The Role of Obesity on Neurovascular Responses to Exercise

BY

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LIST OF ABBREVIATIONS

ADMA   asymmetric dimethylarginine

AIx   Augmentation index

AMP   Adenosine monophosphate

AP   Augmentation pressure

ATP   Adenosine triphosphate

AUC   Area under the curve

bDBP   Brachial diastolic blood pressure

bMAP   Brachial mean arterial pressure

BMI   Body mass index

BP   Blood pressure

bSBP   Brachial systolic blood pressure

cDBP   Central diastolic blood pressure

CFTR   Cystic fibrosis transmembrane conductance regulator

CI   Cardiac index

cMAP   Central mean arterial pressure

CO   Cardiac output

cSBP   Central systolic blood pressure

CVD   Cardiovascular Diseases

CVLM   Caudal ventrolateral medulla

DBP   Diastolic blood pressure

DEXA   Dual-energy x-ray absorptiometry

EDHF   Endothelium-derived hyperpolarizing factors

eNOS	Endothelial nitric oxide synthase
ESP	End systolic pressure
EX	Exercise
FBF	Forearm blood flow
FMD	Flow-mediated dilation
FVC	Forearm vascular conductance
GABA	Gaba-aminobutyric acid
K_{ATP}	ATP-sensitive potassium channels
K_{IR}	Inwardly rectifying potassium channels
H_1	Histaminergic 1 receptors
H_2	Histaminergic 2 receptors
HDL	High-density lipoprotein cholesterol
HR	Heart rate
LBF	Leg blood flow
LBNP	Lower body negative pressure
LDL	Low-density lipoprotein cholesterol
L-NAME	N^G-nitro-L-arginine methyl ester
L-NMMA	L-N^G-monomethyl-L-arginine
LV	Left ventricular
LVC	Leg vascular conductance
MAP	Mean arterial pressure
MBV	Mean blood velocity
MetS	Metabolic syndrome

MSNA	Muscle sympathetic nerve activity
MVC	Maximal voluntary contraction
NA	Nucleus ambiguus
Na+/K+-ATPase	Sodium-potassium ATPase pumps
NE	Norepinephrine
nFBF	Forearm blood flow normalized to lean forearm mass
nFVC	Forearm vascular conductance normalized to lean forearm mass
nLBF	Leg blood flow normalized to lean leg mass
nLVC	Leg vascular conductance normalized to lean leg mass
nNOS	Neural isoform of nitric oxide synthase
NO	Nitric oxide
NTS	Nucleus tractus solitarious
Pb	Forward wave pressure
Pb-T	Time to wave reflection
PEH	Post-exercise hypotension
Pf	Reflected wave pressure
RAAS	Renin-angiotensin-aldosterone system
RER	Respiratory exchange ratio
RI	Reflection index
RM	Repetition maximum
RPE	Ratings of perceived exertion
RVLM	Rostral ventrolateral medulla
SBP	Systolic blood pressure

SEVR	Subendocardial viability ratio
SHR	Spontaneously hypertensive rat
SI	Stroke index
SV	Stroke volume
TPR	Total peripheral resistance
TPRi	Total peripheral resistance index
VEGF	Vascular endothelial growth factors
VO_{2peak}	Peak aerobic capacity

Chapter I: Introduction

A. Introduction to the Problem:

In the U.S., more than one-third of adults are obese [1], and the estimated annual medical cost of obesity is nearly $150 billion [2]. Obesity escalates cardiovascular disease risks [3, 4], which are associated with increased muscle sympathetic nerve activity (MSNA), vascular resistance, and blood pressure (BP) with concomitant reductions in skeletal muscle blood flow [5-7]. Although exercise can restore vascular function and decrease cardiovascular risks [8], the role of obesity on neurovascular responses to exercise remains poorly understood. Mechanisms driving attenuated blood flow associated with obesity are of particular interest. Important questions arise, such as does attenuated blood flow, persist with exercise, or can exercise rescue some or all of the involved responses?

During acute exercise in lean individuals, there is increased vascular conductance (e.g. blood flow normalized to BP) in the exercising muscles as a result of the interaction between locally released vasoactive substances and attenuation of sympathetically-mediated vasoconstriction [9, 10]. This phenomenon, termed "functional sympatholysis," is critical, as blood flow is closely matched to meet the metabolic demands of the contracting muscles while still maintaining BP [9, 11]. In the recovery period following acute exercise, increased vascular conductance continues to persist, resulting in post-exercise hypotension (PEH) that may last several hours. This response plays a role in long-term BP normalization following chronic exercise [12-14]. In obese populations, it is likely that functional sympatholysis may be impaired due to greater sympathetic vasoconstriction [15, 16], and reduced local vasodilatory capacity of both small and large arteries [17-19]. This may result in insufficient blood flow to exercising muscles or alter its neurovascular coupling, contributing to an impaired ability to sustain physical

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activity [20, 21], and reduced work capacity in obese adults [22]. The obesity-related sustained

vasoconstriction and reduced vasodilation may negatively impact the positive effects of exercise

on BP in obese adults. Importantly, obesity-induced neurovascular dysregulation may increase

stress on the cardiovascular system that, when sustained over years, significantly contributes to

the increased cardiovascular risk seen in obesity.

The impact of obesity on functional sympatholysis has surprisingly not been

systematically addressed. Furthermore, few studies have examined PEH in obesity and have

yielded conflicting results [23, 24]. Therefore, the overall aim of our project was to investigate

whether obese adults would exhibit neurovascular dysregulation in response to acute exercise.

The first specific aim was to determine if functional sympatholysis would differ between obese

and lean adults. We hypothesized that obese adults would have a lesser magnitude of functional

sympatholysis during acute exercise compared with lean adults using lower body negative

pressure with and without handgrip exercise as the excitatory tasks. The second specific aim was

to determine if PEH would differ between obese and lean adults. We hypothesized that acute

exercise-induced PEH (using central and brachial BP measures) would be absent in obese adults,

but would be present in lean adults following 60 min of moderate intensity cycling.

B. Significance and Relevance of the Research

The prevalence of obesity is at an all-time high in the U.S. [1, 25]. Obesity is a major contributor to the increased risk of mortality from stroke and cardiovascular diseases [3, 26, 27]. The deleterious effect of obesity on the cardiovascular system is present even at a young age [28]. The annual medical burden of obesity in the U.S. has risen to approximately 10% of all medical spending, or ~$150 billion/year [2]. To date, our understanding of mechanisms related to obesity-induced cardiovascular complications remains poorly understood. There is evolving evidence that vascular function is altered in obese adults, attributable to enhanced sympathetic nerve activity, enhanced vasoconstriction, and reduced vasodilation [15-18, 28]. Although exercise is a recommended lifestyle behavior to combat obesity [29], obesity-induced vascular dysfunction may impair exercise blood flow to contracting skeletal muscles and the ability to sustain physical activity, consistent with reduced work capacity often observed in obesity [20, 21]. Additionally, impaired vasodilation and increased sympathetic vasoconstriction may disproportionately increase BP during and/or after exercise [30], potentiating the risk of sudden cardiovascular events [31].

This study will increase our understanding of the mechanisms of functional sympatholysis and PEH, which will provide novel insight to the control of blood flow and BP regulation, as well as, exercise intolerance associated with obesity. This contribution is significant because it is an important step in the process of understanding: (a) how exercise and physical activity affect hemodynamic responses in obese adults, (b) why BP is elevated more so with sympathoexcitatory stimuli in obese adults, and (c) whether obesity-induced neurovascular dysregulation is related to sympathetic overactivity, impaired vasodilatory capacity, or both. Such understanding will advance the field and lead to subsequent mechanistic studies, which will

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ultimately improve our knowledge of cardiovascular disease risk in obesity. Specifically, future studies will allow for the identification of potential therapeutic targets via exercise and/or pharmacological interventions, which are tailored for obese populations that are likely to prevent or minimize the deleterious effects of obesity on blood flow and neurovascular function. Importantly, this cross-sectional study will provide preliminary data to develop such mechanistic studies that may ultimately influence custom-tailored exercise training strategies that will improve neurovascular coupling in obese adults.

Chapter II: Literature Review

Obesity and Cardiovascular Disease Risks

Obese individuals are at increased cardiovascular risk [3, 26, 27]. The relative risk of mortality from cardiovascular diseases (CVD) is increased by 29% in women and 34% in men with each 5-unit increment of body mass index (BMI) [32]. Importantly, abdominal obesity appears to be an independent risk factor of incident CVD [33], pointing to the importance of fat distribution on CVD risk. In contrast, individuals with lower levels of visceral adiposity are also at lower risk of CVD [34]. There are numerous adverse consequences of obesity on cardiovascular health [4]. Obesity induces alterations in cardiovascular and metabolic systems that promote progression towards incident CVD [33, 35]. For instance, obese individuals may eventually develop conditions such as dyslipidemia, hypertension, insulin resistance, chronic inflammation, and type 2 diabetes, all of which can directly and indirectly jeopardize their cardiovascular structure and function [33, 35]. This is important to help maintain cardiovascular homeostasis and to sustain physical activity [33, 35].

Of particular interest is the impact of obesity on exercise intolerance and poor cardiorespiratory fitness. Indeed, obese individuals have lower levels of peak aerobic capacity (relative to body weight) compared with lean counterparts [36]. Moreover, obese individuals with low fitness have a 5.7-fold higher risk for developing type 2 diabetes compared with lean individuals with high fitness [36]. Importantly, obese individuals with low fitness have a 3.1 times greater relative risk for all-cause mortality (95% confidence interval, 2.5-3.8 relative risk) compared with lean counterparts [37]. Given that both obesity and cardiorespiratory fitness are both independent predictors of CVD [38, 39], it is imperative to understand potential mechanisms underlying exercise tolerance in obesity.

