk)	Duration (min)	Intensity Rating
Clarkson et al.	J Am Coll Cardiol	Healthy Adults	20±0	100	25/0	10	RA	7	--	--
Maiorana et al.	J Am Coll Cardiol	T2DM	52±2	88	16/0	8	RA	3	60	3
Gokce et al.	Am J Cardiol	CAD	59±10	78	40/18	10	A	3	30-40	--
Allen et al.	Med Sci Sport Exer	Healthy Adults	26±6	100	14/14	4	R	5	20	2
Kobayashi et al.	Circ J	CHF	55±2	71	14/14	12	A	2-3	30	2
Walsh et al.	Eur Heart J	HC	55±2	73	11/11	8	RA	3	45-60	3
Walsh et al.	J Appl Physiol	CAD	52±2	100	10/0	8	RA	3	45-60	3
Guazzi et al.	J Appl Physiol	CHF	52±5	100	16/0	8	A	4	40	3
Kelly et al.	J Pediatr	Overweight Children	11±4	45	10/10	8	A	4	50	3
Vona et al.	Am Heart J	AMI	56±6	77	28/24	12	A	3	60	2
Watts et al.	J Am Coll Cardiol	Obese children	14±2	65	23/0	8	RA	3	60	3
Watts et al.	J Pediatr	Obese children	9±2	43	14/14	8	A	3	60	2
Belardinelli et al.	Int J Cardiol	HTN	56±15	100	30/0	8	A	3	70	2
Moriguchi et al.	Hypertens Res	HTN	43±10	72	25/0	12	A	2	60	2
Rakobowchuk et al.	J Appl Physiol	Healthy adults	23±4	100	28/0	12	R	5	60	3
McGowan et al.	Eur J Appl Physiol	HTN	66±6	78	9/9	8	R	3	--	1
Miche et al.	Clin Res Cardiol	T2DM/CHF	67±6	86	20/22	4	RA	3	--	2
Olson et al.	Med Sci Sport Exer	Overweight adults	38±1	0	15/15	52	R	2	--	--
Casey et al.	Exp Biol Med	Healthy adults	21±1	45	24/18	12	R	3	35	3
Casey et al.	Eur J Appl Physiol	Healthy adults	59±5	0	23/0	18	R,A	2	35,35-40	2,3
McGowan et al.	Clin Sci	Healthy adults	28±14	78	9/9	8	R	3	--	1
Westhoff et al.	J Hum Hypertens	HTN	67±5	51	24/27	12	A	3	33	2

Table 3.1 continued. Participant and exercise intervention characteristics of studies included in the meta-analysis.

Study Characteristics						Exercise Intervention Characteristics				
Author	Journal	Population	Age (years)	Males (%)	N (I/C)	Length (wks)	Type	Frequency (sess/wk)	Duration (min)	Intensity Rating
Westhoff et al.	Kidney Blood Press Res	HTN	68±5	50	25/27	12	A	--	--	3
Belardinelli et al.	Circ Heart Fail	CHF	59±11	84	88/0	8	A	3	60,31	3
Sixt et al.	Eur J Cardiovasc Prev Rehabil	IGT/CAD	64±6	74	17/23	4	A	6	--	7
Tinken et al.	J Physiol	Healthy Adults	22±2	100	13/7	8	A	3	30	3
Westhoff et al.	J Hypertens	HTN	53±2	46	12/12	12	A	3	30	2
Baynard et al.	Eur J Appl Physiol	MetSyn	52±1	48	21/0	1	A	10	60	2
Dobrosielski et al.	Med Sci Sport Exerc	Older Adults	81±1	100	12/12	4	R	4	20	2
Munk et al.	Am Heart J	PCI	57±14	83	20/20	24	A	3	60	3
Murphy et al.	Int J Pediatr Obes	Overweight Children	10±2		23/12	12	A	5	30	--
Tjonna et al.	Clin Sci	Overweight Children	14±0		20/22	12	A	2	40	3
Vona et al.	Circulation	AMI	56±6	65	159/50	4	A,R, RA	4	60	2
Wray et al.	Clin Sci	HTN	71±2	100	6/0	6	A	3	60	3
Xiang et al.	Eur J Endocrinol	sHT	53±8	0	53/0	24	A	4-6	40-45	2
Allen et al.	Free Radic Biol Med	PAD	68±10	55	33/0	12	A	3	30-40	3
Credeur et al.	Med Sci Sport Exerc	Healthy adults	22±1	42	12/0	4	R	3	20	2
Desch et al.	Diabetes Obes Metab	CAD	62±6	73	14/12	24	A	7	30	2
Okada et al.	J Atheroscler Thromb	T2DM	62±9	55	21/17	12	RA	3-5	40	--
Tinken et al.	Hypertension	Healthy adults	22±2	100	11/0	8	R	3	60	2
Van Craenenbroeck et al.	Basic Res Cardiol	CHF	61±2	79	21/17	24	RA	3	60	3
Anagnostakou et al.	J Card Fail	CHF	53±10	82	28/0	12	A,RA	3	40	2

Table 3.1 continued. Participant and exercise intervention characteristics of studies included in the meta-analysis.

Study Characteristics						Exercise Intervention Characteristics				
Author	Journal	Population	Age (years)	Males (%)	N (I/C)	Length (wks)	Type	Frequency (sess/wk)	Duration (min)	Intensity Rating
Hermann et al.	Am J Transplant	HTx	53±11	81	14/13	8	A	3	60	3
Kwon et al.	Diabetes Metab J	T2DM	56±9	0	25/15	12	A,R	5	60	2,1
Molmen-Hansen et al.	Eur J Prev Cardiol	HTN	52±8	56	48/25	12	A	3	38,47	3,2
Okamoto et al.	Eur J Appl Physiol	Healthy adults	19±1	77	13/0	10	R	2	--	2
Pierce et al.	Clin Sci	Healthy adults	63±1	42	26/10	8	A	6-7	45	2
Reameriz-Velez et al.	J Obstet Gynaecol Res	Pregnant adults	20±2	0	24/26	16	A	3	60	1
Seeger et al.	Diabetes Obes Metab	T1DM	11±2		7/0	18	A	2	40-60	--
Akazawa et al.	Nutr Res	Healthy adults	59±5	0	11/10	8	A	3	40-60	2
Barone Gibbs et al.	Atherosclerosis	T2DM	58±5	62	49/63	26	RA	3	45	3
Billinger et al.	JNPT	Stroke	61±5	67	9/0	8	A	3	30-40	3
Cornelissen et al.	Eur J Prev Cardiol	CAD	62±1	84	146/0	12	A	3	90	3
Credeur et al.	Atherosclerosis	CHF	62±8	40	10/10	4	R	3	20	2
da Silva et al.	Diabetes Res Clin Pr	MetSyn/ T2DM	58±6	35	20/11	6	A	4	50	1,3
Hopkins et al.	Eur J Prev Cardiol	Twin children	14±1	33	24/0	8	A	3	45	3
Hunt et al.	J Appl Physiol	Healthy adults	26±4	100	9/0	4	R	3	--	1
Naulnim et al.	Am J Cardiol	HTN	60±2			12	A	4	40-50	2
Rakobowchuk et al.	Exp Physiol	Healthy adults	24±3	35	20/0	6	A	3	40	2,3
Swift et al.	Br J Sports Med	Obese adults	57±6	0	132/23	24	A	3-4	--	1
Xiang et al.	Exp Clin Endocrinol Diabetes	HT	45±9	0	86/0	24	A	4	40-45	2
Beck et al.	Exp Biol Med	Prehypertension	21±3	70	28/15	8	A,R	3	60	3
Currie et al.	Med Sci Sport Exerc	CAD	68±8		11/11	12	A	2	30-50,35	2,3

Table 3.1 continued. Participant and exercise intervention characteristics of studies included in the meta-analysis.

Study Characteristics						Exercise Intervention Characteristics				
Author	Journal	Population	Age (years)	Males (%)	N (I/C)	Length (wks)	Type	Frequency (sess/wk)	Duration (min)	Intensity Rating
Kitzman et al.	J Am Coll Cardiol	HF	70±7		24/29	16	A	3	60	3
Mitranun et al.	Scand J Med Sci Sports	T2DM	61±3	35	14/29	12	A	3	40	3
Spence et al.	J Physiol	Healthy adults	27±5	100	23/0	24	A,R	3	60	--

T2DM type 2 diabetes mellitus; T1DM type 1 diabetes mellitus; MetSyn metabolic syndrome; IGT impaired glucose tolerance; CAD coronary artery disease; HF heart failure; CHF chronic heart failure; HC hypercholesterolemia; HTx heart transplant; AMI acute myocardial infarction; PCI percutaneous coronary intervention; HTN hypertension; sHT subclinical hyperthyroidism; HT Hashimoto's thyroiditis; I intervention; C control; A aerobic; R resistance; RA combined aerobic and resistance; Intensity Rating 1 very light-light; 2 moderate; 3 vigorous-near maximal.

3.3.4 Subgroup and Advanced Analyses

Results of subgroup analyses from BAFMD are summarized in Table 3.2.

Overlapping confidence intervals of each with-in comparison suggested no difference in age (≤ 30 versus 31-59 versus ≥ 60 years, $P=0.11$), baseline fitness level ($<50^{\text{th}}$ percentile or $\geq 50^{\text{th}}$ percentile, $P=0.65$) and baseline artery diameter ($<4.00\text{mm}$ or $\geq 4.00\text{mm}$, $P=0.31$). Compared to asymptomatic subjects, diseased patients had a significantly higher BAFMD ET effect (asymptomatic, 3.18, 95%CI, 1.90-4.46 versus diseased, 10.44, 95%CI, 7.53-13.34; $P=0.0007$). When examining disease categories, CV disease ($n=25$, 14.11, 95%CI, 7.63-20.59), overweight/obesity ($n=9$, 9.93, 95%CI, 7.49-12.36) and hypertension ($n=13$, 7.61, 95%CI, 3.82-11.41) groups had significantly greater BAFMD ES than the asymptomatic/healthy category ($n=24$, 3.45, 95%CI, 1.28-4.73) ($P=0.001$). Subjects with a baseline BAFMD $<5\%$ appeared to have a larger ES than those with a baseline BAFMD $\geq 5\%$ ($<5\%$, 8.23, 95%CI, 5.28-11.17 versus $\geq 5\%$, 3.94, 95%CI, 1.82-6.06; $P=0.01$). Methodologically, studies with higher trial quality (>2) ($P=0.003$) showed statistically significant treatment effect, while studies conducted after 2006 (ES, 8.30; 95%CI, 5.76-10.96) tended to have a smaller effect than studies conducted prior to 2006 (ES, 8.70; 95%CI, 8.14-9.26) ($P=0.06$).

ET intervention groups had a significantly greater BAFMD ES than the control groups in regards to modality, length of ET as well as intensity and ET volume subgroups (all, $P<0.0001$; Table 3.2). Within subgroups, very light-light ET intensity was not different than the control group, but ET at a vigorous to near maximal ET intensity did result in a significant improvement in BAFMD (control, -0.42; 95%CI, -2.06-1.21 versus very light-light, 3.63; 95%CI, -0.57-7.83 versus vigorous-near maximal, 9.29; 95%CI, 5.09-13.47).

Furthermore, study groups with an ET duration was ≥ 150 min/wk had a significant improvement in BAFMD compared to those with < 150 min/wk (control, -0.30; 95%CI, -1.99-1.39 versus < 150 min/wk, 4.79; 95%CI 3.08-6.51 versus ≥ 150 min/wk, 11.33; 95%CI, 7.5-15.51; Figure 3.2).

3.4 Discussion

The meta-analyses demonstrated that ET interventions contribute to a significant increase in BAFMD regardless of ET modality, length of ET, intensity or volume of ET, supporting evidence that ET enhances endothelial function¹³. In addition, BAFMD was associated independently with both ET intensity and duration in a dose-response fashion.

Age did not serve as moderator to the changes in BAFMD with ET, suggesting that the vasculature remains modifiable throughout the life-span. The older age groups (> 31 years) tended to have greater BAFMD improvement; suggesting ET can prevent age-related loss in endothelial function. However, since age tended to be a potential moderator ($P=0.11$), it is possible that age specific responses to ET may be masked due to the combining of sexes. Previous research suggests sedentary, middle-aged and older men can prevent age-associated loss in endothelial function with regular aerobic ET³⁰ while healthy postmenopausal women have no improvements in BAFMD with endurance ET³¹. Disease status was found to impact the ES of BAFMD with ET, in particular CV diseases, overweight/obesity and hypertension, likely due to their higher propensity for vascular disease and therefore more potential gain from effective therapies.

Table 3.2. Subgroup analyses of potential moderating factors.

Factor	N	Effect	95% CI	L-Statistic	P-value
Age				4.42	0.11
≤30 years	28	4.63	(2.91, 6.35)		
31-59 years	38	10.68	(7.11, 14.24)		
≥60 years	22	9.17	(2.84, 15.49)		
Disease Status				11.29	0.0007
Asymptomatic/Healthy	25	3.18	(1.90, 4.46)		
Diseased	63	10.44	(7.53, 13.34)		
Baseline Level of Fitness				0.21	0.65
<50 th percentile	52	4.19	(3.31, 7.63)		
≥50 th percentile	6	8.63	(-2.09, 19.35)		
Baseline FMD				6.26	0.01
<5%	54	8.23	(5.28, 11.17)		
≥5%	69	3.94	(1.82, 6.06)		
Baseline Artery Diameter				1.02	0.31
<4.00mm	52	4.80	(2.69, 6.92)		
≥4.00mm	50	7.26	(3.84, 10.67)		
Trial Quality				9.11	0.003
≤2	63	6.54	(4.11, 8.95)		
>2	25	13.01	(8.59, 17.42)		
Year of Publication				3.63	0.06
<2006	16	8.70	(8.14, 9.26)		
≥2006	72	8.30	(5.76, 10.96)		
Type of Exercise				39.34	0.0001
Control	35	-0.61	(-2.11, 1.00)		
Aerobic	58	9.31	(6.08, 12.31)		
Resistance	17	5.80	(1.29, 10.30)		
Aerobic and resistance	13	7.62	(2.33, 12.92)		
Length of Training				37.77	0.0001
Control	35	-0.61	(-2.11, 1.00)		
<12weeks	43	8.09	(5.06, 11.13)		
≥12weeks	45	8.65	(5.23, 12.06)		
Intensity				36.42	0.0001
Control	32	-0.42	(-2.06, 1.21)		
Very light-light	9	3.63	(-0.57, 7.83)		
Moderate	38	8.96	(5.39, 12.53)		
Vigorous-near maximal	34	9.29	(5.09, 13.47)		
Volume				33.22	0.0001
Control	31	-0.30	(-1.99, 1.39)		
<150min/wk	48	4.80	(3.08, 6.51)		
≥150min/wk	43	11.33	(7.15, 15.51)		

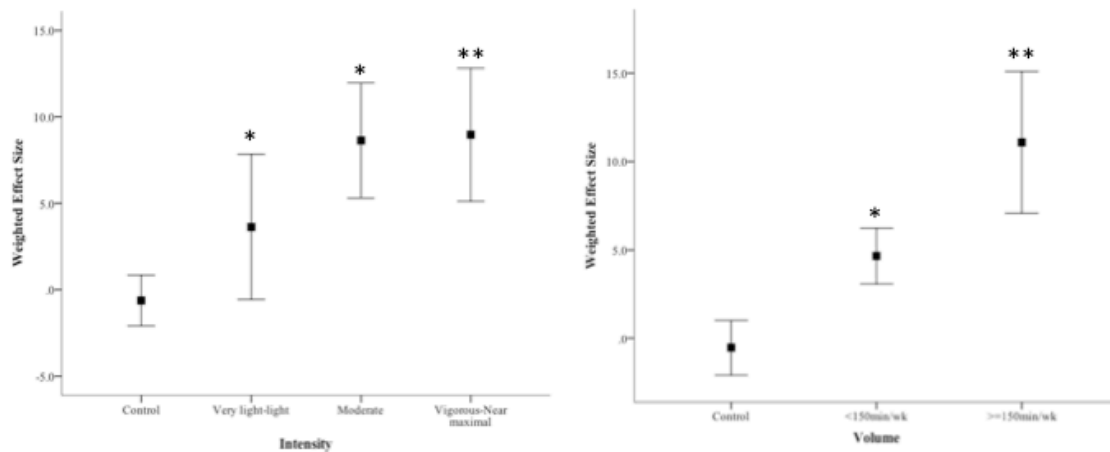


Figure 3.2. Exercise intensity and volume weighted effect sizes displayed as mean and 95% confidence interval. a) Significantly different from control ($P<0.05$). b) Exercise intensity very light-light significantly different from vigorous-near maximal ($P<0.01$) and volume ≥ 150 min/wk significantly different from <150 min/wk ($P<0.01$).