Indeed, the contribution of vascular dysfunction to exercise tolerance has been identified [40], highlighting the importance of obesity-related changes in peripheral circulation. Obesity can induce adaptations in the small resistance and large elastic arteries that favor increased vasoconstriction and decreased vasodilation [40]. Such maladaptations can negatively impact the patterns of blood flow delivery to organs and tissues, coupled with exaggerated BP [40]. Along this line, microvascular and macrovascular endothelial dysfunction are hallmark consequences of obesity and contribute to peripheral vascular dysregulation and exercise intolerance [41, 42]. Because the peripheral vasculature is an area that is particularly vulnerable to the harmful effects of obesity, it is clinically important to understand the potential mechanisms that contribute to peripheral vascular dysfunction in obesity. It is also important to recognize some of these mechanisms may interact in a downward cycle to induce further insult to the vasculature.

Mechanisms of Vascular Dysfunction in Obesity

Vascular Injury

The healthy endothelium plays a-n important role in modulating vascular tone and maintaining vascular homeostasis through release of several vasoactive substances that can induce vasodilation and vasoconstriction [43, 44]. Nitric oxide (NO) is the most important modulator released from the endothelium that can induce vasodilation, as well as prevent atherosclerotic processes [43, 44]. The endothelium also releases other vasodilators, such as bradykinin, prostacyclin, and endothelium-derived hyperpolarizing factors (EDHF) [43, 44]. Bradykinin can stimulate release of NO and prostacyclin and contribute to an inhibition of platelet aggregation and stimulate fibrinolysis via production of tissue plasminogen activator [43, 44]. Prostacyclin can also assist NO in inhibiting platelet aggregation [43, 44]. EDHF can induce

vascular smooth muscle cell hyperpolarization and subsequent vasodilation, even when NO and prostacyclin are pharmacologically inhibited [45].

In contrast, injury to the endothelium causes the cell to release substances that favor vasoconstriction and promotes pro-thrombotic and pro-atherosclerotic processes (e.g. endothelin, angiotensin, vascular cell adhesion molecule, E-selectin) [43, 44]. The release of these substances initiates several cascades of events that lead to increased endothelial permeability, platelet aggregation, and smooth muscle cell proliferation and migration [43, 44]. Importantly, endothelial dysfunction is the earliest manifestation of vascular dysfunction, due to loss of NO bioavailability and is associated with increased risk of CVD [46].

Indeed, endothelial dysfunction may also be present in the obese population [40]. Obese individuals exhibit blunted vasodilation in response to an infusion of an endothelium-dependent vasodilator, acetylcholine [41]. Conversely, impaired endothelium-dependent vasodilation is reversed following vitamin C infusion, highlighting the role of oxidative stress in obesity-related endothelial injury [41]. Using conventional brachial artery flow-mediated dilation (FMD) to noninvasively assess endothelial function, percent FMD (percentage of change in diameter during maximal dilation from baseline diameter) is reportedly lower in young and healthy obese adults compared with lean counterparts (average age ~30-31 years) [47]. Similarly, common femoral artery FMD has been shown to be impaired in obese adults compared with an age-matched lean group, without evidence of impaired endothelial-independent vasodilation (determined using glyceryl-trinitrate) [48]. This highlights the negative impact of obesity on endothelial function in the leg, which plays a major role in human locomotion and exercise capacity [49]. Importantly, the degree of impaired endothelial-dependent vasodilation in both the brachial [50] and the common femoral artery [48] has also been shown to be inversely related to

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visceral obesity, further substantiating the negative effects obesity and fat mass have on cardiovascular function. In contrast to previous studies [41], other evidence supports preserved endothelium-dependent vasodilation in response to an infusion of acetylcholine in young, otherwise healthy obese adults [51-54]. Furthermore, an inverse correlation between BMI and impaired endothelium-dependent vasodilatory response to acetylcholine was abolished following exclusion of participants who were older than 40 years [54]. Taken together, such discrepant findings suggest that endothelial dysfunction is not a common feature of obesity, but may become apparent with a longer duration of obesity, advancing age, and/or presence of CVD.

In addition to endothelial dysfunction, arterial stiffness is another early sign of vascular dysfunction that can predispose one to an increased CVD risk [55, 56]. In general, arterial stiffness is largely determined by the structural adaption of the vascular wall, which results from a loss of elastin relative to an increased collagen content [57]. Interestingly, recent evidence has demonstrated possible crosstalk between endothelial dysfunction and central arterial stiffening, such that any therapeutic intervention that improves endothelial function by enhancing NO bioavailability also reduces central arterial stiffness [58]. Moreover, systemic infusion of L-N^G-monomethyl-L-arginine (L-NMMA) [59] and N^G-nitro-L-arginine methyl ester (L-NAME) [60], to inhibit endothelial NO synthase (eNOS), has been shown to induce arterial stiffness in healthy normal volunteers. Brachial artery FMD has also been positively correlated with arterial stiffness [61]. These findings are of clinical significance and point directly to NO as being a common regulator involved in both vascular tone and large artery stiffness [62].

Indeed, obese individuals who exhibit endothelial dysfunction also have increased central arterial stiffness [63]. Moreover, increased arterial stiffness has been associated with visceral adiposity, highlighting the role of regional fat distribution as a potential determinant of arterial

stiffness [64]. Compared with lean and overweight individuals, obese individuals have greater large artery stiffness, coupled with elevations in brachial, carotid, and aortic BP [65]. Similarly, using magnetic resonance imaging to determine aortic stiffness, apparently healthy obese individuals have higher aortic stiffness than lean individuals [66], which was positively correlated with leptin concentration [66]. Conversely, obese individuals who underwent a 12-week lifestyle modification program (aerobic exercise and dietary intervention) had reduced arterial stiffness (determined by brachial-ankle pulse wave velocity) and plasma asymmetric dimethylarginine (ADMA), which is an endogenous inhibitor of NO synthase [67]. This suggests increased ADMA production may potentially contribute to obesity-related arterial stiffness. Collectively, these studies have demonstrated the presence of endothelial dysfunction and arterial stiffness in obesity. Importantly, endothelial dysfunction and arterial stiffening may act synergistically to compromise vasodilatory capacity and exercise tolerance [68].

Sympathetic Overactivity

The sympathetic nervous system plays an important role in the regulation of cardiovascular function, and sympathetic overactivity has been implicated in the development of CVD [69, 70]. Indeed, obesity has been associated with elevated sympathetic nerve activity [6, 15, 16]. In obese individuals, sympathetic nerve activity is increased in various organs/tissues to drive thermogenesis to help restore energy balance and stabilize body weight; however, prolonged exposure to sympathetic overactivity may eventually lead to the development of obesity-induced hypertension [71].

For instance, a modest weight gain of 5 kilograms has been demonstrated to increase MSNA and resting systolic BP in lean individuals [15]. There is also a positive correlation

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between plasma norepinephrine (NE, a global marker of sympathetic nerve activity) and BP in obese individuals with borderline hypertension [72]. Conversely, the negative impact of obesity on sympathetic nerve activation and the associated increase in BP has been reversed following caloric restriction and weight loss [72]. Interestingly, using the NE spillover technique, one study reported elevated renal NE spillover in normotensive obese individuals, despite similar levels of whole body NE spillover, compared with lean counterparts [73]. This finding demonstrates that obesity-induced sympathetic overactivity may initially be regionalized and that the sympathetic outflow is not uniform [73]. Along these lines, sympathetic overactivity has been associated with subclinical organ damage in young normotensive obese adults [28].

Sympathoexcitatory tasks are important in uncovering further differences between obese and lean individuals and may yield critical information above baseline/resting comparisons. Obese individuals may exhibit exaggerated sympathetic nerve activity and BP responses to sympathoexcitatory tasks, such as mental stress, cold pressor test, or static handgrip exercise [5, 6]. For example, in obese individuals with borderline hypertension, NE and BP responsiveness to isometric handgrip became less exaggerated following diet-induced weight loss, demonstrating a reduced level of sympathetic nerve activity following caloric restriction [72]. These findings indicate that sympathetic nerve activity may be elevated in obese individuals and may lead to exaggerated pressor response when faced with sympathoexcitatory tasks [5, 6, 72]. Interestingly, there was also an inverse relationship between MSNA and endothelial function in normotensive obese individuals [28]. In obese individuals with metabolic syndrome (MetS), endothelial function is improved following central sympathoinhibition with moxonidine [74], which would indicate an interaction between sympathetic nerve activity, NO bioavailability, and vasodilatory capacity.

Whether elevated sympathetic nerve activity may also lead to enhanced sympathetic vasoconstrictor activity in obese individuals remains equivocal. One study demonstrated that despite enhanced MSNA compared with lean counterparts, obese adults exhibited similar magnitude of forearm vasodilation in response to α-adrenergic receptor blockade by phentolamine [75]. In contrast, diet-induced obesity has been demonstrated to enhance sympathetic vasoconstrictor activity in rat mesenteric resistance arteries [76]. In these mesenteric arteries, the enhanced sympathetic vasoconstrictor activity have been shown to result from (1) increased sympathetic perivascular nerve density within the resistance arteries, (2) enhanced synaptic release of and postsynaptic sensitivity/responsiveness to adrenergic and purinergic vasoconstrictors, and (3) reduced postsynaptic sensitivity to vasodilator neurotransmitters [76, 77]. Collectively, these studies suggest obesity may induce alterations at the level of resistance arteries, resulting in increased sympathetic activity and vasoconstrictor responsiveness to sympathetic activation [76, 77].