In clinical studies of diabetes mellitus^{32,33}, obesity¹² and hypertension³⁴, patients have shown a blunted BAFMD. In addition, this study found the greater change in fitness the greater ES of BAFMD, suggesting modifying fitness is important to vascular health. Low cardiorespiratory fitness is an established risk factor for the cardiovascular and total mortality independent of CV risk factors³⁵. Therefore, as ET interventions are applied to patients with CV risk factors it is important to consider ET is important to improving fitness and clinical symptoms and slow the progression of atherosclerosis, as measured by BAFMD.

Evidence supports a significant effect of ET on BAFMD independent of modality or length of ET. The modality with the greatest BAFMD ES was aerobic ET, suggesting regular aerobic exercise may be the most beneficial mode to improve vascular function¹⁵. There were no differences in the magnitude of ES between studies up to 12 weeks versus

studies longer than 12 weeks in length. The ES of BAFMD on ET is associated with both ET intensity and volume suggests a dose-response stimulus. The association with ET intensity may be due to greater shear stimulus triggered by increasing intensity³⁶. This shear stress has been shown to unregulated NO production via NO-synthase and increases the release of NO³⁷. These findings demonstrate high-intensity ET may be beneficial in order to optimally improve vascular function^{38,39}. However, no difference was seen between moderate and vigorous ET intensity, suggesting moderate intensity can sufficiently improve BAFMD. Despite the popular trend of high intensity interval training, there appears to be no benefit of higher, potentially riskier, ET intensity for vascular improvement. Furthermore, maximal effort ET for a sustained period of time has been shown to generate reactive oxygen species, which hinders vascular function^{40,41}. Reduced BAFMD may be the consequence of an increase in circulating vasoconstrictor agonists (i.e. ANG-II, vasopression and endothelin)^{42,43} and increased oxidative stress⁴⁴. NO is believed to play a significant role in buffering against oxygen radicals. If the oxygen stress is elevated as in high intensity exercise, NO's role may shift from a vasodilator to a radical scavenger.

Although we believe this to be the most comprehensive review of evidence supporting the impact of ET-based intervention on vascular function, variations in the methods of BAFMD assessment and reporting of ET characteristics may lead to detectable differences and explain the wide variation in the measurement. Several studies that met the minimum inclusion criteria were of poor methodological quality, and few described the process of random assignment and blinding of the outcome assessment (BAFMD). Additional factors that may potentially influence BAFMD including menopause, diet and smoking could not be examined due to the lack of reporting in the included studies. Future

studies should include age and gender information in their report to further clarify the evidence supporting different BAFMD responses to ET. Interestingly, trials over the last decade (published ≥ 2006) are typically of lower quality, which is potentially a consequence of methodological and physiologic guidelines that have been published and the popularity of the measurement^{8,45}. Few of the included studies were longitudinal; 9 of the 66 studies reviewed were longer than 20 weeks. Further research is necessary to establish optimal ET interventions for improvement in vascular health, measured by BAFMD.

In conclusion, a meta-analysis of 66 studies found ET interventions contribute to a significant increase in BAFMD. Age did not modify BAFMD, suggesting the vasculature is modifiable and age-associated changes in BAFMD can improved with ET. The ES of BAFMD is associated with both ET intensity and duration in a dose-response fashion. Larger effects were seen with higher intensity, longer duration exercise. This meta-analysis provides evidence that ET interventions improve BAFMD, supporting its marked benefits in the primary and secondary prevention and treatment of a variety of CV and metabolic disorders.

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CHAPTER 4. ACUTE EXERCISE ALTERS HEART RATE VARIABILITY TO AN ORAL GLUCOSE TOLERANCE TEST IN OVERWEIGHT MEN: A PILOT STUDY

4.1 Introduction

Overweight/obese individuals display impaired glucose metabolism and autonomic imbalance, measured by heart rate variability (HRV), which is associated with increased risk for cardiovascular morbidity¹. Following glucose ingestion, components of HRV have been shown to shift toward greater sympathetic tone in healthy and obese adults^{2,3}. In obese patients the change in autonomic function is attributed to chronic hyperinsulinemia desensitizing the sinoatrial node to both sympathetic and parasympathetic activity³. In addition, obesity is characterized by increased adiposity, which has correlated with increasing autonomic imbalance⁴ and cardiovascular disease risk¹.

Exercise training has been found to reduce resting sympathetic activity^{5,6}, reduce body fat⁵, and improve glycemic control⁷ in patients with obesity. Although exercise intensity and duration are recognized as factors that affect blood glucose⁸, few studies have examined the impact of acute exercise at different intensities on HRV in response to glucose ingestion. A single bout of aerobic exercise can improve glucose metabolism up to 48-hr after exercise, potentially due to increased glucose uptake to support glycogen resynthesis⁹ or from more complete oxidation of intramuscular lipids and lipid species¹⁰. However, studies of exercise intensity report conflicting results on the impact of exercise intensity on glucose concentrations. Previous research suggests a single bout of aerobic exercise at a higher intensity (as a percentage of maximal cardiorespiratory fitness; $\text{VO}_{2\text{max}}$) improves glucose levels 24-hr after exercise^{11,12}, while others report low intensity exercise is ideal¹³. In addition, exercise results in increased plasma catecholamines, a stimulator of glucose metabolism and sympathetic activity¹⁴. Lower exercise intensity may reduce HRV in

response to glucose ingestion due to lower circulating catecholamines or different substrate utilization. These cellular and molecular adaptations occurring in response to acute exercise may provide insight to how exercise intensity influences mechanisms of glucose and sympathetic regulation.

Lower exercise intensity may provide a similar metabolic and cardiovascular response after exercise and reduce the subjective discomfort that obese individuals typically experience with exercise. To our knowledge, no studies have compared alterations in HRV produced by acute aerobic exercise at a low/moderate intensity compared to high intensity exercise. We hypothesized that a single bout of exercise would increase autonomic response to an OGTT and exercise intensity would mediate the response. The purpose of this study, therefore, was 1) to investigate HRV during an oral glucose tolerance test (OGTT) in sedentary, overweight men and 2) to examine the effect of a single bout of exercise on resting, fasted HRV and HRV in response to 24-hr post-exercise OGTT.

4.2 Methods

4.2.1 Participants

Eight apparently healthy, sedentary, overweight/obese college-aged men gave written informed consent to participate in this study. Individuals who presented with any known cardiovascular disease, type 2 diabetes, resting blood pressure $\geq 140/90$ mmHg, and/or body mass index ≥ 35 kg/m² were excluded. All participants were physically inactive, non-smokers, presently not taking any medications, and had physician approval to participate. This study was approved by the Institutional Review Board at Louisiana State University.

4.2.2 Experimental Protocol

Participants came to the laboratory on six occasions (Figure 4.1). At the first visit, participants performed a maximal cardiorespiratory test ($\text{VO}_{2\text{max}}$) on a cycle ergometer (Velotron, Racermate, Inc., Seattle, WA). Participants rode at an initial workload of 25 Watts for 4-min. The workload increased by 25 Watts every 4-min until respiratory exchange ratio (RER) reached 1.05 after which, the workload increased by 25 Watts every 2-min until volitional exhaustion. Lactate was assessed in the final minute of each stage (Lactate Plus, Nova Biomedical, USA). Throughout the test, respiratory gases were analyzed using a metabolic cart (ParvoMedics Inc., Sandy, UT) and fat oxidation rates were determined using stoichiometric equations¹⁵.

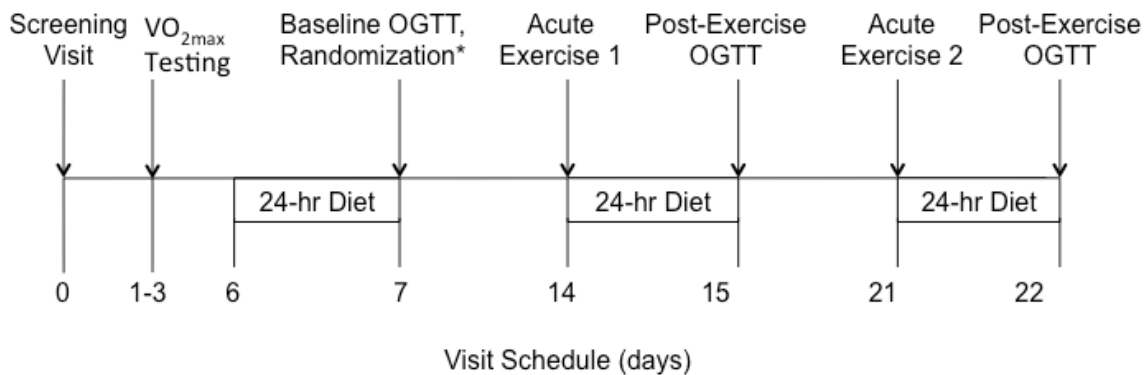


Figure 4.1. Visit schedule. *After confirmation of non-diabetic status, participants underwent 2 acute exercise sessions in random order. The intensity of the acute exercise sessions were 1) maximal fat oxidation (FM) or 2) 5% below lactate threshold (LT). A 24-hr diet was recorded prior to each PGTT and replicated between sessions.

On a separate day, the OGTT was performed between 6:00 and 9:00am after participants fasted overnight for ≥ 10 -hr and refrained from exercise for at least 72-hr. After giving a fasting venous blood sample, participants ingested 75 g of glucose to be consumed in less than 5-min (Azer Scientific, Morgantown, PA). Additional blood samples were taken

at 30, 60, 90 and 120-min during the OGTT. Plasma glucose levels were determined using a standard spectrophotometric assay (Glucose (HK) Assay, Sigma-Aldrich, St. Louis, MO, USA). Respiratory gases were measured 30-min prior to beginning the OGTT and from 30 to 60-min and 90 to 120-min during the OGTT to determine changes in metabolic rate and substrate utilization.

Following the $\text{VO}_{2\text{max}}$ test and baseline OGTT, eight participants underwent two acute exercise sessions in a randomized, counterbalanced, cross-over design separated by at least 1 week. Five of the eight participants had complete HRV during the baseline and both post-exercise OGTTs, therefore only five participants were included in subsequent analysis. Results from the $\text{VO}_{2\text{max}}$ test were utilized to determine the following exercise intensities: 1) maximal fat oxidation (FM) and 2) high carbohydrate oxidation (5% below lactate threshold; LT). The total dose of exercise was held constant at 400 kcal and the exercise time was manipulated in order precisely match the total dose of exercise within an individual. Respiratory gases were measured at 5-min intervals prior to 25%, 50%, 75% and 100% of the exercise bout, equivalent to 100, 200, 300 and 400kcal, respectively, and the time to complete the exercise bout was adjusted accordingly. Dietary intake was recorded 24-hr before the initial OGTT and replicated during the 24-hr period between the exercise session and the post-exercise OGTT. In addition, during the 24-hr period prior to OGTTs, participants were asked to refrain from alcohol and caffeine products and avoid physical activity. Twenty-four hours after the LT or FM exercise session, participants underwent an additional OGTT.

4.2.3 Heart Rate Variability

During the OGTTs and exercise sessions, autonomic function of the heart was evaluated using Zephyr heart rate monitor systems (Zephyr Technologies Corp, Maryland, USA). HRV was assessed throughout the 2-hr OGTT, and measured at the time points 5-min prior to the start of the OGTT and at 30-35-min, 60-65-min and 115-120-min. Data were stored and later analyzed according to the recommendations of the Task Force of the European Society of Cardiology and the North American Society of the Pacing and Electrophysiology¹⁶. Time and frequency domain measurements were calculated from 5-min epochs after correcting for artifact using a low-pass filter in the HRV analysis software (Kubios 2.0, Medical Imaging Group, Kuopio, Finland). The time domain variables considered for this study were the mean RR interval and its standard deviation (RRSD) and the mean squared successive difference (RMSSD). The frequency domains were analyzed with an autoregressive model and included very low frequency (VLF: 0-0.04Hz), low frequency (LF: 0.04-0.15Hz), high frequency (HF: 0.15-0.4Hz), and LF/HF ratio. Each spectral component was also presented in normalized units (nu) by dividing the LF or HF by the total power minus VLF to minimize the VLF component¹⁶.

4.2.4 Statistical Analysis

All statistics were performed using JMP statistical software (SAS Institute Inc., Cary, NJ). One way repeated measures analysis of variance (RM-ANOVA) was used to determine differences in HRV variables in response to glucose ingestion during the baseline OGTT. A Spearman correlation was run to determine whether glucose was associated with HRV. In addition, a two-way RM-ANOVA (exercise treatment x time) was used to determine differences in responses in both the primary outcome variables. Primary outcomes included

time and frequency domains of HRV (mean RR, SDNN, RMSSD, LF_{nu}, HF_{nu}, LF/HF) and plasma glucose. Baseline (non-exercise) and exercise (FM and LT combined) comparisons were evaluated using contrast statements with the one-way RM-ANOVAs. Data were considered significant of $P < 0.05$ all data are reported as mean \pm SD.

4.3 Results

4.3.1 Participants

Participant characteristics for non-exercise (baseline OGTT completers) and exercise subset are presented in Table 4.1. The eight men (range 18-22y) who participated were overweight (BMI 29.5 ± 4.7 , 25.2-33.3) and had relatively low cardiorespiratory fitness (VO_{2max} 33.2 ± 6.8 mL/kg/min). Of the eight men participating in baseline testing (VO_{2max} and OGTT), only five completed both FM and LT exercise bouts with the post-exercise OGTT and HRV measures.

Table 4.1. Anthropometric and physiological characteristics of participants.

	Baseline (non-exercise) n=8	Exercise Subset n=5
Age (years)	20.5 ± 1.5	19.8 ± 1.5
Height (m)	1.81 ± 0.04	1.80 ± 0.03
Weight (kg)	95.9 ± 13.8	87.0 ± 5.5
BMI (kg/m ²)	29.5 ± 4.7	26.8 ± 1.9
VO_{2peak} (mL/kg/min)	33.2 ± 6.8	36.7 ± 6.0
SBP (mmHg)	121 ± 9	116 ± 6
DBP (mmHg)	71 ± 14	66 ± 5
Resting HR (bpm)	71 ± 7	68 ± 5
Fasting plasma glucose (mg/dL)	99.5 ± 9.1	95.7 ± 6.6
Peak plasma glucose (mg/dL)	147.9 ± 57.1	132.4 ± 46.9

VO_{2peak} maximal aerobic capacity; SBP systolic blood pressure; DBP diastolic blood pressure; HR heart rate. Values are mean \pm SD

4.3.2 HRV during Baseline (non-exercise) OGTT

Fasting blood glucose prior to the baseline OGTT was 99.5 ± 9.1 mg/dL, peaked 60-min after glucose ingestion at 147.9 ± 61.3 mg/dL and returned to 115.3 ± 16.5 mg/dL at 120-min post ingestion (0 vs. 60-min, $P < 0.01$; 0 vs. 120-min, $P = 0.83$). Table 4.2 shows time and frequency domain parameters during the baseline (non-exercise) OGTT. There was no change in mean HR or mean RR-interval ($P = 0.18$) over the course of the OGTT. Normalized LF (LF_{nu}) and LF/HF increased 60-min after glucose ingestion (0 vs. 60-min: LF_{nu} 43.4 ± 13.3 vs. 51.8 ± 11.7 , $P < 0.003$; HF_{nu} 57.3 ± 14.2 vs. 48.1 ± 11.7 , $P < 0.007$; LF/HF 0.89 ± 0.64 vs. 1.25 ± 0.84 ; $P < 0.001$) and returned to baseline after 120-min (0-min vs. 120-min: LF_{nu} 43.4 ± 13.3 vs. 44.1 ± 14.4 , $P = 0.62$; LF/HF 0.89 ± 0.64 vs. 1.00 ± 0.95 ; $P = 0.84$). Normalized HF (HF_{nu}) decreased 60-min after glucose ingestion (57.5 ± 14.2 vs. 48.1 ± 11.7 ; $P < 0.001$) and also returned to baseline by 120-min (57.3 ± 14.2 vs. 55.8 ± 14.1 , $P = 0.29$). Higher fasting blood glucose (0-min) was associated with reduced HRV (LF_{nu} $P < 0.009$; HF_{nu} $P = 0.01$; LF/HF $P < 0.009$).

Table 4.2. Time and frequency domain parameters during the baseline (non-exercise) OGTT (n=8).

	0min	60min	120min	P-ANOVA
Mean HR (bpm)	56 ± 9	57 ± 7	58 ± 8	0.18
Mean RR (ms)	1102 ± 177	1060 ± 133	1047 ± 145	0.18
SDNN (ms)	108 ± 27	108 ± 40	107 ± 33	0.99
RMSSD (ms)	109 ± 43	101 ± 50	101 ± 51	0.45
LF_{nu}	43.4 ± 13.3	51.8 ± 11.7^b	44.1 ± 14.4^c	< 0.003
HF_{nu}	57.3 ± 14.2	48.1 ± 11.7^b	55.8 ± 14.1^c	< 0.007
LF/HF	0.89 ± 0.64	1.25 ± 0.84^a	1.00 ± 0.95^c	< 0.01

^aSignificantly different from 0min ($P < 0.005$). ^bSignificantly different from 0min ($P < 0.001$). ^cSignificantly different 60min vs. 120min ($P < 0.05$). Data are mean \pm SD.

4.3.3 Post-exercise HRV Response during an OGTT: Effect of Different Intensities

Five men performed energy expenditure-matched exercise bouts at FM and LT (FM: 404.2 ± 7.3 kcal vs. LT: 405.5 ± 8.4 kcal, $P=0.80$) although the average exercise intensity was different between FM and LT (FM: $41 \pm 12\% \text{VO}_{2\text{max}}$, 1.25 ± 0.21 L/min; LT $68 \pm 10\% \text{VO}_{2\text{max}}$, 2.13 ± 0.55 L/min; $P<0.02$). The average difference between FM and LT exercise intensity was 26.8% (range 9.1%-41.0%). The glucose area under the curve (glucose_{AUC}) was not different between baseline (non-exercise) and LT and FM exercise intensities (Figure 4.2).

LT exercise decreased HF_{nu} ($P<0.01$) and increased LF_{nu} ($P<0.05$) compared to baseline (non-exercise) at 0-min. However, no difference was seen between 24-hr post FM exercise compared to baseline (non-exercise) HRV (LF_{nu} $P=0.32$; HF_{nu} $P=0.32$). After 120-min, both LT and FM exercise had increased LF/HF (0 vs.120-min: LT 0.97 ± 0.33 vs. 1.12 ± 0.32 , FM 0.55 ± 0.32 vs. 1.09 ± 0.22 , $P<0.01$) and LF_{nu} (0 vs.120-min: LT 45.5 ± 5.6 vs. 52.1 ± 6.9 , FM 33.0 ± 11.7 vs. 51.1 ± 5.9 , $P=0.009$) and decreased HF_{nu} (0 vs.120-min: LT 51.9 ± 8.4 vs. 48.1 ± 7.1 , FM 66.9 ± 11.7 vs. 47.7 ± 5.9 , $P<0.01$) compared to baseline (non-exercise) HRV (Figure 4.3).

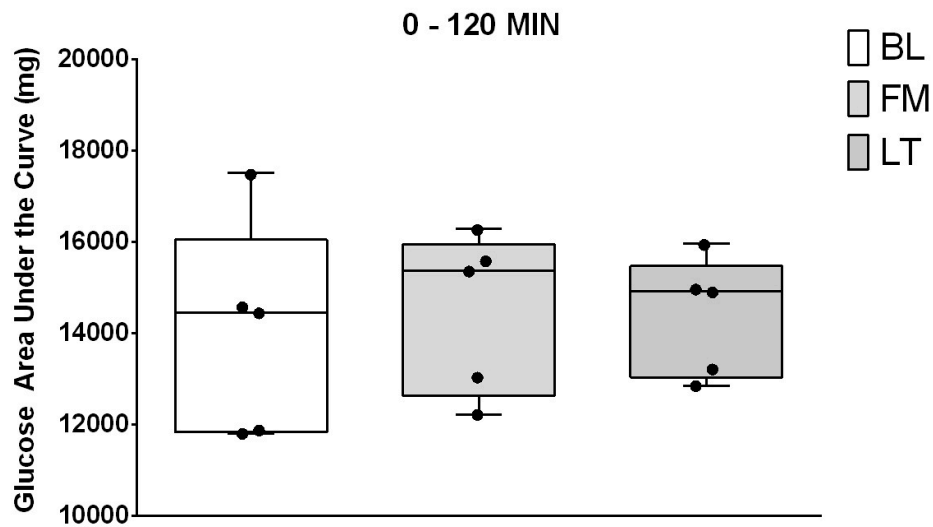


Figure 4.2. Glucose area under the curve ($\text{Glucose}_{\text{AUC}}$) per trial. BL baseline; FM exercise Intensity at maximal fat oxidation; LT exercise Intensity at 5% below lactate threshold. For each box-whisker plot, inside line represents median, upper and lower box limits represent 25th and 75th percentiles, upper and lower bars represent maximum and minimum values, and • indicates individual responses. Data represent subset of $n=5$.

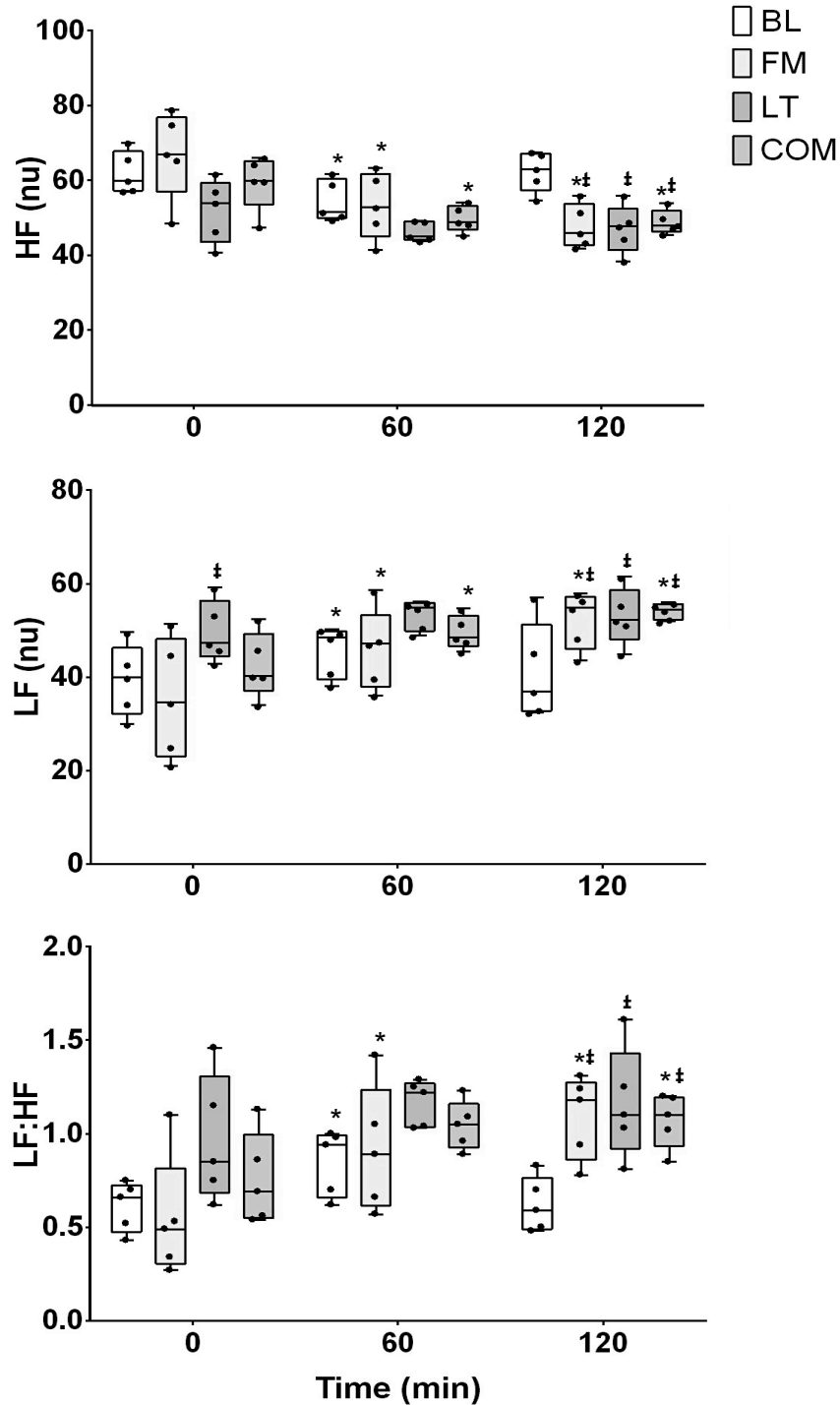


Figure 4.3. Frequency domain HRV parameters over the course of an OGTT per trial. BL baseline; FM exercise Intensity at maximal fat oxidation; LT exercise Intensity at 5% below lactate threshold; COM combined exercise trials. For each box-whisker plot, inside line represents median, upper and lower box limits represent 25th and 75th percentiles, upper and lower bars represent maximum and minimum values, and • indicates individual responses. * Different from baseline (non-exercise) at given time point ($P < 0.05$). † Different from time 0min in the given trial ($P < 0.05$). ‡ Different from FM at given time point ($P < 0.05$).

4.3.4 Post-exercise HRV Response during an OGTT: Exercise Intensities

Combined

Given exercise intensity had minimal effect on HRV during an OGTT, we further examined the effect of an acute bout of exercise by statistically combining the exercise group effects. Fasting blood glucose concentrations at 0-min were not different between baseline (non-exercise) and exercise trials combined (baseline 95.7 ± 6.6 mg/dL vs. 98.0 ± 7.4 mg/dL, $P=0.24$). At 0-min, LF_{nu} (40.5 ± 12.4 vs. 37.9 ± 5.6 , $P=0.64$), HF_{nu} (59.6 ± 12.2 vs. 62.0 ± 5.6 , $P=0.64$) and LF/HF (0.76 ± 0.38 vs. 0.62 ± 0.13 , $P=0.40$) were similar between baseline and exercise combined (COM) (Figure 4.3). At 120-min, a difference was observed between exercise combined (COM) and baseline (non-exercise) for LF_{nu} (BL: 37.6 ± 5.3 , COM: 40.5 ± 12.4 , $P<0.002$), HF_{nu} (BL: 62.4 ± 5.3 , COM: 59.5 ± 12.4 , $P<0.002$) and LF/HF (BL: 0.62 ± 0.14 , COM: 0.75 ± 0.38 , $P<0.005$).

4.4 Discussion

4.4.1 HRV during Baseline (non-exercise) OGTT

The primary aim of our study was to examine the HRV characteristics during a baseline (non-exercise) OGTT and an OGTT 24-hr following a single exercise bout performed at FM and LT intensity in overweight, sedentary men. Our results demonstrated that a significant shift from parasympathetic to sympathetic activity occurred during each OGTT, suggesting glucose ingestion stimulates an unfavorable shift in autonomic balance concordant with increased body mass^{3,17}. This study supports similar findings seen in severely obese individuals^{3,18-20} and pregnant women²¹, where sympathetic nervous system activity predominates in response to glucose ingestion. The HF_{nu} component of HRV, reflecting parasympathetic neural control, decreased during the baseline OGTT while LF_{nu} ,

reflecting parasympathetic and sympathetic control demonstrated the opposite effect. Sympathovagal balance, measured by LF/HF, also shifted toward sympathetic activity. The mechanism behind increased sympathetic activity in response to glucose ingestion may be a consequence of insulin resistance and/or chronic hyperinsulinemia desensitizing the sinoatrial node^{3,20}. Such chronic sympathetic activity may impede parasympathetic stimulation of insulin secretion leading to insulin resistance over time²².

4.4.2 Post-exercise HRV Response during an OGTT: Effect of Different Intensities

Our study demonstrated no significant difference in HRV 24-hr post FM exercise prior to the start of the OGTT (0-min). However, LT exercise prior to the start of the OGTT produced a shift in LF_{nu} , HF_{nu} and LF/HF. A single bout of exercise at low intensity (FM) may not be sufficient to impact HRV 24-hr later. Few studies have examined HRV after 24-hr of recovery and/or utilized more than one exercise intensity^{23,24}. James et al. (2012) suggested greater exercise intensity initially increases sympathetic activity after exercise, but ultimately parasympathetic activity prevailed 24-hr after exercise demonstrating a cardioprotective influence²³. However, James et al. used healthy, fit adults, which may account for a more rapid return to parasympathetic dominance following high intensity exercise, whereas our study examined overweight, sedentary individuals. Interestingly, the shift toward sympathetic dominance before and after exercise may suggest the health of the participants (overweight and sedentary) is contributing to poorer HRV indices. Paolisso et al. (1997) found the magnitude of increase in sympathetic activity due to glucose ingestion was associated with increasing body fat⁴. Future studies should include various

cardiovascular risk populations and time course of HRV to determine if exercise intensity has a prolonged effect on HRV.

At the end of both the FM and LT post-exercise OGTT, LF_{nu} and LF/HF were significantly elevated and LF_{nu} significantly depressed compared to the baseline; HRV remained shifted toward sympathetic function, suggesting acute exercise does not improve the HRV response to glucose ingestion. A potential explanation for these findings is that autonomic imbalance drives an increased secretion of insulin from pancreatic B cells²⁵. Sympathetic nerves release noradrenaline to excite pancreatic alpha cells resulting in increased glucagon secretion. The feedback to maintain glucose homeostasis is to increase insulin secretion from pancreatic beta cells²⁵. Acute exercise may perpetuate over-production of insulin and continue sympathetic dominance in response to glucose ingestion. However, the time course for improved insulin sensitivity, or pancreatic beta cell function, after acute exercise is unknown.

4.4.3 Post-exercise HRV Response during an OGTT: Exercise Intensities Combined

Regardless of exercise intensity, HRV after exercise was not different from baseline (non-exercise) prior to the start of the OGTT (0-min) (Figure 4.3). Although, single bouts of exercise have been found to improve glucose control and insulin sensitivity^{11,12}, in this study no changes in $glucose_{AUC}$ (Figure 4.2) or HRV (Figure 4.3) were seen, suggesting that acute exercise is not sufficient to produce significant improvements in HRV. Other studies have also observed no differences or a return to baseline in HRV 24-hr after exercise^{24,26}. Studies of chronic exercise training have been shown to improve HRV^{6,7} suggesting chronic exercise may be necessary to elicit changes in HRV.

In this study, autonomic balance was assessed with only HRV spectral analysis at intervals during the OGTTs, which limits the inferences that can be made on the response of HRV to glucose ingestion. A larger sample size and lean control group would help confirm the changes in HRV seen in overweight men and also demonstrate whether this population continues to have depressed autonomic nervous system function at rest and after acute exercise during glucose ingestion. In addition, the insulin measurement would provide more insight to the mechanism behind altered HRV during glucose ingestion. Despite these limitations, this study demonstrated meaningful alterations in HRV after exercise and glucose ingestion. Future studies should include time course information on HRV to examine potential prolonged effects of glucose ingestion and exercise at different intensities.

In conclusion, this study demonstrated that glucose ingestion alters HRV in sedentary, overweight men. Higher exercise intensity shifted HRV 24-hr post-exercise to higher sympathetic activity. This study is the first to report that both FM and LT exercise intensity, reflecting low and high exercise intensities, respectively, produce a negative change in HRV response to an OGTT. These findings may have future implications for understanding the impact that acute exercise intensity has on HRV during glucose ingestion and the importance of exercise as a marker of autonomic balance.

4.5 References

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CHAPTER 5. IMPACT OF PROGRESSIVE, CHRONIC DEHYDRATION ON CARDIAC AND SWEAT RESPONSES TO EXERCISE IN A HEATED ENVIRONMENT

5.1 Introduction

Dehydration and heat stress are dual challenges to the cardiovascular system during exercise. The combination of heat stress and dehydration may have concomitant and additive consequences on cardiovascular function and sweat responses^{1,2}. Previous research has demonstrated that dehydration impacts physiologic function and performance with as little as 2% deficit in body water²⁻⁴. Increased core temperature and the subsequent reduced skin blood flow limit the capacity to perform exercise due to the increased demand for adequate sweat rate and dissipation of heat through the skin^{5,6}. When the marked cardiovascular adjustments that occur during exercise are compromised, thermal regulation may suffer. In addition, prior impaired cardiovascular and nervous control may further hinder thermal regulation and skin blood flow during exercise in a heated environment^{7,8}.

Evidence suggests individuals only replace ~70% of total water loss during exercise in the heat⁹; individuals do not ingest sufficient fluid during physical activity to replace water losses and return to a euhydrated state. Therefore, individuals may be persistently dehydrated day to day, causing impairments in cardiovascular performance and function including increased heart rate and blood pressure^{2,5}. Few studies have examined the effects of progressive, chronic dehydration or the long-term health outcomes due to chronic under-consumption of fluids¹⁰. Therefore, the primary aim of this study is to determine whether chronic dehydration, achieved by fluid restriction over a 3-day period, alters cardiovascular function and sweat responses during exercise in a heated environment. In addition, this study determined if an individual's resting cardiovascular health influences their cardiovascular,

blood flow, and sweat responses to exercise in a heated environment. We hypothesized that cardiovascular function and sweat response will be preserved in the euhydrated state and impaired in a hypohydrated state during exercise in a heated environment due to less cardiovascular strain and heat stress during exercise. We expect that the euhydrated state will allow for a lower heart rate, and greater skin blood flow, sweat rate, and temperature regulation during exercise compared with the hypohydrated state. Lastly, we expect resting cardiovascular profiles, evaluated by pulse wave velocity (PWV), brachial artery flow-mediated dilation (BAFMD), and heart rate variability (HRV), would be associated with cardiovascular function and sweat responses pending their hydration status.