There are several mechanisms that may explain enhanced sympathetic nerve activity in obesity. Adiposity is an important modulator of sympathetic nerve activity, and visceral adiposity is positively correlated with elevated MSNA [16]. Moreover, any increase in body weight or percent body fat is positively correlated with the associated increase in MSNA [15]. Conversely, elevated MSNA is not related to subcutaneous adiposity [78], highlighting the role of fat distribution on obesity-induced sympathetic activation. Obesity-related metabolic conditions such as insulin resistance [79], type 2 diabetes [80], hyperleptinemia [81], inflammatory cytokines [82], and dyslipidemia [83] have also been shown as independent contributors to enhanced sympathetic nerve activity. An overactivation of the renin-angiotensin-aldosterone system (RAAS) also contributes to the obesity-induced sympathetic activation in a

positive feedback loop fashion [84]. In obese individuals with obstructive sleep apnea, chronic

peripheral chemoreceptor stimulation induced by hypopneic-apneic episodes has been

demonstrated to markedly increase sympathetic nerve activity [85, 86]. Chronic stress and

associated activation of the hypothalamic-pituitary-adrenal axis levels have also been shown to

augment sympathetic nerve activity in obese individuals [87]. Finally, the arterial baroreflex,

which exerts a profound sympathoinhibitory effect on BP elevations [88], may be impaired in

obese individuals and thus may contribute to obesity-related sympathetic overactivity [89]. Other

neural mechanisms that have been implicated in obesity-related sympathetic overactivity include

increased activity of sympathetic neurons within the rostral ventrolateral medulla in the brain

(RVLM) [90] and impaired metaboreflex (a component of exercise pressor reflex) [91]. Taken

together, obesity has adverse effects on cardiovascular and metabolic regulations, which may

contribute to the enhancement of sympathetic nerve activity and the associated impairment of

vascular function.

Renin-Angiotensin-Aldosterone System

The RAAS plays a crucial role in maintaining water and electrolyte balance, as well as

BP [92]. The RAAS can be found in various tissues, including brain, pancreas, kidneys, heart,

and adipose tissue, which allows it to exert both systemic and local effects [93]. The RAAS

components include angiotensin, renin, angiotensin-converting enzyme, and angiotensin

receptors (both 1 and 2) [93]. Angiotensin is one of the most potent vasoactive substances

produced by the RAAS, which can ultimately induce vasoconstriction and reduce blood flow

[93]. While angiotensin is predominantly produced in the liver in lean individuals, the adipose

tissue appears to be another important contributor to the increasing pool of angiotensin in obese

individuals [92, 94]. In addition to the ability to produce angiotensin via the renin-angiotensin pathway, adipose tissue has been shown to make angiotensin via cathepsins and chymase [95]. In fact, both human [96] and animal [97] studies have shown that obesity induces both the systemic and adipose tissue overactivation of the RAAS, which can be reserved by lifestyle interventions, such as weight loss [96]. Additionally, inflammation of adipose tissue (both visceral and perivascular) appears to be the likely cause of the RAAS activation in obese individuals [98].

Recently, the contribution of adipose tissue RAAS to obesity-induced vascular dysregulation has been extensively evaluated using a high-fat diet in an angiotensin knockout murine model [99]. Despite equivalent increases in weight gain and fat mass with a high-fat diet, only the wild-type mice exhibited a concomitant increase in BP, compared with mice lacking the angiotensinogen gene in the fat cells [99]. Furthermore, while there was no group difference in plasma angiotensinogen protein concentration, the wild-type mice had increased plasma angiotensin II concentrations compared with the knockout mice [99]. These findings demonstrate that the local adipose tissue RAAS may be involved in the obesity-induced hypertension via an increased production of angiotensin II [99]. Most importantly, increased circulating angiotensin II concentrations may induce arterial stiffness, endothelial dysfunction, and vascular insulin resistance in obesity by inhibiting metabolic insulin signaling and increasing the production of vasoconstrictors [100-102].

In addition, there is evidence to suggest that obese individuals may have an increase in aldosterone concentrations [103], which is an important factor implicated in endothelial dysfunction and augmented vascular smooth muscle reactivity [104]. Specifically, aldosterone can activate nicotinamide adenine dinucleotide phosphate-oxidase, which subsequently leads to increased production of reactive oxygen species and reduced NO bioavailability [105].

Conversely, deletion of mineralocorticoid receptors (also known as aldosterone receptors) from the endothelial cells has been shown to improve endothelial function in obese mice [106]. Similarly, when mineralocorticoid receptors have been removed from the vascular smooth cells of mice, there is a reduction in the magnitude of vascular smooth muscle cell contraction and BP elevation induced by an angiotensin II infusion [107]. Taken together, these findings have demonstrated a potential crosstalk between aldosterone and angiotensin II, both of which can induce vascular damage in obese individuals and increase the CVD risk.

Inflammation

Chronic inflammation is a hallmark feature associated with obesity and contributes to the development of vascular dysfunction and increased CVD risk [108]. In obese individuals, adipocyte expansion is a common process that occurs as a result of excess nutrient intake [109]. As the adipocytes hypertrophy, there is a concomitant increase in macrophage infiltration into the adipocytes, leading to local inflammation [109, 110]. With obesity, there is an increased production of pro-inflammatory cytokines, such as tumor necrosis factor-alpha, C- reactive protein, and interleukin-6 [108]. Visceral adipose tissue is said to secrete more pro-inflammatory cytokines than subcutaneous adipose tissue, and increased visceral adiposity is a common feature of obesity [111]. In obese individuals, persistent stress within the adipose tissue due to excess food consumption can lead to adipocyte apoptosis, thus promoting further macrophage infiltration, in an attempt to eliminate the apoptotic adipocytes, and greater release of pro-inflammatory cytokines [112]. The release of these pro-inflammatory cytokines into the circulation allows these cytokines eventually to reach the liver, stimulate hepatic inflammation, and contribute to further systemic inflammation [110].

In the vasculature, pro-inflammatory cytokines can activate kinases that phosphorylate serine residues of insulin receptor substrates 1 and 2 [108]. When these serine residues are phosphorylated, a downstream insulin signaling pathway becomes suppressed, and a growth factor signaling pathway becomes promoted, ultimately promoting the initiation and pathogenesis of vascular insulin resistance [108]. In obesity, pro-inflammatory cytokines and the associated oxidative stress are involved in the inhibition of eNOS, which leads to the reduced NO bioavailability and impaired endothelial function [40]. Pro-inflammatory cytokines can also induce increases in vascular permeability, endothelial cell adhesion molecules, and thrombus formation [40]. Furthermore, pro-inflammatory cytokines have been associated with impaired capillary recruitment and diminished vasodilatory capacity within the small resistance arteries [40]. Taken together, obese individuals may exhibit chronic low grade inflammation induced by excess adiposity, which may lead to an impairment of vascular function, seen as reduced vasodilation and increased vasoconstriction.

Collectively, it can be concluded from the information explained above that excess adiposity may induce vascular damage via several mechanisms including impaired endothelial function, sympathetic overactivation, RAAS, and inflammation. These maladaptations may lead to impaired vasodilatory capacity and increased vasoconstriction in both small resistance and large elastic arteries, which may contribute to impaired blood flow and BP regulation in response to physical activity. Importantly, impaired peripheral vascular function may explain why obese individuals have poor exercise tolerance.

Neural and Local Vascular Control for the Cardiovascular and Hemodynamic Responses to Exercise

Appropriate adjustments of the cardiovascular system are essential for the sustainment of physical activity and exercise [113]. Such adjustments, via neural and local vascular mechanisms, ensure a sufficient delivery of blood flow enriched with oxygen and nutrients to, and the removal of metabolic byproducts from the contracting skeletal muscles [113]. In fact, during exercise, skeletal muscle blood flow increases in an intensity-dependent manner, from resting values of 5-10 mL/min (per 100 grams of tissue) to 250-400 mL/min (per 100 grams of tissue), with blood flow being greater in high oxidative tissue than low oxidative tissue [113, 114]. The increase in skeletal muscle blood flow during dynamic exercise is dependent on the amount of muscle mass involved and is a result of the interaction between vasodilation and vasoconstriction, so that skeletal muscle blood flow is increased without comprising mean arterial pressure (MAP) [113, 115, 116]. This concept has been demonstrated by studies that examine the influence of muscle mass on skeletal blood flow and blood pressure adjustments during dynamic exercise [115-117].

For instance, when strenuous arm exercise is superimposed upon leg exercise, leg blood flow and oxygen uptake become reduced, whereas MAP is maintained. [116], suggesting an increase in vasoconstriction and vascular resistance in the exercising legs to prevent a fall in MAP, while facilitating blood flow redistribution. While peak values of cardiac output and oxygen uptake are achieved during combined arm and leg exercise, skeletal muscle blood flow is reduced to both upper and lower extremities due to increased sympathetic vasoconstriction during high intensity exercise [117]. This is necessary to avoid a dramatic drop in BP due to high levels of vasodilation [117]. Thus, it is clear that during exercise, it is important to have a

balance between sympathetic vasoconstriction and vasodilation so that vascular conductance, skeletal muscle blood flow, and BP are adjusted to match oxygen delivery to the increased metabolic demand of the exercising skeletal muscles [117, 118]. Conversely, an insufficient matching of blood flow delivery and demand from the skeletal muscle contributes to exercise intolerance, coupled with an exaggerated pressor response, and may induce incident cardiovascular events, especially in populations at high risk [9].

Neural Mechanisms of Circulation

The autonomic nervous system is comprised of the sympathetic and parasympathetic arms, both of which play a key role in matching the cardiovascular and hemodynamic adjustments during exercise to meet the increased metabolic demand of the skeletal muscles [119]. Such adjustments include a tonic reduction in the parasympathetic nerve activity that leads to increased heart rate (HR), cardiac contractility, stroke volume, and cardiac output [119]. A simultaneous increase in sympathetic nerve activity to the heart contributes to increased HR and cardiac contractility, whereas an increase in sympathetically mediated vasoconstriction in the non-exercising muscles and visceral organs, such as splanchnic blood flow, contributes to the redistribution of blood flow towards the active skeletal muscles in an exercise intensity-dependent manner [119]. The key neural mechanisms involved in the cardiovascular and hemodynamic adjustments to exercise include central command, exercise pressor reflex, arterial baroreflex, and cardiopulmonary baroreflex [119, 120].

Central Command

Initially termed as "cortical irradiation" and later as "central command [121, 122]," this concept describes the descending signals, a feed forward mechanism, originating from the higher brain centers (the cerebral cortex and/or subcortical nuclei) that activates the cardiovascular responses to exercise [120], with limited evidence suggesting its involvement in feedback mechanisms as well [123, 124]. The role of central command in mediating cardiovascular responses, such as increases in HR, BP, and MSNA to exercise has been demonstrated in several studies using neuromuscular blockade, electrical stimulation, and even hypnosis [120, 125-127]. For example, the influence of central command on the cardiovascular and hemodynamic responses can be studied using an infusion of tubocurare [126]. One study demonstrated that tubocurare enhances the exercise-induced increase in BP, without affecting the increase in heart rate [126]. The results from this study suggest that central command has minimal influence on HR, but that it is responsible for the increase in BP, likely via changes in peripheral vascular resistance, to match the increasing metabolic demand at the onset of exercise [126].

Interestingly, central command can also be activated without concomitant parallel motor activation [124, 127], and the magnitude of cardiovascular responses to exercise can also be altered by changing perceived levels of effort during constant muscular force production [127]. When subjects are instructed to imagine an uphill grade on a constant cycling load, HR and BP become elevated in the same direction as the regional cerebral blood flow, showing simultaneous activation of specific brain regions and cardiovascular responses necessary to meet the perceived metabolic demands [127]. Conversely, an imagined downhill grade reduces regional cerebral blood flow, but does not concomitantly reduce HR or BP, suggesting that cardiovascular

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responses are required to sustain the actual metabolic needs despite decrease in effort sense [127].

While central command modulates HR and BP responses during light exercise intensities, it appears to influence increases in MSNA only during higher exercise intensities [125]. Specifically, neuromuscular blockade with curare augments the increase in MSNA during isometric handgrip exercise only at 75% of maximal voluntary contraction (MVC) [125]. However, MSNA does not increase when exercising at 25% or 50% of MVC and is not affected by the neuromuscular blockade, suggesting that central command influences MSNA responses only at high exercise intensity, but not at mild or moderate exercise intensity [125].

With regards to obesity, evidence from animal research has demonstrated an altered regulation of sympathetic outflow stemming from the brain region [90, 128, 129]. Specifically, augmented activity of the sympathetic neurons within the RVLM and elevations in resting BP have been reported in obese Sprague-Dawley rats [90], obese Zucker rats [129], and in Long Evan rats [128]. In these studies, the augmented activity of the sympathetic neurons within the RVLM were attributed to (1) reduced gaba-aminobutyric acid (GABA)-mediated inhibition of the RVLM [90, 129], (2) increased angiotensin receptor responsiveness [129], (3) enhanced leptin receptor activation [128], and (4) increased responsiveness to glutamine-induced activation of the RVLM [130]. Taken together, animal studies support the notion of exaggerated sympathetic outflow driven by a central mechanism in obesity; however, little is known if obesity alters influences from central command at rest and during exercise in humans, but data support increased MSNA and BP in human during perturbations, such as mental stress [5].

Exercise Pressor Reflex

The exercise pressor reflex is a feedback mechanism that also modulates cardiovascular adjustments to exercise [131, 132]. Skeletal muscle afferents, which contain both mechanically and metabolically sensitive fibers, provide a feedback mechanism to the higher brain in response to mechanical (deformation induced by pressure or stretch) and metabolic (metabolites released within the contracting muscles) stimuli [132]. The mechanically sensitive fibers are comprised of thinly myelinated group III afferent neurons, whereas the metabolically sensitive fibers contain unmyelinated group IV neurons [132]. These group III afferents are thought to respond at the immediate onset of muscle contraction in response to stretch, with the first impulse of discharge being as quick as 200 milliseconds [133]. The rate of discharge from these group III afferents has been shown to increase in response to tetanic contractions as peak tension develops and to decrease as the contracting muscles start to fatigue [133]. Additionally, their rate of discharge can be synchronized with the changing rate and muscular contraction [134]. Interestingly, group III afferents have also been shown to respond to metabolic byproducts from muscular contractions including bradykinin, potassium, arachidonic acid, a thromboxane A_2 mimetic, lactic acid, histamine, and serotonin [132], suggesting that these afferents might possess the ability to respond to both chemical and mechanical stimuli.

Unlike the group III afferents, the group IV afferents do not respond immediately at the onset of muscular contractions, as they require a sufficient amount of time for metabolites to be produced and to accumulate [132], with latencies of approximately 5-30 seconds [133]. These group IV afferents are said to be more responsive to a static muscular contraction only when muscles are under perfused, but are less responsive when muscles are normally perfused [135]. The group IV afferents are stimulated by similar metabolic factors as the group III afferents, and

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these factors include, but are not limited to, bradykinin, potassium, lactic acid, arachidonic acid, prostaglandin E_2, a thromboxane A2 mimetic, histamine, and serotonin [132]. Evidence also suggests that adenosine may stimulate the group IV afferents [136]; however, this theory has been challenged by work by Maclean et al. [137] who demonstrated that an infusion of adenosine into a femoral artery does not further increase MSNA response when it is trapped in the leg by cuff occlusion. However, when the cuff is released, MSNA becomes elevated, suggesting that adenosine acts on central mechanism, rather than a peripheral mechanism to cause an increase in MSNA [137]. In addition to sensitivity to chemical stimuli, the group IV afferents may also be slightly sensitive to mechanical stress, as it has been shown to fire one or two impulses at the onset of muscular contraction [134]. Thus, it can be concluded that both group III and group IV skeletal muscle afferents are responsive to both mechanical and chemical stimuli and contribute to the exercise pressor reflex.

The important role of the exercise pressor reflex in cardiovascular adjustments to exercise has been demonstrated by an early work from Alam and Smirk [131]. In this seminal study, circulatory arrest was induced using an arm cuff, which were inflated above systole immediately before cessation of muscular contractions [131]. As a result, BP measured in an arm post-exercise remains elevated, whereas BP falls immediately following cuff release [131]. The work from this seminal study has led to several subsequent studies that examine how components of exercise pressor reflex, together, or in isolation, influence the cardiovascular adjustments to exercise [138-141]. When the influence from the exercise pressor reflex is minimized using epidural anesthesia (Lidocaine or Bupivacaine injected at L3-L4), the increases in HR and BP during electrically-stimulated muscular contraction become attenuated [138]. This demonstrates that this feedback response originates from the contracting muscles and is necessary for the

cardiovascular adjustments to dynamic exercise [138]. It should be noted, however, that the use

of lidocaine may also attenuate the discharge of motor nerves, resulting in increased central

command to maintain the same force as before drug infusion [132]. Regardless of involvement

of central command, the cardiovascular responses are still substantially attenuated with lidocaine

injection [138].

Interestingly, one study examined the role of the metaboreflex on cardiovascular

responses to exercise via arterial cuff occlusion before cessation of static contractions of the

quadriceps femoris in combination with blockade of central command [139]. Reportedly, HR and

BP, which normally decrease after exercise, become substantially elevated with arterial cuff

occlusion, an effect that is seen both with and without neuromuscular blockade [139]. This

suggests that the exercise pressor reflex (specifically the metabosensitive component), like

central command, is of importance and may be redundant in nature [139]. Moreover, the use of

arterial cuff occlusion to trap metabolites within the previously contracting muscles has been

shown to maintain approximately ~85% of the exercise-induced increases in BP and MSNA, and

this response is intensity-dependent [142]. In contrast to the results from the metaboreflex

studies, one study performed either sustained passive stretch of the triceps surae (via dorsiflexion

of foot) for one minute on subjects in an attempt to isolate the mechanoreflex contribution to HR

and BP responses [140]. Passive stretch increased HRbut not BP, suggesting that these

mechanosensitive afferents may contribute only to the initial increase in HR at the onset of

muscle contraction without affecting BP [140]. When passive stretch is performed during

parasympathetic blockade using glycopyrrolate, cardiac acceleration becomes blunted,

suggesting that passive stretch may activate a specific group of mechanosensitive afferents that

selectively inhibit cardiac parasympathetic nerve activity [141]. Despite these findings, it is clear

that both components of the exercise pressor reflex participate in the cardiovascular adjustments in response to exercise.

With regards to obesity, few studies have investigated the role of exercise pressor reflex in modulating cardiovascular adjustments to perturbations [6, 91, 143, 144]. In normotensive obese women, involuntary contractions induced by whole-body vibration have been shown to elicit exaggerated BP response, suggesting an augmented mechanoreflex [143]. Another study demonstrated a smaller increase in MSNA during post-exercise ischemia in normotensive obese women compared with lean women, suggesting diminished muscle metaboreflex [6]. However, the diminished muscle metaboreflex was improved following weight loss induced by caloric restriction with and without exercise training, with obese women demonstrating similar increases in MSNA during post-exercise ischemia compared with lean women [91]. In contrast, young obese adults did not exhibit any alteration in MSNA or BP responses to mechanoreflex (static handgrip) and metaboreflex activation (post-exercise ischemia), despite the presence of MetS [144]. As such, these discrepant findings make it difficult to conclude that obesity *per se* alters exercise pressor reflex and thus warrant additional investigations into this topic.

Arterial Baroreflex

Located in the carotid arteries and aorta, the arterial baroreceptors play a key role in a negative feedback mechanism by evoking rapid cardiovascular adjustments in response to beat-to-beat changes in BP, by alternating sympathetic nerve activity and vascular tone at rest and during exercise (Figure 1) [145-148]. Impulses from the carotid arterial baroreceptors are transmitted to the higher brain via a branch of glossopharyngeal nerve (also called the Hering nerve), whereas impulses from the aortic baroreceptors travel via the vagus nerves and converge

within the nucleus tractus solitarious (NTS) of the medulla oblongata [145-148]. Within the NTS, these neural signals will be integrated and translated, and result in alterations of sympathetic and parasympathetic nerve activation to the target organs (i.e. heart and blood vessels), in order to ensure appropriate cardiovascular and hemodynamic adjustments to exercise [145].