5.2 Methods

5.2.1 Participants

Eighteen participants (6 male, 12 female) aged 20.6 ± 1.2 y) participated in this study. High-risk individuals as categorized by the American College of Sports Medicine (ACSM), including those with known cardiovascular, pulmonary or metabolic disease, or sings/symptoms suggestive of disease were excluded¹¹. In addition, participants taking any medications that potentially influence fluid balance or cardiovascular function. This study was approved by the Louisiana State's University's Institutional Review Board. All participants provided written informed consent.

5.2.2 Experimental Design

A counterbalanced, cross-over design was used where participants were randomized to two intervention periods and a total of 5 laboratory visits (Figure 5.1). Following an initial screening, participants underwent baseline and cardiovascular testing. Participants spent 4 days tracking daily urine color and thirst, which consisted of normal behaviors of food and

fluid intake (tracking) (Figure 5.1). The randomly assigned intervention period was 3 days of either hydration (HYD) or dehydration (DEH) where participants were encouraged to drink or restrict fluids to achieve each state (intervention). On the fourth day of the intervention period, participants performed exercise testing standardized to the same time of day to reduce effects of diurnal variation. The exercise tests were separated by at least 1 week. To reduce the impact of menstrual cycle on hydration status, female participants performed laboratory visits within the first two weeks after the end of their last menstrual cycle.

5.2.3 Experimental Protocol

Participants arrived between 8:00-10:00am for the initial screening visit and baseline testing after an overnight fast of ≥ 10 hours. In addition, participants were asked to avoid alcohol and exercise for at least 48 hours prior to the screening visit (Figure 5.1). If participants signed the informed consent, they proceeded to anthropometrics, resting vitals, fasting blood and urine samples, and cardiovascular assessments.

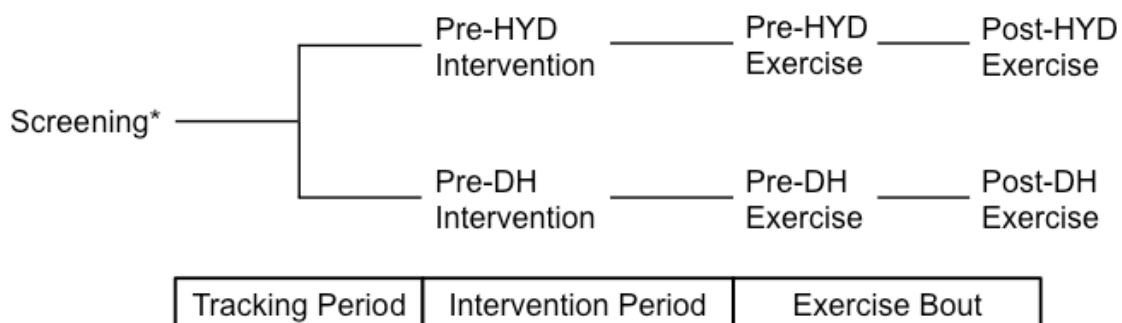


Figure 5.1. Schedule of study visits. Tracking periods include normal participant behavior for daily fluid and food intake. Hydration and dehydration interventions were randomized and counter-balanced with an exercise test following each period.

Each tracking periods consisted of the 4 days that occurred immediately prior to the intervention periods. During the tracking periods, participants were instructed to follow normal food and fluid intake behaviors and record their perceptions of urine color and thirst¹². During the intervention periods, participants continued to record food and fluid intake, perceived urine color, and thirst. Participants were encouraged not to exercise strenuously and to avoid alcohol and prolonged outdoor activities. However, given the effect of caffeine withdrawal on cardiovascular parameters¹³, and the lack of data to support that caffeine promotes dehydration¹⁴, normal intake of caffeinated beverages was allowed. At the start of the intervention period a fasting blood and urine sample was provided. The hydration status intervention procedures are described as:

HYD Trial: Participants were asked to drink adequate fluids prior to the exercise test in order to promote proper hydration indicated by perceived urine color (<3 out of 8) and thirst (<3 out of 10). The U.S. military currently recommends this method to estimate hydration status has used this method and has been extensively researched in active military¹².

DEH Trial: Participants were asked to dehydrate by restricting fluid intake to achieve a given thirst level (perceived thirst of 7 out of 10) and urine color (>4 out of 8). If a participant's thirst exceeded a rating of 7, they were instructed to drink just enough fluids to alleviate the sensation of thirst. In addition, participant's fluid intake during meals was restricted to 1 cup (250mL) of fluid and complete fluid restriction night before the exercise test to produce ~2% decrease in body weight.

Each exercise test consisted of a 30-min steady-state bout of exercise on a cycle ergometer in a heated environment ($30.2 \pm 0.8^{\circ}\text{C}$, $26.5 \pm 7.4\%\text{RH}$). During the steady-state

exercise bout, pedal rate on the cycle ergometer was set to 60 RPM and a resistance factor necessary to elicit an individualized estimated metabolic rate of $35.0 \pm 0.9 \text{ W/m}^2$ using ACSM cycle equations¹⁵. No fluids were allowed during the exercise tests. Upon entering the lab participants voided, provided a urine sample, and nude body weight was recorded. A rectal thermometer was self-inserted to a depth of 10 cm past the anal sphincter and a heart rate monitor affixed. Lastly, a pre-exercise blood sample was taken. During the exercise test, skin temperatures (T_{sk}) and blood flow (SkBF) were continuously monitored as well as substrate metabolism using a metabolic cart. Sweat samples from the upper back were used to assess local sweat rate (LSR) and electrolyte losses. At the end of the exercise bout, participants provided blood and urine samples and a nude weight. The post-exercise urine sample was provided prior to the post-exercise body weight.

5.2.4 Experimental Measurements

5.2.4.1 Anthropometrics and Cardiovascular Measures

Anthropometric measures included height, weight and estimation of body surface area using standard equations¹⁶. Body weight was measured to determine changes incurred by changing hydration status. Cardiovascular testing included heart rate variability (HRV), pulse wave velocity (PWV) and brachial artery flow-mediated dilation (BAFMD).

HRV was examined according to the Task Force for Pacing and Electrophysiology¹⁷. Briefly, a heart rate monitor (Zephyr Technology Corp., Annapolis, MD) was worn during the resting and exercise visits to capture R-R intervals and was analyzed using Kubios software 2.0 (Biosignal Analysis and Medical Imaging Group, Kuopio, Finland). Frequency components of HRV included LF and HF expressed in normalized units (nu), calculated by dividing the LF and HHF by the total spectral power minus the VLF, and low to high

frequency ratio (LF/HF). Time domain measures included standard deviation of the NN intervals (SDNN) and the square root of the mean squared difference of NNs (RMSSD).

Vascular function was assessed by BAFMD following current guidelines¹⁸. With participants in the supine position and a 3-lead ECG placed on the wrists and leg to monitor cardiac cycle, BAFMD will be induced by 5-min of forearm occlusion by inflation of a pneumatic cuff (Hokanson) to 200 mmHg, positioned approximately 1 cm distal to the olecranon process. Using ultrasonography (Logiq e Ultrasound, GE Healthcare, WI), the brachial artery was imaged for 30 seconds prior to cuff release and for 3-min following return to normal, unrestricted blood flow. BAFMD was calculated as the percent change in brachial artery diameter. All ultrasound images were stored for subsequent analysis (ImageJ software, National Institutes of Health).

Blood pressures (systolic, diastolic, mean and pulse pressure) and systemic arterial stiffness (augmentation pressure and index) were measured with applanation tonometry (SphygmoCor, Version 8.0, AtCor Medical). Augmentation index (Aix) was calculated by the difference in the second systolic peak and diastolic pressure divided by the difference between the first systolic peak and diastolic pressure ($\times 100\%$)¹⁹.

5.2.4.2 Blood and Urine Sampling

A 5ml venous blood sample was drawn into lithium-heparin tubes. Whole blood was immediately analyzed for contents of hemoglobin spectrophotometrically using the cyanmethemoglobin method (Sigma-Aldrich, MO) and hematocrit using the microcapillary technique in triplicates. Thereafter, blood was centrifuged and analyzed in triplicates for plasma electrolytes, including sodium (Na^+), potassium (K^+), and chloride (Cl^-), and plasma osmolality (Posm) using a vapor pressure osmometer (Wescor Elitech Group, Logan, Utah).

Urine samples were analyzed for color, urine specific gravity (USG) (hand refractometer, NSG Precision Cells, Inc., Farmingdale, NY, USA), urine electrolytes and osmolality (Uosm). Perceived thirst and urine color were recorded on logs using a thirst scale (1 not thirsty at all, 10 very, very thirsty) and a urine color scale¹².

5.2.4.3 Skin Temperature and Blood Flow

Skin thermometers (Biopac Systems, Santa Barbara, CA) were placed on 4 sites: the mid-thigh, chest, mid-biceps and calf²⁰. Weighted skin temperature (T_{sk}) was estimated using the equation of Ramanathan (1964)²¹. Total body temperature (T_b) was calculated from rectal (T_{re}) and T_{sk} ²² and then used to calculate the rate of heat storage (HS) using previously published equations²³. The change of T_{sk} was calculated by the difference in skin temperatures at that start of exercise (T_0) and maximal T_{sk} (T_{max}). SkBF was measured in perfusion units continuously during exercise using a laser Doppler flowmeter (Perimed, Stockholm, Sweden). The laser Doppler probe was affixed to the flexor aspects of the forearm (muscle belly of the brachioradialis) using adhesive and surgical tape. SkBF slope was determined by the difference between perfusion units at the onset of SkBF rise and the plateau of perfusion units, divided by the time.

5.2.4.4 Sweat Response and Substrate Metabolism

During exercise, local sweat rates (LSR) of the upper back were measured during exercise with the technical absorbent technique²⁴. LSR is reported as the difference in pre and post patch weight, divided by the surface area (cm^2) and duration of application (30-min) ($\text{mg}/\text{cm}^2 \cdot \text{min}$)²⁴. Sweat collected from the electrolyte-free absorbent patch was centrifuged and analyzed for electrolyte concentrations using ion selective electrodes (EasyLyte, Medica Corp., MA). Respiratory gases were monitored during exercise sessions using an integrated

oxygen/carbon dioxide analyzer calibrated with standard gas mixtures (TruOne 2400, ParvoMedics, Inc., Sandy, UT).

5.2.5 Statistical Analysis

Statistics were performed in JMP statistical software 12 (SAS Institute Inc., Cary, NC). One-way and two-way (trial by time) repeated measures analysis of variance (ANOVA) were performed to determine differences between HYD and DEH and across the tracking, intervention, and exercise time points. Significant main or interaction effects were further evaluated using Student's *t* analyses where appropriate. Linear and multiple regressions were used to determine relationships between resting cardiovascular measures and changes in T_{sk} , $SkBF$ and heat storage by trial before and after adjusting for weight, exercise heart rate and VO_2 , and gender. Data are displayed as mean \pm standard deviation and significant differences declared at $P < 0.05$.

5.3 Results

5.3.1 Participants

Participant screening characteristics are displayed in Table 5.1. Participants ($n=18$, 6 male, 12 female) were normal body weight (67.0 ± 10.6 kg, body mass index [BMI] 23.6 ± 3.4 kg/m²) and had a normal systolic (109 ± 9 mmHg) and diastolic (72 ± 7 mmHg) blood pressure. Males were larger than females (body surface area [BSA] 1.92 ± 0.08 vs. 1.69 ± 0.12 m²). Resting cardiovascular function was assessed by HRV, PWV and BAFMD and represented in Table 5.2. According to previously published research, participants had within normal range cardiovascular health profiles²⁵⁻²⁷.

Table 5.1. Screening participant characteristics.

	Male (n=6)		Female (n=12)		All (n=18)
Age (y)	20.3±1.9	(19-24)	20.8±0.8	(19-22)	20.6±1.2
Height (m)	1.74±0.07	(1.68-1.86)	1.66±0.09	(1.56-1.85)*	1.68±0.09
Weight (kg)	77.8±7.9	(62.9-84.8)	61.6±7.2	(76.9-53.3)*	67.0±10.6
BMI (kg/m ²)	25.4±3.2	(25.7-30.1)	22.5±2.6	(18.9-28.7)*	23.6±3.4
BSA (m ²)	1.92±0.08	(1.79-2.04)	1.69±0.12	(1.54-2.02)*	1.76±0.16
Systolic BP	108±9	(96-122)	109±9	(90-122)	109±9
Diastolic BP	71±10	(58-80)	73±5	(62-80)	72±7

BMI body mass index; BSA body surface area; BP blood pressure. Mean ± standard deviation (range). *Different from men (P<0.05).

Table 5.2. Screening cardiovascular measures.

Heart Rate Variability		
Resting HR (bpm)	64 ± 8	(44-74)
Mean RR (ms)	972.2 ± 132.2	(830.8-1374.8)
SDNN (ms)	102.9 ± 52.5	(31.6-252.7)
RMSSD (ms)	88.8 ± 47.4	(30.5-172.9)
LF _{nu}	49.1 ± 13.7	(23.0-74.5)
HF _{nu}	50.9 ± 13.7	(25.5-77)
LF/HF	1.13 ± 0.70	(0.30-2.92)
Vascular Measures		
Aortic Systolic BP (mmHg)	92 ± 7	(81-107)
Aortic Diastolic BP (mmHg)	71 ± 7	(60-84)
Mean Arterial Pressure (mmHg)	80 ± 6	(70-94)
AP (mmHg)	0.1 ± 3.7	(-10.5-7)
AIx (%)	2.9 ± 11.8	(-18.5-32)
PWV (m/s)	6.88 ± 1.19	(4.4-9.2)
Resting artery diameter (mm)	3.54±0.68	(2.51-5.01)
Peak artery diameter (mm)	3.79±0.71	(2.70-5.25)
Absolute change (mm)	0.24±0.08	(0.11±0.39)
BAFMD (%)	6.67±2.33	(2.92-11.16)

HR heart rate; RR R-to-R interval; SDNN standard deviation of RR intervals; HF_{nu} high frequency normalized units; LF_{nu} low frequency normalized units; LF/HF low-to-high frequency ratio; BP blood pressure; AP augmentation pressure; AIx augmentation index; PWV pulse wave velocity; FMD flow-mediated dilation. Mean ± standard deviation (range).

5.3.2 Screening and Pre-intervention Visits

Weight changes, urine markers and blood markers are displayed in Table 5.3. No difference was observed between tracking period and the start of both HYD and DEH intervention periods in perceived urine color ($P=0.89$) or perceived thirst ($P=0.14$). Over the course of the tracking periods, weight ($P=0.87$), USG ($P=0.50$) and Posm ($P=0.13$) did not change. Urine color decreased during the tracking period prior to HYD intervention period (Screening vs. Pre-HYD intervention, 4 ± 1 vs. 3 ± 1 , $P<0.05$) where as the DEH trial was not different from screening (Screening vs. Pre-DEH intervention, 4 ± 1 vs. 4 ± 1 , $P=0.59$). Plasma potassium decreased during the tracking period prior to DEH intervention period, but prior to HYD intervention period (Screening vs. Pre-DEH intervention, 3.9 ± 0.4 vs. 3.8 ± 0.3 , $P<0.05$; Screening vs. Pre-HYD intervention, 3.9 ± 0.4 vs. 3.9 ± 0.2 , $P=0.23$). The intervention periods produced a $0.2\pm 1.0\%$ (range: -1.5 - 2.3%) increase in body weight during the HYD trial compared to $-1.0\pm 1.4\%$ (range: -4.0 - 0.6%) in the DEH trial (pre-intervention to pre-exercise body weight).

During the randomized intervention periods, two female participants did not follow hydration status guidelines of fluid intake to achieve either a HYD or DEH state. To confirm their hydration status was different from the assigned hydration intervention, weight, urine and blood markers were measured at the pre-exercise visit. After confirmation, the two participants were excluded from further analyses.

Table 5.3. Baseline and pre-intervention start urine and blood markers (n=18).