During exercise, arterial baroreceptors regulate BP by resetting in order to operate at a higher prevailing BP during exercise in an intensity-dependent manner [145-148]. Inputs from central command, the exercise pressor reflex, and the cardiopulmonary reflex influence the exercise-induced resetting of arterial baroreflex [145-148]. In humans, the arterial baroreflex can be studied using indirect techniques: the Oxford technique and the variable pressure neck collar [145]. The Oxford technique stimulates the carotid and aortic baroreceptors by arterial infusion of pharmacologic agents, phenylephrine and nitroprusside, to raise and lower BP, respectively [145]. In contrast, the variable pressure neck collar technique mechanically manipulates the carotid baroreceptors to induce hypotension (via neck pressure) and hypertension (via neck suction) [145, 149]. No matter the technique, a baroreflex stimulus-response curve can be generated and allows for a greater understanding of arterial baroreceptors' function at rest and during stimulation [145, 149]. The commonly studied sigmodal curves are: the carotid-cardiac (carotid baroreflex-HR) and the carotid-vasomotor (carotid baroreflex-mean arterial pressure) reflex function curves, both of which demonstrate HR and mean arterial pressure changes in response to neck pressure and neck suction [145, 149]. Additionally, a diastolic BP and MSNA curve has also been utilized to demonstrate the arterial baroreflex control of MSNA [150].
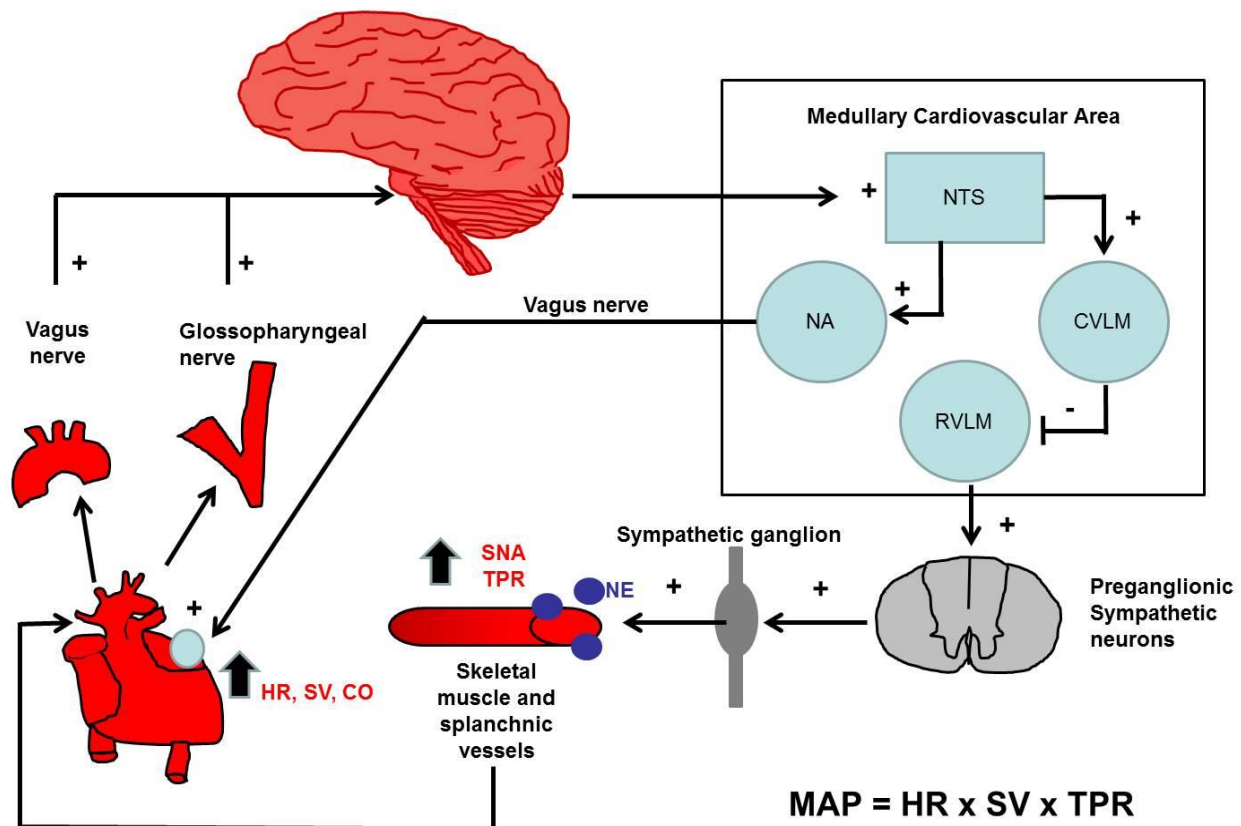
Figure 1. Arterial Baroreflex Control of Blood Pressure. The arterial baroreceptors respond to stretch induced by increased blood pressure. The neural impulses travel from these afferents to provide an excitatory input to the nucleus tractus solitarious (NTS) within the medulla oblongata. Barosensitive NTS neurons stimulate the interneurons in the caudal ventrolateral medulla (CLVM), resulting in an inhibition of sympathoexcitatory neurons within the rostral ventrolateral medulla (RVLM). When the sympathoexcitatory neurons in the RVLM are excited, they induce more release of norepinephrine (NE), increasing sympathetic nerve activity (SNA) and total peripheral resistance (TPR). In addition, barosensitive NTS neurons send neural input directly to a group of vagal preganglionic neurons housed within the ventrolateral portion of the nucleus ambiguus (NA). These vagal preganglionic neurons project to the heart's ganglion neurons and induce changes in heart rate (HR), stroke volume (SV), and cardiac output (CO). The changes in HR, SV, and TPR lead to alterations in mean arterial pressure (MAP) at rest and during perturbations (e.g. exercise). Adapted from Benarroch EE. The arterial baroreflex: functional organization and involvement in neurologic disease. *Neurology.* 71(21): 1733-1738, 2008.

Based on the baroreflex stimulus-response curves (carotid to HR or mean arterial pressure), there is a vertical upward shift and a lateral rightward shift on the response arm to higher operating pressures (Figure 2), essentially allowing the baroreflex to operate functionally at the prevailing exercise-induced increase in BP [145, 151]. In fact, this resetting of both the carotid-cardiac and the cardiac-vasomotor reflex function curves can be present until 100% of peak aerobic capacity, demonstrating that the resetting is intensity-dependent [152]. Additionally, one study investigated how the arterial baroreflex controls MSNA by assessing the relationship between spontaneous variations in MSNA and diastolic blood pressure [150]. From rest to steady-state dynamic exercise (50% of peak aerobic capacity), there is a continuous rightward shift in the arterial baroreflex control of MSNA, which occurs around the exercise-induced increases in BP and MSNA [150]. Moreover, the resetting of the baroreflex function curves occur without a change in sensitivity during dynamic exercise [150, 152] as well as during isometric exercise [153]. Additionally, previous studies have demonstrated that the carotid baroreflex regulates BP primarily by modulating changes in total vascular conductance at rest and during exercise [154]. It should be noted, however, that approximately 25% of the BP changes mediated by the carotid at rest are attributed to changes in cardiac output [154], whereas during exercise, changes in MSNA are mediated by the baroreflex in order to ultimately regulate the total vascular conductance [150]. Collectively, these studies have demonstrated the importance of arterial baroreflex in mediating cardiovascular adjustments via alterations in cardiac output and total vascular conductance at rest and during exercise.

Evidence from animal [155] and human [88] studies have demonstrated that the arterial baroreflex may be altered in obesity. For instance, the range at which the arterial baroreflex can induce changes in sympathetic nerve activity, BP, and HR become significantly depressed in

obese Zucker rats [155]. Nevertheless, the arterial baroreflex maintained the ability to reset upwards and rightwards to operate at a higher prevailing BP during somatic afferent nerve stimulation [155]. In normotensive obese humans, an improved arterial baroreflex control of HR and MSNA in response to changes in BP induced by infusion to phenylephrine and nitroprusside has been demonstrated following weight loss induced by caloric restriction [156]. In this study, weight loss was also accompanied by overall reductions in resting MSNA, BP, and plasma NE concentrations in obese humans [156]. Additionally, aerobic exercise training has also been shown to improve resting baroreflex sensitivity in obese individuals [157]. Taken together, any impairment in arterial baroreflex induced by obesity may be reversible following weight loss and exercise training.
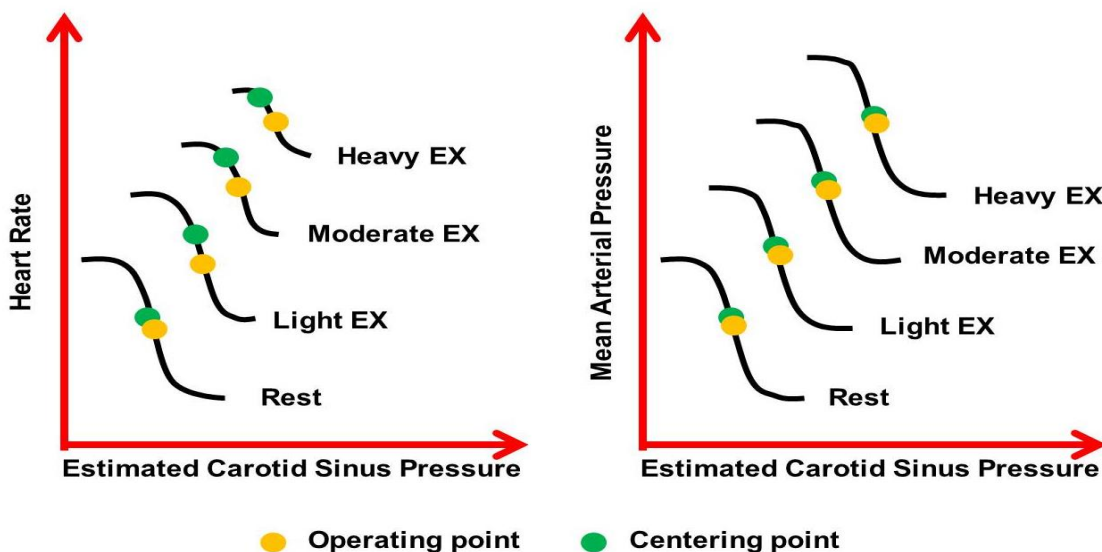
Figure 2. The Resetting of Baroreflex during Exercise. The resetting of the carotid baroreflex-heart rate (left) and the carotid baroreflex-mean arterial pressure (right) curves occurs during exercise (EX) in an intensity-dependent manner without any change in sensitivity (gain). The operating point is the blood pressure point before stimulus. The centering point indicates the point of equal depressor and pressor response to a given blood pressure. Adapted from Fadel PJ *et al*. Human investigation into the arterial and cardiopulmonary baroreflexes during dynamic exercise. *Ex Physiol.* 97(1): 36-50, 2012.