	Screening	Pre-HYD Intervention	Pre-DH Intervention	ANOVA (P-value)
Weight (kg)	66.9 ± 10.6	66.9 ± 10.7	66.9 ± 10.7	0.87
Perceived Thirst	3 ± 1	3 ± 1	3 ± 1	0.14
Perceived Urine Color	3 ± 2	3 ± 1	4 ± 1	0.89
Urine Markers				
Urine Color	4 ± 1	3 ± 1*	4 ± 1	0.13
USG	1.019 ± 0.007	1.020 ± 0.005	1.019 ± 0.007	0.50
Urine Osm (mOsm/kg)	423.4 ± 376.0	583.2 ± 387.9	465.1 ± 412.7	0.28
Urine [Na ⁺] (mmol/l)	127.7 ± 65.3	128.5 ± 57.5	120.6 ± 64.2	0.86
Urine [Cl ⁻] (mmol/l)	133.9 ± 65.8	153.2 ± 65.0	131.9 ± 76.8	0.37
Urine [K ⁺] (mmol/l)	57.8 ± 46.2	73.9 ± 44.6	63.4 ± 52.8	0.42
Blood Markers				
Plasma Osm (mOsm/kg)	292.2 ± 11.8	288.4 ± 11.7	293.0 ± 9.8	0.15
Plasma [Na ⁺] (mmol/l)	141.8 ± 1.8	141.8 ± 1.8	140.5 ± 4.6	0.73
Plasma [Cl ⁻] (mmol/l)	103.8 ± 2.3	102.2 ± 12.5	103.8 ± 2.1	0.77
Plasma [K ⁺] (mmol/l)	3.9 ± 0.4	3.9 ± 0.2	3.8 ± 0.3*	0.02
Hematocrit (%)	48 ± 5	47 ± 5	47 ± 4	0.33
Hemoglobin (g/dL)	17.2 ± 3.3	15.9 ± 2.2*	15.4 ± 2.7*	0.02

USG urine specific gravity; Osm osmolality. *Different from baseline P<0.05.

5.3.4 Pre- and Post-Exercise Visits

5.3.4.1 Exercise Trials

Participants' wattage for workload during the exercise trials was 35.0±0.9W/m² (1.1±0.1Kp). Oxygen consumption (HYD vs. DEH: VO₂ 1.15±0.26L/min vs. 1.14±0.16L/min; P=0.84) and respiratory exchange ratio (RER) (HYD vs. DEH: RER 0.89±0.04 vs. 0.87±0.05; P=0.20) were similar between exercise trials. Ambient temperature (HYD vs. DEH, 30.1±0.9°C vs. 30.4±0.8°C, P=0.37) and relative humidity (HYD vs. DEH, 27.4±7.4°C vs. 25.6±7.6%, P=0.20) were also not different between exercise trials. Heart rate after 10-min of exercise was not different between trials, but after 20-min and at 30-min heart rate was greater in the DEH trial compared to the HYD trial (20-min: 150±27 vs. 142±23bpm, P=0.02; 30-min: 153±26 vs. 144±23bpm, P=0.02, resp.).

5.3.4.2 Blood and Urine Markers

Table 5.4 represents weight, urine and blood markers from the start of the intervention period (Pre-intervention) to the start and end of exercise (Pre- and Post-exercise) for 16 participants (6 male, 10 female). Over the course of the DEH intervention (Pre-intervention vs. Pre-exercise), weight decreased (67.9 ± 11.0 vs. 66.2 ± 10.8 kg, $P < 0.001$), urine color increased (4 ± 1 vs. 5 ± 2 , $P = 0.01$), and Posm increased (294.4 ± 9.7 vs. 299.2 ± 8.5 mOsm/kg, $P = 0.03$). The HYD intervention (Pre-intervention vs. Pre-exercise), weight (67.7 ± 11.1 vs. 67.8 ± 10.5 kg, $P = 0.88$), urine color (3 ± 1 vs. 3 ± 1 , $P = 0.59$), and Posm ($289.8.4 \pm 11.6$ vs. 290.6 ± 10.5 mOsm/kg, $P = 0.16$) were not different from pre-intervention.

At the start of exercise (Pre-exercise), significant differences between HYD and DEH trials were seen in weight (68.8 ± 10.5 vs. 66.2 ± 10.8 kg, $P < 0.005$), USG (1.016 ± 0.008 vs. 1.023 ± 0.007 , $P < 0.001$), urine color (3 ± 1 vs. 5 ± 2 , $P < 0.001$) and perceived thirst (3 ± 1 vs. 5 ± 1 , $P < 0.001$). Posm was also different between trials prior to exercise (HYD vs. DEH, 282.2 ± 10.5 vs. 301.3 ± 7.6 mOsm/kg, $P < 0.002$).

Table 5.4. Pre-intervention and pre- and post-exercise urine and blood markers (n=16).

	Pre-Intervention		Pre-Exercise		Post-Exercise		ANOVA (P-value)
	HYD	DH	HYD	DH	HYD	DH	
Weight (kg)	67.7±11.1	67.9±11.0	67.8±10.5	66.2±10.8 ^{*b}	68.7±10.6	66.1 ±10.8 ^{*c}	<0.008
	Δ -0.2 (-0.6-0.3)		Δ 0.6 (0.2-1.1)		Δ 0.6 (0.2-1.1)		
Perceived Thirst	3±1	3±1	3±1	5±1 ^{*b}	---	---	<0.004
Perceived Urine Color	3±1	4±1	3±1	6±1	---	---	<0.004
Urine Markers							
USG	1.022±0.004	1.020±0.007	1.016±0.008 [*]	1.023±0.007 ^b	1.015±0.009 [*]	1.024±0.007 ^{*c}	<0.001
	Δ 0.002 (-0.001-0.006)		Δ -0.009 (-0.013- -0.005)		Δ -0.01 (-0.015- -0.005)		
Urine Color	3±1	4±1	3±1	5±2 ^{*b}	3±1	4±1 ^c	0.02
	Δ -0.4 (-1.1-0.4)		Δ -2 (-2.9- -1.0)		Δ -1 (-1.8- -0.2)		
Uosm (mOsm/kg)	647.5±361.7	512.3±414.3	210.0±238.6 [*]	223.2±273.3 [*]	204.4±240.4 [*]	247.3±341.3 [*]	0.09
	Δ 135.2 (-116.3-386.7)		Δ -100.4 (-318.8-117.9)		Δ -125.3 (-340.3-89.7)		
Urine [Na ⁺] (mmol/L)	131.0±60.7	120.6±67.1	139.2±70.9 [*]	165.1±74.2 ^{*b}	117.1±68.5 [*]	144.0±60.3 ^{*c+}	0.01
	Δ 10.5 (-22.5-43.5)		Δ -43.2 (-89.5-3.1)		Δ -57.2 (-86.0- -28.3)		
Urine [Cl ⁻] (mmol/L)	158.6±67.1	133.8±78.7	163.9±82.2	192.9±76.7 ^{*b}	152.3±84.6 ⁺⁺	188.1±76.0 ^{*c}	0.01
	Δ 24.8 (-19.5-69.2)		Δ -48.7 (-97.4-0.0)		Δ -79.4 (-131.8- -26.9)		
Urine [K ⁺] (mmol/L)	78.8±45.1	68.1±54.3	84.8±59.4	91.0±59.9	87.3±57.7	103.6±56.5 [*]	0.54
	Δ 10.6 (-22.9-44.3)		Δ -13.8 (-59.1-31.4)		Δ -16.6 (-72.5-39.3)		

USG urine specific gravity; Uosm urine osmolality. Mean ± standard deviation, Δ change (95% confidence interval). ^{*}Different from baseline P<0.05. ⁺Different from pre- to post-exercise (P<0.05). ^bDifferent between pre-exercise trials (P<0.05). ^cDifferent between post-exercise trials (P<0.05).

Table 5.4 continued. Pre-intervention and pre- and post-exercise urine and blood markers (n=16).

	Pre-Intervention		Pre-Exercise		Post-Exercise		ANOVA (P-value)
	HYD	DH	HYD	DH	HYD	DH	
Blood Markers							
Posm (mOsm/kg)	289.8±11.6	294.0±12.2	282.2±10.5*	301.3±7.6 ^{*b}	292.3±10.3*	298.1±8.7	<0.001
	Δ -4.6 (-9.41-0.16)		Δ -11.2 (-19.0- -3.3)		Δ -4.0 (-11.9-3.8)		
Plasma [Na ⁺] (mmol/L)	141.2±1.9	141.6±1.6	141.6±1.5	142.9±2.0 ^b	141.9±1.3	142.9±2.7	0.19
	Δ 0.3 (-0.7-1.3)		Δ -1.3 (-2.3- -0.2)		Δ -0.1 (-2.2-1.6)		
Plasma [Cl ⁻] (mmol/L)	105.0±4.1	103.7±2.0	103.7±1.3	106.3±6.2	103.7±1.5	105.9±6.5 ^{*c}	0.26
	Δ 1.1 (-1.5-3.6)		Δ -1.5 (-3.4-0.5)		Δ -3.4 (-8.3-1.6)		
Plasma [K ⁺] (mmol/L)	3.92±0.28	3.79±0.30	3.97±0.31	4.03±0.59	4.03±0.27 ⁺⁺	4.16±0.52 ⁺⁺	0.06
	Δ 0.13 (-0.00-0.26)		Δ -0.13 (-0.49-0.22)		Δ -0.11 (-0.47-0.26)		
Hematocrit (%)	47±5	47±4	49±8	46±4	49±7	46±3 ^c	0.36
	Δ 0.5 (-1.7-2.8)		Δ 2.9 (-0.8-6.7)		Δ 3.2 (-0.1-6.5)		
Hb (mg/dL)	16.1±2.1	15.7±2.7	16.9±4.0	16.4±3.3	16.9±3.9	16.1±3.2	0.32
	Δ 0.4 (-0.9-1.7)		Δ 0.7 (-2.1-3.5)		Δ 1.2 (-1.7-4.1)		

Posm plasma osmolality; Hb hemoglobin. Mean ± standard deviation, Δ change (95% confidence interval). *Different from baseline P<0.05. ⁺Different from pre- to post-exercise (P<0.05). ^bDifferent between pre-exercise trials (P<0.05). ^cDifferent between post-exercise trials (P<0.05).

5.3.5 T_{sk} , SkBF and Sweat Responses to Exercise and Relationships to Cardiovascular Function

T_{sk} , SkBF (n=12) and LSR (n=10) measurements are displayed in Table 5.5. Complete T_{sk} data was determined in 12 participants, and sweat electrolytes were assessed in 10 participants (5 male, 5 female); 6 participants did not produce enough sweat to measure electrolytes. LSR was not different between exercise trials (HYD vs. DEH, 4.4 ± 3.3 vs. 4.3 ± 3.7 g/cm²/min). T_{sk} after 30-min of exercise was not different between HYD and DEH trials (T_{sk} P=0.47, T_b P=0.72). There were also no observed differences between HYD and DEH temperature change at T_{sk} or T_b (Figure 5.2). Mean HS was higher in the DEH trial compared to the HYD trial (48.3 ± 31.9 vs. 45.9 ± 25.4 , P=0.73), but the peak HS was higher in the HYD trial compared to the DEH trial (189.5 ± 65.8 vs. 150.9 ± 67.8 , P=0.09). SkBF tended to be greater in the HYD trial compared to the DEH trial (SkBF slope: HYD vs. DEH, 3.5 ± 2.6 vs. 2.4 ± 1.1 , P=0.05; SkBF Change: HYD vs. DEH, 370.0 ± 156.3 vs. $169.0 \pm 130.4\%$, P=0.05).

Table 5.5. T_{sk} , SkBF (n=12) and sweat (n=10) responses to exercise.

	HYD			DH		ANOVA (P value)	
Skin Temperatures							
Mean HS (W/m ²)	45.9	±	25.4	48.3	±	31.9	0.73
Peak HS (W/m ²)	189.5	±	65.8	150.9	±	67.8	0.09
T _{sk}							
T ₀	32.2	±	0.7	32.6	±	0.5	
T _{max}	33.7	±	0.9	33.8	±	0.9	
Change	1.49	±	0.59	1.29	±	0.85	0.23
T _b							
T ₀	36.5	±	3.5	36.2	±	2.5	
T _{max}	37.0	±	3.4	36.3	±	0.37	
Change	0.61	±	0.31	0.65	±	0.46	0.65
Skin Blood Flow							
SkBF Start (PU)	8.0	±	2.9	9.5	±	3.5	0.17
SkBF End (PU)	38.3	±	22.6	32.2	±	8.5	0.25
SkBF Slope	3.5	±	2.6	2.4	±	1.1	0.05
SkBF Change (%)	370.0	±	156.3	169.0	±	130.4	0.05
Sweat Response							
LSR (g/cm ² /min)	4.4	±	3.3	4.3	±	3.7	0.93
Sweat [Na ⁺] (mmol/L)	56.3	±	28.7	59.9	±	26.1	0.71
Sweat [Cl ⁻] (mmol/L)	56.2	±	28.5	54.7	±	30.5	0.86
Sweat [K ⁺] (mmol/L)	7.6	±	3.0	7.9	±	4.1	0.97

HS heat storage; T_{sk} weighted skin temperature; T_b body temperature; T_0 temperature at time zero; T_{max} temperature peak; LSR local sweat rate.

After collapsing the exercise trials together (n=24), a linear regression showed an increase in BAFMD was related to an increase change in T_{sk} (P=0.04) and T_b (P=0.006) during exercise. Increased LF/HF was related to increased peak heat storage (P=0.03). Lastly, no relationships between SkBF, LSR and the resting cardiovascular measures were determined.

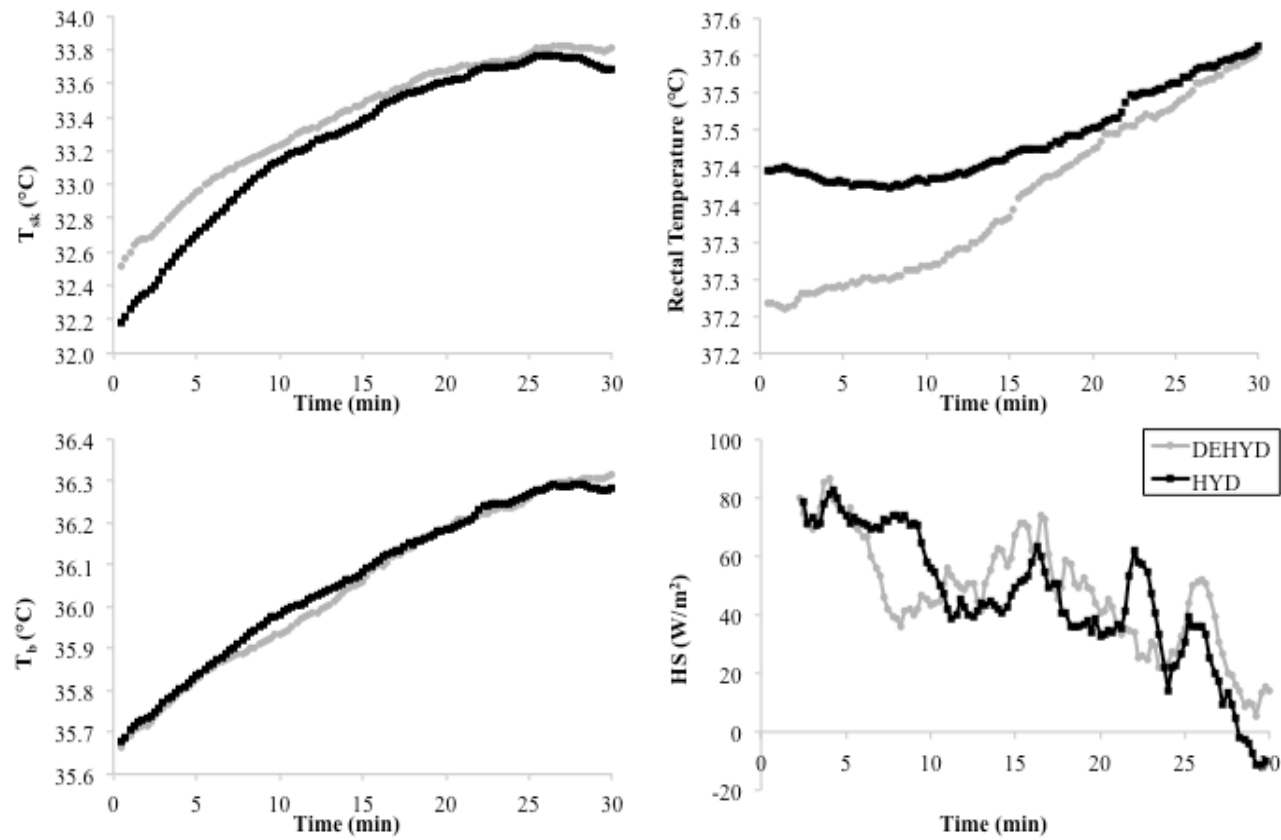


Figure 5.2. Weighted skin temperature (T_{sk}), rectal temperature, total body temperature (T_b) and 2-min average heat storage (HS) during 30-min exercise bouts ($n=12$). Black squares represent HYD trial and gray circles represent DEH trial.

5.4 Discussion

This study demonstrated that 3 days of chronic, progressive dehydration altered weight, urine and blood markers of hydration, and altered cardiovascular response to exercise in a heated environment. In addition, BAFMD was related to the change in weighted skin temperature and body temperature during exercise in the heat, suggesting resting vascular function plays a role in the rate in which body temperature increases. Increased LF/HF at rest was associated with increased peak heat storage, suggesting greater sympathetic tone at rest leads to a greater rate in which heat is stored during exercise. Together these results suggest that chronic, progressive dehydration can produce significant alterations in hydration markers and cardiovascular responses to exercise in the heat may be influenced by resting cardiovascular health.

From screening through the tracking periods, participants maintained the same hydration status as measured by weight, perceived thirst and urine color, and urine specific gravity suggesting participants started hydration interventions in similar states (Table 5.2). Furthermore, the similar states produced pre-intervention suggested that the second randomized tracking period was sufficient time to return to screening hydration status. Although urine color pre-HYD intervention was significantly different from screening, this measure is subjective and may not represent a meaningful change.

According to current recommendations, the combination of weight, urine color and thirst provide a strong indication of a hypohydrated state^{11,28}. During the HYD and DEH interventions, participants achieved a small change in body weight (HYD trial $0.2 \pm 1.0\%$ vs. DEH trial $-1.0 \pm 1.4\%$). In comparison, the majority of studies involving dehydration achieve a decrease of 3% in body weight^{1,3}. Despite these small changes, perceived thirst, weight and

urine color were different between pre-HYD and pre-DEH trials, suggesting participants achieved a euhydrated and hypohydrated state, respectively. In addition, plasma osmolality and urine specific gravity were also different between pre-HYD and pre-DEH trials, suggesting participants were in a dehydrated state.

The exercise bouts for both HYD and DEH trials were performed in similar environments ($30.2 \pm 0.8^\circ\text{C}$, $26.5 \pm 7.4\%\text{RH}$). When exercise was performed in the DEH trial, heart rate was significantly higher ($P=0.02$), suggesting a hypohydration is more taxing on the cardiovascular system compared to a euhydrated state². SkBF (% change) tended to be blunted during the DEH trial (Table 5.5). Dehydration and heat stress have been shown to independently and together reduce SkBF and heat dissipation²⁹ and ultimately may impair aerobic performance³⁰. Interestingly, heat storage and body temperature were greater initially in the HYD trial compared to the DEH trial (Figure 5.2). Heat storage and body temperature tended to be greater in the DEH trial compared to HYD trial at the end of 30-min, suggesting thermoregulatory mechanisms are prompted manage heat stress more efficiently when euhydrated compared to dehydrated. However, in this apparently healthy population, the environment and work rate may not have produced enough stress on the cardiovascular system to significantly alter SkBF and sweat rate. Previous studies demonstrate sweat rate is varies widely depending on exercise intensity and environment³¹. In addition, light clothing was worn during exercise trials in order to permit heat loss, but the participants' fitness level may have impacted their sweat rate. Those with increased aerobic fitness may be more likely to tolerate heat exposure, therefore respond by sweating more³².

Increased BAFMD was related to both increased weighted skin temperature and body temperature. With improved resting BAFMD, the vasculature may have greater ability to

dilate during exercise in the heat and in turn raise body temperature to a greater degree by increasing skin blood flow. Carter et al. (2014) demonstrated repeated increases in core temperature through local heating resulted in increased BAFMD, suggesting thermoregulation-mediated changes in blood flow can improve conduit vascular function³³. Increased cardiovascular function, as measured by BAFMD, may be a useful biomarker in the ability to thermoregulate during exercise with heat stress. In addition, increased sympathetic activity, measured by LF/HF, was related to the peak heat storage. Those with poorer autonomic balance may have a more difficult time adapting to exercise in the heat while in a dehydrated state due to increasing the rate in which they store heat. In rats subjected to heat stress with and without dehydration, dehydrated rats had increased HF, suggesting higher sympathetic activity when dehydrated³⁴. Similarly, Crandall et al. (2000) found increased HF and reduced vagal tone when participants were subjected to whole body heat stress³⁵. Competing metabolic and thermoregulatory demands during exercise and heat stress may cause the sympathetic innervation of the skin to constrict in order to redirect blood flow to the core³⁶. Therefore, increase the rate in which heat is stored.

A limitation of this study was participant compliance with the interventional protocol. Hydration and/or dehydration was self-determined, which may have limited the magnitude of change in body weight and hydration status. Yet, blood and urine markers of hydration status were influenced with the small changes produced. This study also delivered a low environmental stress and work rate during the exercise bouts compared to current literature^{1,2,7}. In this study, greater differences between HYD and DEH exercise trials may have been observed had the bout been longer to pose more stress.

In conclusion, progressive, chronic dehydration produced changes in weight, urine and blood markers, and cardiovascular response to exercise in a heated environment. Improved vascular cardiovascular function measured by BAFMD may be related to the ability to better deliver blood flow to the skin during exercise in the heat; however, resting indicators of increased sympathetic activity may be related to increased rate of heat storage during exercise. Future research should continue to examine the impact of resting cardiovascular function on cardiovascular and thermoregulatory responses to exercise, particularly in a dehydrated state.

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CHAPTER 6. CONCLUSION

The purpose of this dissertation was to study non-invasive markers of cardiovascular and autonomic function in response various conditions including exercise, glucose ingestion and dehydration. The meta-analysis (chapter 3) was designed to target the effects of exercise training on endothelial function, as measured by BAFMD. In the 66 studies analyzed (1865 trained, 635 control), BAFMD proved to be a useful biomarker in detecting changes in function resulting from exercise training ($P < 0.0001$). Aerobic exercise tended to have the greatest effect on BAFMD (ES 9.30; 95%CI, 6.29-12.31). In addition, greater exercise training intensity and volume appeared to play an important role in the magnitude of change in BAFMD. This study sheds light on the impact of exercise training on vascular function and may help future studies to better prescribe exercise to improve vascular health in both healthy and diseased individuals. Future studies should implement BAFMD as a useful clinical tool to assess vascular function, which is capable of detecting changes in response to exercise training.

In chapter 4, participants shifted to higher sympathetic activity (increased LF_{nu} and LF/HF), suggesting glucose ingestion may increase autonomic dysfunction. Secondly, a single bout of low- or high-intensity exercise altered the autonomic response to an OGTT, leading to increased LF_{nu} , LF/HF and decreased HF_{nu} ($P < 0.01$). This study was unique in that autonomic balance, measured by HRV, was assessed in response to and OGTT after two different exercise intensities that were set not as an arbitrary relative intensity ($\%VO_{2max}$), but according to the intensity relative to each individuals ability to oxidize fat (FM) or work at lactate threshold (LT). Future research should continue to investigate the potential changes in HRV after exercise in individuals with chronic exposure to high blood glucose and/or

insulin. Acute exercise and exercise training may produce different alterations in HRV, especially depending on a diseased state.

Lastly, chapter 5 aimed to determine if resting cardiovascular function influenced cardiovascular and sweat response to exercise in the heat when euhydrated and hypohydrated. After the hydration interventions, weight ($P<0.005$), USG ($P<0.001$), urine color ($P<0.001$), perceived thirst ($P<0.001$) and plasma osmolality ($P<0.002$) were different between HYD and DEH trials. This suggests the interventions produced significantly altered hydration statuses. DEH trials produced elevated heart rate and blood pressure compared to the HYD trial, suggesting more cardiovascular strain occurs when in a hypohydrated state. BAFMD was associated with increased core temperature ($P=0.006$) suggesting greater ability to dilate vasculature may allow for increased blood flow to the skin. In addition, increased sympathetic activity at rest (increased LF/HF) may impact the rate of heat storage ($P=0.03$). This study was unique in that progressive, chronic dehydration induced changes in blood and urine markers, thirst and weight. Secondly, this study is the first to our knowledge to examine the impact of resting cardiovascular function on responses to exercise in the heat in young, healthy college-aged participants. This research may lead to using the cardiovascular health profile to predict changes in cardiovascular, thermoregulatory and sweat responses during exercise. Future research should continue to examine the dual impact of heat stress and dehydration during exercise on cardiac and sweat responses, particularly in both healthy and diseased participants due to the effects health status may have.

In summary, cardiovascular and autonomic functions are intertwined systems that are alerted depending on health status. Exercise training may mitigate dysregulation of these systems, while glucose ingestion, dehydration and heat stress may complicate their

responses. As cardiovascular health persists as a public health issue, exercise training may be a promising intervention to improve the measureable outcomes of the cardiovascular and autonomic system.

APPENDIX. STUDY FORMS

1.1 LSU IRB Approval

ACTION ON PROTOCOL APPROVAL REQUEST



Institutional Review Board
Dr. Dennis Landin, Chair
130 David Boyd Hall
Baton Rouge, LA 70803
P: 225.578.8692
F: 225.578.5983
irb@lsu.edu | lsu.edu/irb

TO: Neil Johannsen
Kinesiology

FROM: Dennis Landin
Chair, Institutional Review Board

DATE: August 18, 2015

RE: IRB# 3630

TITLE: Impact of progressive, chronic dehydration on cardiac and sweat responsiveness to exercise in a heated environment

New Protocol/Modification/Continuation: New Protocol

Review type: Full ☒ Expedited ☐ **Review date:** 8/7/2015

Risk Factor: Minimal ☐ Uncertain ☒ Greater Than Minimal ☐

Approved ☒ **Disapproved** ☐

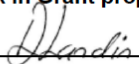
Approval Date: 8/14/2015 **Approval Expiration Date:** 8/13/2016

Re-review frequency: (annual unless otherwise stated)

Number of subjects approved: 40

LSU Proposal Number (if applicable):

Protocol Matches Scope of Work in Grant proposal: (if applicable) _____

By: Dennis Landin, Chairman 

PRINCIPAL INVESTIGATOR: PLEASE READ THE FOLLOWING –
Continuing approval is **CONDITIONAL** on:

1. Adherence to the approved protocol, familiarity with, and adherence to the ethical standards of the Belmont Report, and LSU's Assurance of Compliance with DHHS regulations for the protection of human subjects*
2. Prior approval of a change in protocol, including revision of the consent documents or an increase in the number of subjects over that approved.
3. Obtaining renewed approval (or submittal of a termination report), prior to the approval expiration date, upon request by the IRB office (irrespective of when the project actually begins); notification of project termination.
4. Retention of documentation of informed consent and study records for at least 3 years after the study ends.
5. Continuing attention to the physical and psychological well-being and informed consent of the individual participants, including notification of new information that might affect consent.
6. A prompt report to the IRB of any adverse event affecting a participant potentially arising from the study.
7. Notification of the IRB of a serious compliance failure.
8. SPECIAL NOTE:

**All investigators and support staff have access to copies of the Belmont Report, LSU's Assurance with DHHS, DHHS (45 CFR 46) and FDA regulations governing use of human subjects, and other relevant documents in print in this office or on our World Wide Web site at <http://www.lsu.edu/irb>*

1.2 Consent Form

CONSENT TO PARTICIPATE IN A RESEARCH STUDY INFORMED CONSENT

Title of Study: Impact of progressive, chronic dehydration on cardiac and sweat responsiveness to exercise in a heated environment

We give you this consent form so that you may read about the purpose, risks and benefits of this research study.

- The main goal of a research studies is to gain knowledge that may help future patients.
- You have the right to refuse to take part, or agree to take part now and change your mind later on.
- Please review this consent form carefully and ask any questions before you make a decision.
- Your participation is voluntary.
- By signing this consent form, you agree to participate in the study as it is described.

1- Investigators:

The following investigator will be available for questions about this study:

Principal Investigator: Neil M. Johannsen, Ph.D.
Phone: 225-578-5314
Email: Njohan1@lsu.edu

Co-Investigators: Brian Harrell, M.D. Cuneaux@gmail.com
Kate Early, M.A. Kausti4@lsu.edu
Matthew Scott, M.S. Mscot26@lsu.edu

Neil Johannsen, Ph.D. directs this study. Dr. Brian Harrell serves as the medical oversight for this study. The study will take place over a period of 3 visits.

2- Performance Site:

This study will take place at Louisiana State University-Baton Rouge Campus.

3- Purpose of the Study:

This study is designed to investigate whether chronic hypohydration, achieved by fluid restriction over a 3-day period, alters cardiovascular function and sweat responsiveness during exercise in a heated environment.

4- Participant Inclusion:

Study staff will discuss with you the requirements for participation in this study. It is important that you are completely truthful with the staff about your health history. You should not participate in this study if you do not meet all the qualifications.

You are eligible for this study if you are:

- Capable and willing to give written informed consent, understand exclusion criteria and accept the randomized assignment
- Male or Female aged 18-45 years
- Healthy (no uncontrolled disease) with a BMI between $<35 \text{ kg/m}^2$
- Willing to participate in a 2-week research study
- Willing to be cleared to participate in the study by their primary physician if deemed necessary by study staff
- Willing to record food and fluid intake for the duration of the trial
- Willing to undergo resting cardiovascular measures including electrocardiogram (EKG), pulse wave velocity analysis, brachial artery flow mediated dilation determination, and heart rate variability assessment
- Willing to answer questionnaires about their typical sleep patterns, thirst, and anxiety levels
- Willing to participate in 2 exercise tests in a heated environment; once after 3 days of drinking normal fluids and once after 3 days of restricting fluid intake
- Willing to have blood drawn through an IV catheter
- Willing to have the sweat rate determined on your upper back
- Willing to have local skin blood flow rate determined
- Willing to wear physiological monitors for the duration of the study (~14 days)
- Willing to insert a rectal thermometer (self-insertion) and attach skin temperature probes prior to and maintain during the exercise tests

You are **NOT** eligible for this study if you have any of the following conditions:

- HIV, Hepatitis B, or Hepatitis C
- Uncontrolled CVD/ arrhythmia
- COPD, Emphysema, Exercise Induced asthma/bronchospasm
- Cerebral Palsy, Multiple Sclerosis, Amyotrophic lateral sclerosis, Cystic Fibrosis
- Diabetes (Type 1 or 2); fasting glucose $>125 \text{ mg/dL}$
- Systolic blood pressure $\geq 160 \text{ mmHg}$ and/or diastolic blood pressure $\geq 100 \text{ mmHg}$
- Uncontrolled thyroid disorder (controlled = 6 months of medication)
- Epilepsy
- Osteo/Rheumatoid arthritis
- Unresolved orthopedic injury of any kind
- Any other medical, psychiatric or behavioral factors that in the judgment of the Principle Investigator that may interfere with study participation or the ability to follow the protocol

Medications/Non-Drug Therapies:

- Blood pressure medication
- Diuretics
- Beta-blocker
- Antipsychotic

- Other medications that may affect fluid balance, thirst, or heat tolerance

Lifestyle:

- Consume > 3 drinks/day of any alcoholic beverage
- Smoker (Former smokers must be smoke free for 12 months)
- Donated blood within the past 8 weeks

5-Study Procedures:

The study for which you are volunteering will take about 2-3 weeks to complete and will include 3 phases:

1. Screening and Baseline Testing (One visit)
2. Tracking (Two, 4 day) and Intervention (Two, 3 day) Periods
3. Exercise Testing (Two visits)

Study Timeline



Screening and Baseline Testing Visit – 1-2 hours

(Fasting visit – Please do not eat food or drink anything but water for 10 hours and no alcohol or exercise for before this appointment)

At this visit, our study staff will explain the informed consent to you and the procedures that will be performed. If you choose to sign the informed consent, the following tests and procedures will be performed to determine if you qualify to participate in this research study:

- You will be asked questions about your:
 - Demographics
 - Medical history
 - Medication use
- You will be asked to perform the following medical assessments:

- o Blood pressure and heart rate- by a cuff placed around your arm and a monitor worn around your chest
- o Height and weight
- o Blood sample- a blood sample will be drawn by venipuncture from your arm. About 1-2 teaspoons of blood will be collected and stored if you consent
- o Urine sample- a urine sample will be taken and assessed for hydration status
- o Cardiovascular tests- your pulse will be traced and a cuff temporarily inflated around your forearm while looking at your upper arm with ultrasound

Tracking Period – Two periods of 3-5 days each

During the tracking period you will be asked to maintain your normal food and fluid intake. This time will serve as a washout period between study interventions.