Cardiopulmonary Baroreflex

The cardiopulmonary baroreceptors are located within the walls of heart's atria and ventricles, coronary and pulmonary arteries, and the great veins, and are sensitive to mechanical stretch [120, 158]. This stretch is induced by fluctuations in central blood volume and pressure and can respond to these fluctuations by altering sympathetic nerve activity [120, 158]. Limited evidence suggests that the cardiopulmonary receptors play a pivotal role in the cardiovascular and hemodynamic adjustments via changes in MSNA and BP [120]. Specifically, during an onset of mild-intensity dynamic exercise in an upright position, the cardiopulmonary baroreceptors become activated and exert an inhibitory influence, resulting in a reduction in MSNA compared with pre-exercise value [159]. In this study, the reduction in MSNA is likely the result of increased venous return upon muscle pump activation, which leads to increased central venous volume and pressure, thereby activating the cardiopulmonary baroreceptors and their inhibitory influence [159]. Conversely, when exercise was performed in the supine position, MSNA did not further decrease during exercise, likely because the central blood volume and cardiac filling were already elevated in this position and that the cardiopulmonary baroreceptors were loaded and thus inactivated [159]. Along this line, an unloading of the cardiopulmonary baroreceptors using lower body negative pressure (LBNP, -5 mmHg) has also been shown to remove such inhibition (or inactivate the cardiopulmonary baroreceptors) and reflexively provoke greater increases in the vasoconstrictor and pressure responses to exercise [160, 161].

Evidence suggests that the cardiopulmonary baroreflex resetting is present during exercise (Figure 3), such that it will reset to the new operating point that is associated with changes in cardiac filling volume and pressure during exercise [158]. In addition to influencing the prevailing exercise BP, changes in central blood volume have been shown to contribute to the

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resetting of the arterial baroreflex [162]. For instance, an addition of leg cycling to arm cranking has been shown to lower BP below that of arm cracking alone, causing the carotid baroreflex-vasomotor curve to change its operating point to a lower BP level [163]. The resetting of the carotid baroreflex-vasomotor curve is likely due to increases in central blood volume and venous return (due to muscle pump activation) [163], thus suggesting an interaction between cardiopulmonary baroreflex and arterial baroreflex. Collectively, the cardiopulmonary baroreflex can exert an important modulatory effect on the cardiovascular adjustments to exercise. Conversely, exaggerated sympathetic nerve activity and pressor responses during exercise may occur if the cardiopulmonary baroreflex becomes impaired [164]. This can happen directly via diminished inhibitory neural inputs to the sympathetic control centers and indirectly via its interaction with the arterial baroreflex [164].

Limited evidence is available regarding how obesity may alter the cardiopulmonary baroreflex [165]. In rats, high-fat diet-induced obesity caused subsequent increases in renal sympathetic nerve activity compared with rats fed on a normal diet [165]. During a cardiopulmonary baroreceptor challenge, induced by saline infusion to cause volume expansion, reductions in renal sympathetic nerve activity were only observed in normal rats (-66%), but not in obese rats, suggesting depressed cardiopulmonary baroreflex function [165]. Interestingly, subjecting obese rats to renal denervation improved cardiopulmonary baroreflex responsiveness during volume expansion challenge, indicating that deranged neural signals from the kidneys may contribute to dysregulated cardiopulmonary baroreflex in obese rats [165]. Whether such derangement exists in human obesity remains poorly understood.
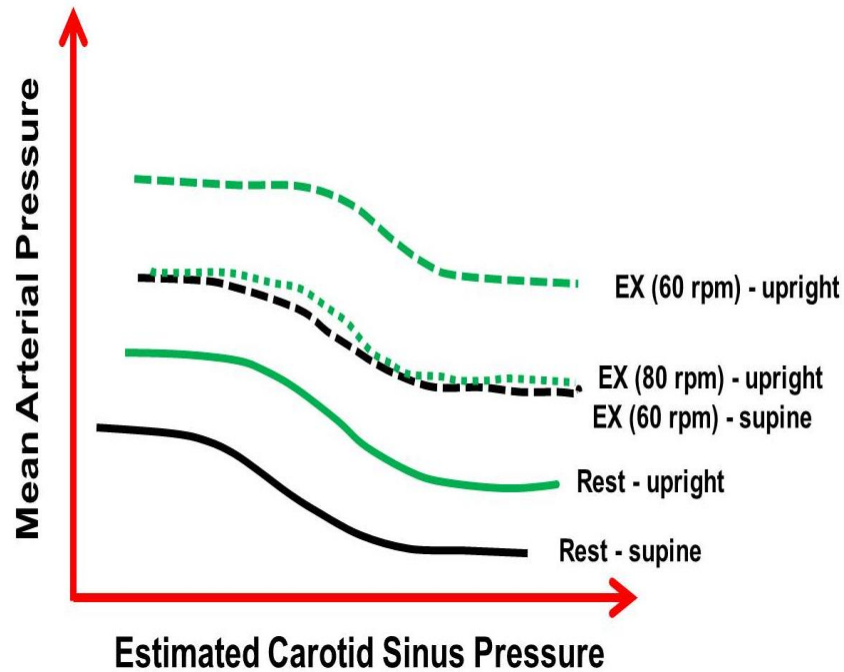
Figure 3. The Influence of the Cardiopulmonary Baroreflex on the Resetting of the Carotid Arterial Baroreflex during Exercise. The resetting of the cardiopulmonary baroreflex is influenced by central blood volume. Central blood volume can be increased by changing from upright to supine position and by increasing pedaling frequency, which enhances muscle pump. This results in a lower magnitude of blood pressure elevation and the carotid baroreflex resetting during cycling exercise. Adapted from Ogoh S *et al*. Cardiopulmonary baroreflex is reset during dynamic exercise. *J Appl Physiol.* 100(1): 51-59, 2006.

Local Vascular Control of Circulation

During dynamic exercise, an increase in sympathetic nerve activity causes the blood vessels in the non-exercising skeletal muscles and the visceral organs to constrict, whereas this sympathetic vasoconstriction is attenuated in the exercising skeletal muscles [113, 119]. The attenuation of the sympathetic vasoconstriction by muscle contraction is a phenomenon termed "functional sympatholysis" [166]. Functional sympatholysis is a critical component allowing for proper delivery of blood flow to the exercising tissues and requires carefully balanced actions of vasodilation and vasoconstriction.

Indeed, the blunting of sympathetic vasoconstriction is not related to the mechanical effects of muscle contraction *per se* [167], but is rather mediated, in part, by locally released vasoactive substances, which contribute to the diminution of the vasoconstrictor responsiveness to α-adrenergic receptor activation [9, 119]. For instance, one study demonstrated that adding forearm isometric exercise to reflexively increase sympathetic vasoconstriction did not alter blood flow delivery to the exercising leg muscles, despite significant increases in MSNA [168]. In humans, the blunting of sympathetic vasoconstriction has been shown to involve both post-junctional α_1- and α_2-adrenergic receptors [169].

Evidence from animal studies suggests that exercise intensity appears to play a role in the activation of α_1/α_2-adrenergic receptors [170-174]. For instance, α_2-adrenergic receptors within the arterioles of rat skeletal muscle are more sensitive to attenuation by metabolites released during moderate intensity exercise, whereas the blunting of α_1-adrenergic receptors tends to occur as exercise intensity increases [170]. Similarly, studies using dogs have demonstrated that α_1-adrenergic receptor responsiveness is attenuated only during high intensity exercise, whereas α_2-adrenergic receptor responsiveness is blunted at mild exercise [173, 174]. To date, it remains

unclear whether exercise intensity also plays a role in the activation of α_1/α_2-adrenergic in humans. Regardless, it is evident that both α_1/α_2-adrenergic receptors are involved in modulating the vascular responses to exercise.

While functional sympatholysis serves as a protective mechanism to ensure the matching of blood flow delivery and metabolic needs of the contracting skeletal muscles, this influence is incomplete [9, 119]. This is because a level of sympathetic vasoconstriction is needed in order to maintain exercise BP, especially during intense exercise that involves large muscle groups [9, 119]. Additionally, using positron emission tomography to monitor tissue-specific blood flow, inhibiting α-adrenergic receptors via an infusion of phentolamine has been shown to increase blood flow to inactive muscles within the exercising leg, as well as to the non-exercising leg [175]. The redistribution of blood flow towards the non-active tissues is also accompanied by reductions in oxygen extraction within the exercising leg, demonstrating the importance of sympathetic vasoconstrictor tone in the regulation of exercise hyperemia [175]. Thus, functional sympatholysis is critical for the maintenance and sustainment of physical activity, especially because impaired functional sympatholysis may lead to malperfusion, exaggerated pressor response, and diminished exercise capacity [9].

Putative Mediators of Functional Sympatholysis

To date, several studies have attempted to elucidate potential vasodilatory mediators for exercise hyperemia, which include, but are not limited to: NO, prostaglandins, lactate, temperature, pH, hydrogen ion, oxygen, potassium, EDHF, adenosine, and adenosine triphosphate (ATP) [176]. Despite a number of mediators, there is no consensus available as to

which one may exert the strongest sympatholytic effect, but some evidence points to NO or ATP as being potentially sympatholytic [177-183].

Evidence suggests that NO may play a role in the attenuation of sympathetic vasoconstriction, because antagonizing NO synthase, using L-NAME, has been shown to enhance sympathetic vasoconstriction induced by lumbar sympathetic nerve stimulation in rats' contracting hind limb [179]. In rats, NO appears to attenuate sympathetic vasoconstriction via ATP-sensitive potassium (K_{ATP}) channels [179]. In spontaneously hypertensive rats, exercise training (3 months of voluntary wheel running) has been shown to reverse an impaired functional sympatholysis through a NO-dependent mechanism [184]. Furthermore, human and mouse studies of Duchenne muscular dystrophy have demonstrated impaired functional sympatholysis when a neuronal isoform of NO synthase (nNOS) is absent or reduced [177, 178]. This suggests that the binding of NO to nNOS may, in part, mediate the attenuation of sympathetic vasoconstriction [177, 178]. Similarly, another study demonstrated that both selective nNOS (S-methyl-L-thiocitrulline) and non-selective NO synthase (L-NAME) blockades diminished the attenuation of sympathetic vasoconstriction (induced via lumbar) during exercise in healthy rats [185].