- You will be asked to complete the following during the 3-5 day period:
 - o Food and fluid intake log
 - o Sleep questionnaire- each morning fill out a survey of how you felt you slept the night before
 - o Thirst and urine color scale- each time you void your bladder you will record your urine color and report how thirsty you feel on a categorical scale (1 not thirsty at all, 10 very, very thirsty)
 - o Wearable device- 1 to 2 devices worn around your wrist similar to a watch

Intervention Period – Two periods of 3 days each

Hydration Trial Intervention Period

For this intervention period you will be asked to maintain a hydrated state. You are encouraged to drink fluids to keep your urine color and thirst level indicative of a hydrated state. Additionally, during this time we ask you avoid strenuous physical activity and outdoor activities as well as alcohol consumption. You may continue your caffeine intake during this time.

- You will be asked to complete the following during the hydration intervention period:
 - o Food and fluid intake log
 - o Sleep questionnaire- each morning fill out a survey of how you felt you slept the night before
 - o Thirst and urine color scale- each time you void your bladder you will record your urine color and report how thirsty you feel on a categorical scale (1 not thirsty at all, 10 very, very thirsty)
 - o Heart rate monitor- worn around your chest
 - o Wearable device- 1 to 2 devices worn around your wrist similar to a watch

- Urine sample- a urine sample will be taken and assessed for hydration status

Dehydration Trial Intervention Period

During this intervention period you will be asked to modify your normal food and fluid intake. You will be asked to restrict your fluid intake to achieve a given thirst level and urine color. If you exceed the target restriction on these scales, you should drink fluids to alleviate the sensation of thirst or any symptoms (dizziness, nauseousness, cramping) you may have. As in the hydration trial, you will be asked to avoid strenuous physical activity and outdoor activities as well as alcohol consumption. You may continue your caffeine intake during this time.

- You will be asked to complete the following during the dehydration intervention period:
 - Food and fluid intake log
 - Sleep questionnaire- each morning fill out a survey of how you felt you slept the night before
 - Thirst and urine color scale- each time you void your bladder you will record your urine color and report how thirsty you feel on a categorical scale (1 not thirsty at all, 10 very, very thirsty)
 - Heart rate monitor- worn around your chest
 - Wearable device- 1 to 2 devices worn around your wrist similar to a watch
 - Urine sample- a urine sample will be taken and measured for hydration status

Exercise Testing Visits – Two visits of 1 day each

(Non-Fasting visit – Please refrain from alcohol and exercise for 48 hours and caffeine for 5 hours before this visit. Please eat a small meal 2-3 hours before the visit)

You will be asked to return to the Clinical Exercise Testing Laboratory at LSU to complete the Exercise Testing Visits after each interventional period. The following tests will be performed:

- Blood pressure and heart rate – by a cuff placed around your arm and monitor worn around your chest
- Wearable device- 1 to 2 devices worn around your wrist similar to a watch
- Weight
- Questionnaires about exertion and feelings during exercise
- Respiratory measures – described below
- Blood samples – An IV will be inserted in your arm vein to draw blood before and after the test. About 5 teaspoons of blood will be collected each trial and measured for hemoglobin and hematocrit. The remaining blood will be stored for this study and for future research if you consent
- Urine samples- a urine sample will be taken and hydration status will be assessed

- Sweat sample- sweat will be collected in small chamber on your back and assessed for electrolytes
- Body temperature- skin and rectal temperature will be recorded continuously during exercise for safety
- Skin blood flow- a thumb sized sensor will be placed on your forearm to record blood flow changes during exercise

During the exercise tests, the volume of oxygen and carbon dioxide you are breathing will be measured continuously using an instrument known as a metabolic cart. The test requires that you breathe through a mouthpiece, similar to a snorkel, with a nose clip on. The mouthpiece is attached to a long tube, which is then attached to the metabolic cart. The breath gases collected during the exercise test allow us to accurately measure your energy expenditure and type of fuel (carbohydrate or fat) you are using at each stage of the test. Your heart rate and blood pressure will be monitored throughout the test.

The exercise tests will be performed in a heated environment. If you show signs and symptoms of physiological distress, feel dizziness and nausea, or your rectal temperature exceeds 40°C, exercise will be discontinued immediately.

6- Risks/Discomforts:

VO_{2max} and exercise testing in the heat: All exercise testing is completed in accordance with the American College of Sports Medicine's Guidelines for Exercise Testing and Prescription as well as the American Heart Association. There is minimal risk of injury or a cardiovascular event during testing. We believe the use of a highly trained staff, a pretest review of participant associated risk factors including the Physical Activity Readiness Questionnaire (PAR-Q) and medical screening by your doctor, and well-defined emergency procedures minimize the risk of an event during testing. During testing you may experience temporary discomfort during blood pressure recordings due to the pressure of the blood pressure cuff on the arm. The exercise will be discontinued if your core temperature measured by rectal temperature exceeds 40°C. You may also experience muscle fatigue, weakness, soreness and/or muscle pulls or tears. All tests are conducted in the presence of trained staff. In addition, all staff are trained in BLS (basic life support-CPR) and/or ACLS (advanced cardiac life support). In the event of an emergency, you would be treated appropriately and transported to the nearest acute care medical-surgical facility via Emergency Medical Services.

Cardiovascular testing: Inflation of the blood pressure cuff around the arm may cause some discomfort and pain during the ultrasound test. The temporary numbness and tingling in the hand is similar to the sensation of having your hand "fall asleep". If the discomfort is too severe, you may stop the test by notifying the technician to stop and the test will be immediately terminated. There is no known risk from the use of ultrasound to measure the size of the blood vessel in the arm from previous tests performed by the laboratory technician or reported in the literature.

ECG: There are no risks associated with this test. There is a small possibility there may be some redness or irritation if you happen to be allergic to the adhesive on the electrodes.

IV procedure and blood draws: There may be minimal discomfort and bruising and/or bleeding where the needle is inserted for finger prick blood sampling. There is a possibility of pain, bruising, and/or infection at the site of the needle insertion for the IV line for the oral glucose tolerance test. Aseptic (sterile) technique to minimize this risk. Dr. Johannsen will perform any blood draws, who has been trained to perform insertions under the supervision of a licensed physician.

Body composition: The measurement of skinfold thickness using skinfold calipers requires a firm squeezing and subsequent measurement of the skin. This measurement may cause slight discomfort and possible bruising of the measured site. However, if undue bruising, soreness, or if more than slight swelling occurs, please notify the Principal Investigator.

Hydration/Dehydration Intervention:

Risks associated with overhydration and dehydration include fatigue, irritability, headache, dizziness, abnormal heart rate and blood pressure. Modest dehydration (<2%) is commonly experienced day-to-day with no associated symptoms. However, if you begin to feel symptoms, you are advised to decrease or increase your fluid intake depending on your intervention trial.

Sweat sampling:

The medical grade glue used to hold the small chamber on your back may cause skin irritation. Collection of sweat is non-invasive and is routinely employed in clinical practice.

Body temperature:

There are no known risks.

Loss of Confidentiality: Completing questionnaires may result in a breach in confidentiality of personal data. Participants will be assigned ID numbers and information that could identify a participant will not appear in publications.

Unknown risks: In addition to the risk listed above, you may experience a previously unknown risk or side effect.

7- Benefits:

We cannot promise any benefits from your being in the study. However, possible benefits include:

- Information about your general health
- Knowledge of your cardiovascular fitness
- An understanding of your exercise performance and physical activity

8-Alternatives to Participation:

There are no alternatives to the study described in this consent. You have the choice at any time not to participate in this research study. If you choose not to participate, any benefits to which you are entitled will not be affected in any way.

9-Injury/Illness or Questions:

If you have any questions about your rights as a research volunteer, you should call Dennis Landin, Ph.D., Institutional Review Board Office at 225-578-8892. If you have any questions about the research study or think you have a research-related injury or medical illness, contact Neil Johannsen, Ph.D. at 225-578-5314 during regular working hours.

10-Privacy:

Every effort will be made to maintain the confidentiality of your study records. Results of the study may be published; however, we will keep your name and other identifying information private. Other than as set forth above, your identity will remain confidential unless disclosure is required by law.

11-Early Study Withdrawal:

Neil Johannsen, Ph.D. can withdraw you from the study for any reason or for no reason. You may withdraw from the study at any time without penalty. Possible reasons for withdrawal include injury, the presence of an old or existing injury that may be deemed risky, sufficient medical history deemed too risky for testing. The sponsor of the study may end the study early.

12-Additional Information:

During the course of this study there may be new findings from this or other research that may affect your willingness to continue participation. Information concerning any such new findings will be provided to you.

13-Charges for Participation:

None

14-Payments for Participation:

You will earn \$150 upon completion of the study.

15- Compensation for study-related injury or medical illness:

No form of compensation for medical treatment or for other damages (i.e., lost wages, time lost from work, etc.) is available from Louisiana State University. In the event of injury or medical illness resulting from the research procedures in which you participate, you will be referred to a treatment facility. Medical treatment may be provided at your own expense or at the expense of your health care insurer (e.g., Medicare, Medicaid, Blue Cross-Blue Shield, Dental Insurer, etc.) which may or may not provide coverage.

16- Signatures:

The study has been discussed with me and all my questions have been answered. I understand that additional questions regarding the study should be directed to the study investigators. I agree with the terms above and acknowledge that I have been given a copy of the consent form.

Printed Name of Volunteer

Signature of Volunteer

Date

Date of Birth of Volunteer

Signature of Person Administering Informed Consent

Date

Neil M. Johannsen, Ph.D.
Principal Investigator

17- Tissue/Specimen Storage for Future Research or Use

Biospecimens for future research:

You are being asked to allow some of your blood to be stored and used for research at a later time. These bodily materials are called biospecimens. The donation of biospecimens in this study is optional. No matter what you decide to do, it will not affect your study participation. You will still be allowed to take part in the study even if you don't want your specimens to be collected and used for future research. Some biospecimen samples will be stored and used for the study and other biospecimen samples will be stored for future studies. The collection of samples may give scientists valuable research material that can help them to develop new diagnostic tests, new treatments, and new ways to prevent diseases. If you agree to have your samples stored, you can change your mind up until the end of the study.

The samples will be stored indefinitely. If you agree to donate your samples, they may be given to other investigators for future research as well. The future research may take place at Louisiana State University and may involve Louisiana State University Researchers in this study. The future research may not take place at Louisiana State University and may not be reviewed by Louisiana State University's Institutional Review Board. For privacy and confidentiality, your biospecimens will be labeled with a unique series of letters and numbers. Louisiana State University will store your biospecimens with this unique identifier and the minimum number of personal identifiers to meet laboratory standards. The research done with your specimens may help to develop new products in the future, or may be used to establish a cell line or test that could be patented or licensed. You will not receive any financial compensation for any patents, inventions or licenses developed from this research.

Making your choice about future research:

Please read about each biospecimen below. It is your choice which samples will be collected, stored and used for future research for this study or future studies. After reading about each below, sign next to "Yes" or "No" to show your choice about the collections for this research study and for future research studies.

Blood

If you give permission, approximately 4 tablespoons of blood will be collected and stored by this study. Your stored samples may be tested at Louisiana State University or other locations used in future research. Do you give permission for your blood to be collected and used in future research by this study?

Yes, I give permission

SIGNATURE

No, I do not give permission

SIGNATURE

1.3 Study Protocol

Study Protocol

I) Title

Impact of progressive, chronic dehydration on cardiac and sweat responsiveness to exercise in a heated environment

II) Overview

Exercise in a heated environment has detrimental effects on cardiovascular function, core body temperature regulation and performance because of the increased demand for adequate sweat rate and heat dissipation through the skin(7, 19). The combination of heat stress and dehydration may have an additive negative consequence on cardiovascular function and sweat responses(6, 8). Dehydration has been found to impact physiologic function and performance with as little as 2% deficit in body water(3, 8, 17). Early research demonstrates individuals only replace ~70% of total water loss during exercise in the heat(9), suggesting individuals do not ingest sufficient fluid to replace water losses and return to a euhydrated state after exercising. Therefore, it is conceivable that individuals may be persistently dehydrated day to day, causing impairments in performance and function. Few studies have examined the effects of progressive, chronic dehydration or the long-term health outcomes due to chronic under-consumption of water(2).

III) Purpose

The primary aim of this study is to determine whether chronic hypohydration, achieved by fluid restriction over a 3-day period, alters cardiovascular function and sweat responsiveness during exercise in a heated environment in apparently healthy men and women aged 18 to 45 years compared to a euhydrated state. In addition, we will test whether an individual's resting cardiovascular function influences their cardiovascular, blood flow, and sweat responses to exercise in a heated environment.

IV) Inclusion/Exclusion

The target study group will be 40 healthy men and women aged 18 to 45 years. We will recruit approximately equal men and women. High-risk individuals as categorized by the American College of Sports Medicine (ACSM), including those with known cardiovascular, pulmonary, or metabolic disease, or sign/symptoms suggestive of disease will be excluded(18). Potential participants will be included or excluded based on the following criteria:

a) Inclusion Criteria

- Men and women aged 18-45 years
- Healthy (No Known Disease/No uncontrolled Disease)
- Body mass index (BMI) < 35 kg/m²
- Willing to participate in a 2-week research study
- Willing to be cleared to participate in the study by their primary physician if deemed necessary by study staff
- Willing to record food and fluid intake for the duration of the trial
- Willing to undergo resting cardiovascular measures including electrocardiogram (EKG), pulse wave velocity analysis, brachial artery flow mediated dilation determination, and heart rate variability assessment
- Willing to answer questionnaires about their typical sleep patterns, thirst, and anxiety levels
- Willing to participate in 2 exercise tests in a heated environment; once after 3 days of drinking normal fluids and once after 3 days of restricting fluid intake
- Willing to have blood drawn through an IV insertion
- Willing to have the sweat rate determined on your upper back
- Willing to have local skin blood flow rate determined

- Willing to wear physiological monitors for the duration of the study (~14 days)
- Willing to insert a rectal thermometer (self-insertion) and attach skin temperature probes prior to and maintain during the exercise tests

b) Exclusion Criteria

i) Diseases

- HIV
- Hepatitis B
- Hepatitis C
- Uncontrolled CVD/ arrhythmia
- COPD
- Emphysema
- Exercise Induced asthma/bronchospasm
- Cerebral Palsy
- Multiple Sclerosis
- Cystic Fibrosis
- Amyotrophic lateral sclerosis
- Diabetes Mellitus
- Uncontrolled Thyroid disorder (controlled = 6 months of medication)
- Epilepsy
- Osteo/Rheumatoid arthritis
- Heat Intolerance
- Unresolved orthopedic injury of any kind
- Any other condition or known disease that can be exacerbated by exercise in a heated environment

ii) Medications/Non-Drug Therapies

- Diuretics
- Beta-blocker
- Antipsychotic
- Other medications that may affect fluid balance, thirst, or heat tolerance

iii) Lifestyle

- Consumption > 3 drinks/day of any alcoholic beverage
- Smoker (Former smokers must be smoke free for 12 months)
- Donated blood within the past 6 weeks

V) Primary Study Outcomes

This study will aim to assess cardiovascular function, sweat responsiveness and hydration status during exercise in a heated environment following a euhydrated state or progressive, chronic hypohydration state. In addition, this study will aim to study the potential effect of the resting cardiovascular profile on cardiovascular and sweat responsiveness during exercise in a heated environment. The following procedures and techniques of the measurements to be utilized in this study are described below:

a) Cardiovascular techniques

Cardiovascular measurements taken during exercise in a heated environment will include heart rate and heart rate variability (HRV) and skin blood flow. HRV will be examined according to the Task Force for Pacing and Electrophysiology(1). A Zephyr heart rate monitor (Zephyr Technology, MD) will be used to capture R-R intervals during rest and exercise and be analyzed using Kubios software (available at <http://kubios.uef.fi>). High-frequency, low-frequency and very-low-frequency components of HRV will be assessed from the HR data(13). Additionally,

time domain measures including standard deviation of the NN intervals (SDNN) and the square root of the mean squared difference of NNs (RMSSD) will be calculated(13). Skin blood flow will be measured continuously during exercise using a laser Doppler flowmeter and the associated software (Perimed, Stockholm, Sweden). The laser Doppler probe will be affixed to the flexor aspects of the forearm using adhesive and surgical tape. Recordings will be stored in the software for further analysis.

Resting cardiovascular measures including HRV, pulse wave velocity (PWV) and brachial artery flow-mediated dilation (BAFMD) will be performed between 7-10am. Participants will be asked to fast for at least 12-hours prior to resting cardiovascular testing and avoid alcohol and exercise for at least 48-hours prior. Vascular function will be assessed by BAFMD following current guidelines(20). With participants in the supine position and a 3-lead ECG placed on the wrists and leg to monitor cardiac cycle, BAFMD will be induced by 5 min of forearm occlusion by inflation of a pneumatic cuff (Hokanson) to 220 mmHg, positioned approximately 1 cm distal to the olecranon process. This gives the sensation of the hand "falling asleep" or tingling. Using ultrasonography (Logiq e Ultrasound, GE Healthcare, WI), the brachial artery will be imaged for 30 sec prior to cuff release and for 3 min following return to normal, unrestricted blood flow. BAFMD will be calculated as the percent change in brachial artery diameter. All ultrasound images will be stored for subsequent analysis (Vascular Tools 5, Medical Imaging Applications, LLC).