In contrast to the previous studies [177-179, 184], one study suggested that NO is not fully responsible for functional sympatholysis [186]. Specifically, inhibition of NO synthase with L-NAME abolished the blunting of α_1-adrenergic receptor responsiveness to phenylephrine during heavy exercise, but did not alter the blunting of α_2-adrenergic receptor responsiveness to clonidine (α_2-adrenergic receptor agonist) in dogs [186]. Specifically, it has been shown that NO synthase inhibition using intra-arterial infusions of either L-NMMA, or L-NAME, decreases femoral blood flow at rest by 50%, but has only minimal effects of the magnitude of increase in

femoral blood flow during exercise [187]. While it may be tempting to completely rule out NO, there may be a compensatory mechanism that involves EDHF, which is produced by cytochrome P450 2C9 (CYP 2C9), and has been shown to induce hyperemia independently of NO and prostaglandins [188]. Along this line, simultaneous inhibition of NO synthase (via L-NMMA) and cytochrome P450 2C9 (CYP 2C9; via sulfaphenazole) reduces femoral blood flow by 16%, whereas blood flow is unaffected during blockade of NO synthase or CYP 2CP alone [189]. This suggests that one mediator becomes responsible for vasodilation when the other becomes compromised to ensure maintenance of skeletal muscle blood flow delivery [189].

The importance of NO and prostaglandins in regulating skeletal muscle blood flow has been further confirmed with reductions in leg blood flow (-33%) and leg vascular conductance (-36%) following double blockade with L-NMMA and indomethacin (prostaglandin antagonist) [190]. Interestingly, no further reductions in leg blood flow and leg vascular conductance were seen when NO, prostaglandins, and EDHF (via tetrathylammonium chloride) are simultaneously blocked [190]. This suggests that EDHF might not be involved in the compensatory mechanism during reduced bioavailability of NO and prostaglandins, whereas NO and prostaglandins appear to play a crucial role in exercise hyperemia [190]. Collectively, these studies demonstrate that NO serves an important role in functional sympatholysis by acting synergistically with other mediators, such as prostaglandins.

In addition to NO, ATP (whose half-life is only <1 second [191]) has also been proposed as another putative mediator of functional sympatholysis [180-182]. An intra-arterial infusion of ATP into the femoral artery has been shown to attenuate the sympathetically mediated vasoconstriction induced by tyramine infusion (which is used to evoke the release of NE from the sympathetic nerve endings) in the exercising leg [182]. Similarly, during handgrip exercise,

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ATP infusion has been shown to blunt the α_1-adrenergic mediated vasoconstriction (via phenylephrine infusion) by11% and the α_2-adrenergic mediated vasoconstriction (via dexmedetomidine infusion) by 13% [192]. The sympatholytic effect of the circulating ATP likely occurs within the vascular endothelium, as ATP cannot readily cross the endothelium into the interstitium [193]. Moreover, extraluminal ATP can induce vasoconstriction when bound to the ligand-gated P2X purinergic receptors that can be found on the vascular smooth muscle [194]. Additionally, ATP binding to G-protein-coupled P2Y purinergic receptors on the vascular endothelium can cause vasodilation, possibly via release of EDHF, NO, and prostaglandins [195, 196]. Interestingly, it appears that the sympatholytic effects of ATP in the skeletal muscle vasculature are induced by ATP itself and not by its dephosphorylated metabolites including adenosine, adenosine monophosphate (AMP), and adenosine diphosphate [197].

Sources of ATP include sympathetic nerves, endothelial cells, skeletal muscle cells, and red blood cells; however, the observed increase in plasma ATP during exercise is likely from red blood cells [198]. Indeed, red blood cells can produce ATP via glycolysis and can release ATP in response to mechanical deformation, as well as to reductions in oxygen and pH [180, 181]. In both humans and rats, adenylyl cyclase and cyclic AMP (cAMP) have been shown to induce increased activity of the cystic fibrosis transmembrane conductance regulator (CFTR), by acting through a cAMP-dependent protein kinase, which leads to a subsequent release of ATP from the red blood cells [199]. Once ATP is released, ATP can induce vasodilation via several mechanisms. While studies have shown that ATP acts as a vasodilator via actions of EDHF, NO, and prostaglandins [195, 196], one study suggests only minimal contributions of NO and prostaglandins on ATP-mediated vasodilation [200]. Specifically, combined inhibition of eNOS (L-NMMA) and cyclooxygenase (ketorolac) does not have any effect on the vasodilatory

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response to intra-arterial ATP infusion in humans, in which the vasodilatory response was determined using forearm venous occlusion plethysmography [200]. However, in the same study, when the vasodilatory response to ATP infusion was determined by Doppler ultrasound, the combined blockade reduced the vasodilatory response to low dose ATP by -31% and high dose ATP by -25% [200]. This suggests a modest role of NO and prostaglandins and that ATP may induce vasodilation via endothelium-independent pathways [200].

Along this line, one study has demonstrated that ATP induces vasodilation largely via the activation of sodium-potassium ATPase pumps (Na+/K+-ATPase) and inwardly rectifying potassium (K_{IR}) channels in humans [201]. In this study, combined inhibition of Na+/K+-ATPase pumps (via ouabain infusion) and K_{IR} channels (via barium chloride infusion) reduced the vasodilatory effect of ATP by approximately 56% on average, suggesting that ATP mediates vasodilation primarily by inducing vascular hyperpolarization upon K_{IR} channels activation [201]. Interestingly, although both the Na+/K+-ATPase pump and K_{IR} channels may contribute to the attenuation of sympathetic vasoconstriction [201], one study reported that combined blockades of Na+/K+-ATPase pumps and K_{IR} channels do not augment α1-adrenergic mediated vasoconstriction (via phenylephrine infusion) during muscle contraction [202]. Collectively, these studies have demonstrated that an attenuation of sympathetic vasoconstriction during exercise likely results from complex local mechanisms that can interact and compensate for one another to ensure the matching of blood flow delivery to the increased metabolic demands of muscles.

Functional Sympatholysis in High Disease Risk Populations

The topic of functional sympatholysis has been investigated in several populations at high cardiovascular disease risk, because impaired functional sympatholysis can contribute to malperfusion, exaggerated BP response, and exercise intolerance [9]. In hypertension, the ability to blunt the sympathetic vasoconstriction in an active muscle becomes impaired, but such impairment can be improved by antioxidant supplementation, suggesting the role of oxidative stress in modulating the ability of locally released vasoactive mediators to blunt sympathetic vasoconstriction [203]. Similarly, oxidative stress has also been implicated in impaired functional sympatholysis in an animal model of heart failure, a condition that is also reversed by infusion of L-arginine or a superoxide scavenger, tempol, or tiron [204].

In addition to oxidative stress, impaired functional sympatholysis in hypertensive individuals appears to be modulated via angiotensin-dependent increases in sympathetic vasoconstriction, a condition that is reversed following treatment with angiotensin receptor blocker irbesartan [205]. Furthermore, in estrogen-deficient postmenopausal women, transdermal oestradiol replacement therapy has been shown to improve functional sympatholysis, suggesting the potential role of estrogen in modulating sympathetic vasoconstriction and why pre-menopausal women are protected from vascular dysregulation [206]. Interestingly, functional sympatholysis is not impaired in patients with type 2 diabetes who have intact endothelium-dependent vasodilatory responses to acetylcholine infusion [207]. This suggests the presence of impaired endothelial function may be requisite for impaired functional sympatholysis. Taken together, these studies suggest that functional sympatholysis is a complex phenomenon that involves several mechanisms whose alterations may require treatments specific to the disease state and causes of increased sympathetic vasoconstriction.

Obesity and Muscle Blood Flow

Previous work has evaluated skeletal muscle blood flow in the upper and lower limbs of obese individuals using strain-gauge venous plethysmography [5, 6, 41, 208-212], ultrasonography [18, 51, 213-220], and Doppler flowmetry [221]. At rest, skeletal muscle blood flow in the *forearm* of obese individuals has been reported to be lower [5, 7], or similar [51, 209, 211, 212, 214, 215, 217, 221], or higher [18, 216], when compared with lean counterparts. Furthermore, compared with obese individuals with MetS, resting *forearm* blood flow appears to be similar [216] or lower [51, 217] in obese individuals without MetS. These findings suggest that the presence of MetS may alter metabolic demand or blood flow distribution in resting forearm blood flow in obese individuals. However, resting *femoral* blood flow has been demonstrated to be lower [213] or similar [215, 220] in obese individuals compared with lean counterparts. Additionally, obese individuals have lower resting calf blood flow than do lean individuals [208]. Such discrepancies in findings of resting upper and lower limb blood flow are likely attributable to differences in mechanisms controlling skeletal muscle blood flow [222], different methods of blood flow determination, as well as different ages across various studies. In those with impairment of resting blood flow, regardless of upper or lower limb, factors such as endothelium-dependent and independent vasodilatory dysfunction, oxidative stress, sympathetic nerve activity, and endothelin-1 have been shown as potential culprits that contribute to the impairment [5, 41, 209, 220].

Despite an abundance of evidence concerning resting skeletal muscle blood flow, there are a limited number of studies that have comprehensively evaluated skeletal muscle blood flow during exercise in obese individuals, and the results have been equivocal [6, 18, 213, 215, 216, 218, 220]. In essence, although obese individuals exhibit impaired rapid onset of exercise-

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induced vasodilation in an exercise intensity-dependent manner [18], steady-state exercise skeletal muscle blood flow and vasodilatory capacity appears to be lower [213, 220], similar [6, 215, 217], or higher [216] in obese individuals compared with lean counterparts. It should be noted again that these discrepancies may be due to differences in age groups, methods of blood flow measurement, and selection of upper versus lower limb. The presence of MetS may further increase exercise blood flow compared with obesity alone [217], although a similar magnitude of exercise blood flow has also been reported in obese individuals with or without MetS [216]. Despite discrepant findings, it is important to note that impairment of exercise-induced increases in skeletal blood flow may compromise the ability to sustain physical activity and may increase risk for sudden cardiovascular events in individuals at high cardiovascular risk, such as obese individuals [9].