Blood pressures (systolic, diastolic, mean and pulse pressure) and systemic arterial stiffness (augmentation pressure and index) will be measured with applanation tonometry (SphygomoCor, Version 8.0, AtCor Medical). Participants will lie on a bed while a 3-lead ECG is placed on the wrists and leg to monitor the cardiac cycle. A pen-like transducer detects peripheral waveforms traces of the radial and carotid arteries, which is used to derive the central arterial waveform using a validated transfer function. Peripheral and central augmentation indices are calculated by the difference in the second systolic peak and diastolic pressure divided by the difference between the first systolic peak and diastolic pressure ($\times 100\%$)(21).

b) Sweat response techniques

Sweat rates of the upper back will be measured during exercise using ventilated capsules secured with surgical adhesive. Anhydrous air will be pushed through the capsules at 1.8 L/min and vapor concentration will be measured in the outflowing air. Local sweat rate is reported as the product of vapor concentration and flow rate of anhydrous air ($\text{mg}/\text{cm}^2 \cdot \text{min}$)(12).

Sweat samples will be collected during practice with an adhesive patch attached to the body. The water-proof patch contains an absorbent cotton material that will be placed against the skin thus absorbing the sweat produced. The absorbent material will be weighed before application and after removal to estimate sweat rate. The material will then be centrifuged and the resultant sweat will be analyzed for electrolytes (sodium, potassium, and chloride).

During the exercise testing, rectal and skin temperature will be continually monitored. Participants will insert a rectal temperature probe to a depth of 10 cm past the anal sphincter (BioPac Systems Inc., CA). Skin temperatures will be monitored at 5 sites, the mid-thigh, chest, forearm, mid-biceps and calf(15). Average body temperature will be calculated from rectal and skin temperatures and used to calculate the rate of heat storage using previously published equations(5). The exercise testing will be discontinued if a participant's rectal temperature exceeds 40°C or the participant is symptomatic for heat exhaustion.

c) Hydration status

Blood will be drawn under fasting conditions and during exercise via a Teflon indwelling catheter will be inserted into an antecubital vein. Blood drawn during the study will be immediately analyzed for hemoglobin (cyanmethemoglobin technique) and hematocrit (microcapillary technique) to determine changes in plasma volume, placed in lithium heparin tubes, centrifuged, and analyzed for plasma osmolality and electrolyte concentrations (sodium, potassium, and chloride). The remainder of the blood sample will be stored at -80°C for future analyses. Urine samples taken during the study will be assessed for electrolytes and osmolality and discarded after analysis.

Sweat samples during exercise will be collected from the upper back, chest and forearm. Sweat volume will be estimated using the change in body weight adjusted for metabolic carbon and respiratory water loss and urine output(16). The sweat samples will be used to determine the average sweat electrolyte losses.

VI) Secondary Study Outcomes

a) Anthropometric assessments

Anthropometric measures will be obtained at screening and will consist of body height, weight, waist circumference, and body composition by skin fold thickness. Height will be measured using a standard stadiometer and fasting body weight will be recorded to the nearest 0.05 kg after voiding and wearing light clothing. Waist circumference will be measured using the recommendations of the Airlie Conference. Body fat percent will be measured by 3-site skinfold thickness the arm, hip and thigh for women and chest, abdominal, and thigh for men. Each site will be measured twice and the average skin fold thickness will be used to estimate body density, and therefore, body fat percentage using standard equations(14). If any 2 measurements are different by > 5mm, a third measurement will be taken at that site.

b) Exercise tests in a heated environment

Each exercise test will consist of a 30-minute steady-state bout of exercise on a cycle ergometer (Monark, Sweden) in a heated environment (~30°C, ~50%RH). Prior to exercise and immediately after, participants will rest quietly in the seated position for 10 minutes. During the steady-state exercise bout, pedal rate on the cycle ergometer will be set to 60 RPM and a resistance factor necessary to elicit an individualized estimated metabolic rate of 35 W/m² using ACSM cycle equations(18). This workload is standard in hydration literature and is considered moderate intensity exercise across the age and sex normative data for the participants.

c) Substrate metabolism

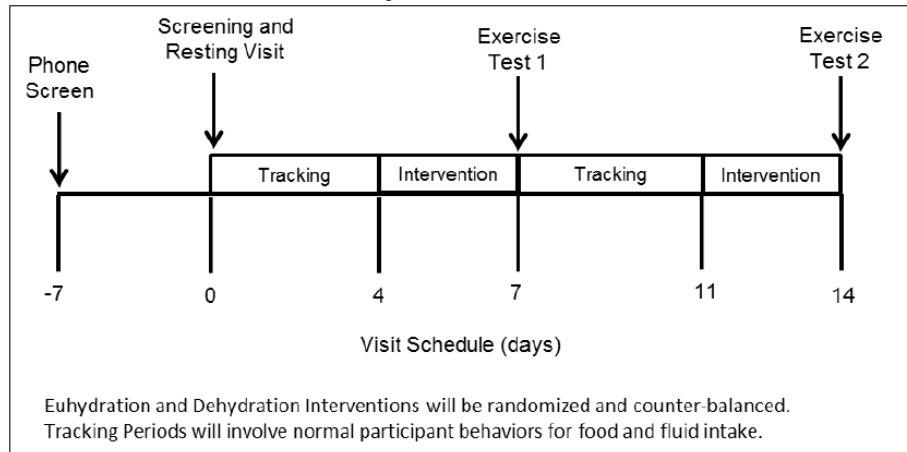
Respiratory gases will be monitored during exercise sessions using an integrated oxygen/carbon dioxide analyzer calibrated with standard gas mixtures (TruOne 2400, ParvoMedics, Inc., Sandy, UT). Metabolic rate will be used to determine dry and wet heat exchanges using known partitioned calorimetric equations. Stoichiometric equations will be used to determine fat and carbohydrate oxidation rates as well as caloric expenditure rates and the time to finish the total exercise dose (11).

VII) Study Timeline and Visits

Participants enrolling in the study will be required to make 3 visits to the Clinical Exercise Testing and Metabolic and Vascular Testing Laboratories at LSU (**Figure 1**. schematic overview of study design). The study is a counterbalanced, cross-over design where study participants will be assigned to the intervention periods in a random order. We estimate that each participant will take approximately 2 to 3 weeks to complete the full study protocol. The time obligation entails a screening visit, 2 randomly assigned intervention periods, and 2 exercise tests

(described in detail below). **Table 1.** provides a schedule of assessments previously described in primary and secondary outcomes.

Figure 1. Schematic Overview of Study



a) Recruitment and Initial screening

We will recruit for this study using word of mouth and strategically placed fliers on the Louisiana State University campus. Additionally, electronic media will be used for recruitment of this study to be seen or heard by prospective participants. Advertisements and/or videos to be used on social media include networking and video sharing sites (e.g. Facebook, Twitter, YouTube) and interactive websites. Recruitment ads will include the following information: the title of the study, name of the PI, a clear statement of the research goal and contact information. Each ad will clearly state that this research is voluntary and will not be published until it has received appropriate IRB review and approval. No protected health information will appear in these advertisements. Video or photographs of participants may appear in advertisements only if they have signed the informed consent and photography release waiver.

We expect interested individuals to contact researchers via phone or email. An initial contact will be made through phone and/or email to interested individuals. Potential participants will be given a full description of the study (study-related procedures, risk, benefits) and time to ask questions. If individuals appear to meet inclusion/exclusion criteria and are still interested in volunteering for this study, the first study visit will be scheduled.

b) Screening visit and Baseline testing (1-2 hours)

During the Screening Visit, participants will be offered the informed consent and, if signed, will undergo medical screening, anthropometrics, vitals, fasting blood draw and urine sampling, and cardiovascular assessments.

- Informed consent. The informed consent will be explained to participants by study staff. Once procedures have been explained and volunteers have had the opportunity to ask questions, they will have the option to sign the informed consent.
- Medical screening. All participants will complete a PAR-Q, medical history questionnaire, and medication inventory.

- Anthropometrics. Body height, weight, and body composition by skin fold thickness will be assessed. Body fat percent will be measured by 3-site skinfold thickness the arm, hip and thigh for women and chest, abdominal, and thigh for men.
- Blood sampling. Participants will have a fasting (>10 hours) blood drawn for hematocrit and hemoglobin, electrolyte panel, and osmolality determination.
- Urine sampling. Participants will give a fasting urine sample for determination of osmolality, specific gravity, and electrolytes.
- Resting vitals. Heart rate and blood pressure will be assessed.
- Cardiovascular assessments. Pulse wave analysis (PWV), brachial artery flow mediated dilation (BAFMD) and heart rate variability (HRV) will be assessed in the supine position after 30 minutes rest. PWV, BAFMD and HRV will be used to determine arterial stiffness, vascular function, and sympathovagal balance determination, respectively.

c) Tracking periods (3-5 days)

Two tracking periods will last 3-5 days and will involve recording normal food and fluid intake and recording perceptions of thirst, urine color and sleep quality. The 2 tracking periods will happen immediately prior to the 3 day intervention periods as shown in **Figure 1**. The tracking periods will serve as the washout periods between the 2 interventions and exercise testing sessions.

- Food and Fluid Intake. Participants will record daily food and fluid intake on an app.
- Questionnaires. Perceived thirst and urine color will be recorded after each voiding of the bladder using a categorical scale (1 not thirst at all, 10 very, very thirsty) and color scale, respectively. A sleep diary will also be provided and completed each morning.

d) Interventional period (3 days)

The Intervention Periods will last for 3 days immediately prior to the exercise testing sessions as shown in **Figure 1**. During the intervention periods, participants will alter their hydration status following the described procedures below:

- Euhydrated Trial:** During the euhydrated trial, participants will be asked to drink adequate fluids for 3-days prior to the exercise test in order to promote proper hydration. Participants will be encouraged to drink fluids to attain a urine color indicative of a hydrated state. The U.S. military currently uses this method to assure proper hydration in troops and has been extensively researched (4). If a urine color suggestive of a hypohydrated state is found, participants will be instructed to drink additional fluids. During the 3 day intervention period, participants will be encouraged not to exercise strenuously and to avoid alcohol and prolonged outdoor activities. However, given the effect of caffeine withdrawal on cardiovascular parameters, and the lack of data to support that caffeine promotes dehydration, normal intake of caffeinated beverages will be allowed.
- Hypohydrated Trial:** For the hypohydrated trial, participants will dehydrate over a period of 3-days by restricting fluid intake to achieve a given thirst level (perceived

thirst of 7 out of 10). If a participant's thirst exceeds a rating of 7, they will be instructed to drink just enough fluids to alleviate the sensation of thirst. Past research has shown that relying on thirst to gauge the amount of fluid needed to restore adequate hydration only results in ~70% of the fluid replaced that is necessary(9, 10). In addition, participant's fluid intake during meals will be restricted to 1 cup of fluid and complete fluid restriction will be required the night before the exercise test. We expect this method of dehydration to produce ~3 to 5% of body weight over the 3 day period. A similar restriction on strenuous exercise, alcohol and outdoor activities to the euhydrated trial will be encouraged and normal caffeine intake will be allowed.

During the intervention periods participants will be asked to record food and fluid intake and record their sleep quality, perceived thirst and urine color as in the tracking period. At the start of the intervention period, participants will enter the lab and give a urine sample.

- Food and Fluid intake. Participants will record daily food and fluid intake on an app.
- Questionnaires. Perceived thirst and urine color will be recorded after each voiding of the bladder using a categorical scale (1 not thirsty at all, 10 very, very thirsty) and color scale, respectively. A sleep diary will also be provided and completed each morning.
- Urine sampling. Participants will give a urine sample for determination of electrolyte concentrations, osmolality, and specific gravity.
- Blood sampling. Participants will have a fasting (>10 hours) blood drawn for hematocrit and hemoglobin, electrolyte panel, and osmolality determination.
- Wearable devices. Participants will wear a Zephyr Bioharness for the duration of the intervention period. This device will continuously detect heart rate and HRV, breathing rate, calculated core temperature, and hydration status.

e) Exercise testing (1-2 hours)

The exercise tests will be conducted after the 4 day tracking period and 3 day intervention period and, therefore, will be separated by at least 1 week. As outlined in the intervention section above, participants will be required to control their fluid intake and physical activity for the 3 days prior to the exercise tests. Each exercise test will consist of a 30-minute steady-state bout of exercise on a cycle ergometer in a heated environment (~30°C, ~50%RH), and the following assessments performed. No fluids will be allowed during the exercise tests.

- Steady-state exercise. The steady-state exercise bout will consist of 30 minutes of exercise on a cycle ergometer.
- Body weight. Body weight will be measured before and after the steady-state exercise bout to determine overall fluid balance and dehydration level (change in body weight). Body weight will be measured after urinating and toweling dry.
- Resting vitals. Heart rate and blood pressure will be assessed before, during and after exercise.

- Blood sampling. Prior to the steady-state exercise bout, a Teflon indwelling catheter will be inserted into an antecubital vein for blood sampling. Ten milliliters of blood will be drawn before and after the steady-state exercise bout.
- Urine sampling. Urine will be collected before and after the steady-state exercise bout.
- Body temperature. Rectal and skin temperatures will be continually monitored throughout the entire exercise test. The exercise test will be discontinued if a participant's rectal temperature exceeds 40°C or the participant is symptomatic for heat exhaustion.
- Wearable devices. Participants will wear a Zephyr Bioharness for the duration of the exercise test. This device will continuously detect heart rate and HRV, breathing rate, calculated core temperature, and hydration status.
- Skin blood flow. Skin blood flow will be measured during exercise using laser Doppler flow on the forearm. The laser Doppler probe will be affixed to the flexor aspects of the forearm using adhesive and surgical tape.
- Local sweat rate. Sweat rates of the forearm and upper back will be measured during exercise using ventilated capsules secured with surgical adhesive.
- Sweat sampling. Samples of sweat from the upper back, chest, and forearm during the entire steady-state exercise bout. The sweat samples will be used to determine the average sweat electrolyte losses.
- Substrate metabolism. Participant's caloric expenditure rate and substrate utilization will be monitored for the duration of the steady-state exercise bout using a metabolic cart.
- Other measurements. Ratings of perceived exertion, thirst (visual analogue scale), and psychological affective responses (feeling and felt arousal) will be assessed before the steady-state exercise bout, every 10 minutes during steady-state exercise, and immediately after the exercise bout.

VIII) Statistical Analysis

Statistics will be performed in JMP statistical software (SAS Institute Inc., Cary, NC). Data analysis will generally follow CONSORT recommendations using General Linear Models and one- and two-way repeated measures analysis of variance (ANOVA) co-varied as needed depending on normality distributions of cohort data at baseline. Significant effects will be further evaluated using Tukey's HSD post-hoc analyses where appropriate. Data will be reported as mean±SD and differences declared at $P<0.05$.

IX) Expected Outcomes

We hypothesize that cardiovascular function and sweat responsiveness will be greater in the euhydrated state compared with the hypohydrated state during exercise in a heated environment due to less cardiovascular strain and heat stress during exercise. We expect that the euhydrated state will allow for a lower heart rate and greater HRV, skin blood flow, sweat responsiveness, and temperature regulation during exercise compared with the hypohydrated state. We expect resting cardiovascular profiles evaluated by PWV, BAFMD and HRV will influence cardiovascular function and sweat responsiveness more after chronic, progressive

dehydration. Individuals with lower cardiovascular function may have impaired ability to make bodily adjustments necessary during exercise in heated environments. Lastly, hydration status tracked by dietary and fluid intake logs will be indicative of the intervention given. We expect sleep quality to decay in a dehydrated state.

Table 1. Schedule of Assessment

	Screening Visit	Tracking Period	Intervention Period	Exercise Testing
	Visit 1			Visits 2 and 3
Procedure (Time)	Day 0	Day 0 to 3 and 7 to 10	Day 4 to 6 and 11 to 13	Day 7 and 14
Informed Consent	X			
PARQ and Medical History	X			
Medication Inventory	X			
Height	X			X
Weight	X			
Body Composition	X			X
Vital Signs	X			X
Wearable Devices		X	X	X
Cardiovascular Measures	X			
Questionnaires	X	X	X	X
Food and Fluid Record		X	X	
Randomization	X			
Altered Fluid Intake Intervention			X	
Exercise Test (30 min, moderate)				X
Rectal and Skin Temperatures				X
Local Sweat Rates				X
Skin Blood Flow				X
Blood Samples	X		X	X
Urine Samples	X		X	X
Sweat Samples				X
Substrate Metabolism				X

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VITA

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