As previously mentioned, preserved functional sympatholysis is an important component of blood flow regulation during exercise and allows for the normal matching of blood flow to the increased metabolic demand even in the face of increased sympathetic vasoconstrictor influences [9]. To date, studies on functional sympatholysis in obesity are scarce, with two known studies conducted in obese individuals with MetS [219] or overweight individuals with type 2 diabetes [207]. In obese individuals with MetS, there is increased vasoconstrictor responsiveness to α-adrenergic receptor stimulation, coupled with higher resting MSNA, compared with lean counterparts [219]. Forearm blood flow and vascular conductance are well maintained during exercise despite increased sympathetic vasoconstrictor influence, indicating preserved functional sympatholysis [219]. Similarly, overweight individuals with type 2 diabetes exhibit a lack of impaired functional sympatholysis in the leg, which is attributed to maintained endothelial function [207]. Despite their findings, it remains inconclusive whether

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functional sympatholysis is preserved or impaired in obese individuals who lack overt CVD. This is an important piece of information, as an early detection of neurovascular abnormality may offer not only insight into the disease progression, but also a potential therapeutic target for interventions. This warrants the need for future studies to explore such a topic in healthy obese individuals.

Post-Exercise Hypotension (PEH)

An acute bout of aerobic exercise has been shown to elicit a sustained reduction in blood pressure (BP) following exercise cessation that can last nearly 2 hours in normotensive individuals or more than 12 hours in hypertensive individuals, a phenomenon termed "post-exercise hypotension (PEH)" [12, 13, 223]. There are two ways to represent PEH in the literature, with some studies looking at BP reductions from pre-exercise values during 1-2 hours post-exercise [224, 225] and other studies comparing 24-hour ambulatory BP on a pre-exercise day with a post-exercise day [226, 227]. It should be noted that reductions in average 24-hour ambulatory BP are typically driven by the large reductions in BP a few hours following exercise [228].

PEH has been described as a persistent elevation in vascular conductance that is not offset by an increase in cardiac output [12, 223]. In brief, there is an increase in venous pooling during passive recovery post-exercise, as a result of an overall increase in vasodilation that is not limited to sites of the previously exercised muscles, coupled with an absence of muscle pump action [12]. The venous pooling and plasma volume loss due to sweating contributes to declines in central venous pressure and cardiac filling pressure [12]. Despite the decreases in central venous pressure and cardiac filling pressure, stroke volume remains normal due to a lower afterload, which occurs simultaneously with increased cardiac contractility and rate, all of which results in an increased cardiac output during exercise recovery [12]. It should be noted that both forearm and leg vascular conductance generally increase in parallel with systemic vascular conductance, suggesting that vasodilation is not restricted to the sites of active skeletal muscles [229]. Additionally, mechanisms of PEH may vary depending on populations studied; for instance, endurance trained men do not exhibit an increase in post-exercise systemic vascular

conductance and their cardiac output is often decreased post-exercise [230]. L. Hill (1897) was among the first to have documented the BP reductions during the first 90 min following a 400 yard dash [231]. Subsequent to this study, W. Fitzgerald (1981) reported a reduction in labile hypertension following 25 min of jogging at 70% of VO_{2peak}, which then has sparked many subsequent studies in PEH [232]. Both central (i.e. neural) and local mechanisms have been suggested to be involved in PEH [13, 233], such that PEH results from an integrative interactions of reductions in sympathetic nerve activity, reduced sympathetic vascular transduction, and local vasodilatory influences [13].

Mechanisms of Post-Exercise Hypotension

Central Mechanisms of Post-Exercise Hypotension

There is a plethora of evidence supporting the role of a central mechanism in mediating PEH, but it is currently unresolved as to which specific receptor or site of interaction plays the most important role in PEH. To date, the primary central mechanism in mediating PEH has focused upon neural inputs from the skeletal muscle afferents and baroreceptors into the NTS within the medulla oblongata [233]. Within the NTS, glutamate is the primary excitatory neurotransmitter that mediates fast synaptic transmission when bound to the ionotropic glutamate receptors, whereas GABA is the inhibitory neurotransmitter that mediates fast inhibitory transmission upon binding onto $GABA_A$ receptors [233]. When the NTS neurons within the caudal ventral lateral medulla (CVLM) become excited, they provide tonic inhibitory (GABAergic) inputs into the sympathetic neurons within the RVLM, which plays a major role in modulating the sympathetic pre-ganglionic outputs in the intermedial lateral cell column in the spinal cord [233]. Blockade of the $GABA_A$ receptors in the RVLM has been shown to increase

the resting activity of sympathetic neurons within the RVLM, which then augments overall

sympathetic nerve activity and arterial BP [234, 235]. Essentially, the magnitude of sympathetic

neural activity is an integrative result of a balance between the excitatory and inhibitory flow to

and from the NTS [236]. To put this into perspective, elevated BP activates the baroreceptors,

which augments the firing activity of the NTS, resulting in increased GABAergic neuronal

activity within the CVLM, decreased firing activity of the RVLM, and a reduction in

sympathetic nerve activity [233]. The end result is a reduction in BP [233].

Given that the NTS is the first central site that receives and integrates neural signals and

facilitates cardiovascular adjustments, it is likely that the NTS may also be implicated in PEH

(Figure 4) [233]. Specifically, the thinly myelinated and unmyelinated (groups III and IV)

muscle afferents, which originate from the contracting skeletal muscles, become activated upon

muscle contraction and acidification [233]. Then, the neural outputs from these skeletal muscle

afferents travel via the dorsal horn of the spinal cord in order to convey this neural information

into the NTS [233]. Upon activation, these skeletal muscle afferents also synthesize and release a

neurotransmitter called substance P at neurokinin-1 receptors on GABAergic interneurons

expressed in the NTS, which is thought to be involved in the resetting of the baroreflex to a

higher level [233, 237]. The action of substance P is localized to excite the inhibitory

GABAergic interneurons, which then release GABA onto the $GABA_A$ receptors of these

barosensitive neurons within the NTS, thus reducing the firing rate of the second order

barosensitive neurons [233]. The second order barosensitive neurons, which normally convey

neural information to the CVLM, becomes less excited, which results in less inhibition of the

RVLM and increases sympathetic nerve activity during exercise [233]. When substance P's

release is pharmacologically blocked, isometric muscle contraction-induced elevations in BP

become abolished [238]. Collectively, substance P appears to be an important player and contribute to the exercise pressor reflex response.

Interestingly, the prolonged activation of the neurokinin-1 receptors by the release of substance P from the muscle afferent fibers during exercise triggers the receptors to undergo internalization to reduce inhibitory transmission in the NTS after exercise [239]. As a result, the GABAergic interneurons provide less inhibitory input to the second order barosensitive neurons in the NTS [240]. This disinhibition of the NTS neurons result in a higher excitatory output to the CVLM, a greater inhibition to the RVLM, and a subsequent reduction in sympathetic nerve activity and PEH [233]. In the spontaneously hypertensive rat (SHR) model, the pharmacologic blockade of neurokinin-1 receptors prior to exercise results in a 37% attenuation of PEH, without any effect on exercise BP [241]. Thus, it appears the substance P-neurokinin-1 receptor mechanism must be functional for PEH to occur. Similarly, the magnitude of PEH is reduced when SHRs are subjected to the $GABA_A$ receptor antagonism, suggesting the involvement of $GABA_A$ receptor in mediating the reduced sympathetic outflow and the generation of PEH [242]. In addition to the substance P-neurokinin-1 receptor mechanism, central arginine vasopressin has also been implicated in PEH, such as central blockade of arginine vasopressin V_1 receptor attenuates PEH in SHRs [243]. PEH has also been shown to be attenuated following antagonism of the opioid receptors by naloxone in humans and animals [223, 244, 245], a depletion of brain serotonin with parachlorophenylalanine [246], as well as a blockade of cardiac afferents whose action are mediated via opioid receptors within the medial NTS [247].

Alterations in centrally-mediated mechanisms have been shown to reset the arterial baroreflex to defend the lower BP after exercise in humans and animals [242, 248, 249]. In humans, an acute bout of aerobic exercise has been shown to reset the arterial baroreflex to a

lower level after exercise, coupled with reduced sympathetic outflow, associated with the baroreflex-sympathetic nerve activity relationship curve shifting downward and leftward without any alteration in baroreflex sensitivity [248]. In humans, a reduction in sympathetic nerve outflow has been demonstrated with a 30% reduction in MSNA post-exercise [248]. Conversely, although arterial baroreflex resetting to a lower level has also been demonstrated in rats, resulting in lower renal [249] and lumbar [242] sympathetic nerve activity, baroreflex sensitivity appears to be attenuated, demonstrated by a significantly reduced gain and range during PEH [242]. Moreover, reductions in BP and HR following exercise become attenuated in rats that underwent sinoaortic denervation [250]. Thus, any impairment in the arterial reflex may contribute to the inappropriate cardiovascular adjustments post-exercise.

In addition to central mechanisms, the sympathetic vascular transduction into vascular resistance appears to be altered post-exercise as well [248]. Specifically, there is less of an increase in vascular resistance with any given increase in muscle sympathetic activity induced by isometric handgrip exercise [248]. Blunted sympathetic vascular transduction is likely explained by either pre-synaptic reuptake of neurotransmitters or pre-synaptic inhibition of release of neurotransmitters [251]. In contrast, post-synaptic vascular responsiveness still remains intact, because α-adrenergic infusions (α_1-agonost phenylephrine and α_2-agonost clonidine) has been shown to evoke similar reductions in forearm vascular conductance after exercise [251]. In addition, vascular conductance after exercise, which is greater than that observed after α-adrenergic receptor antagonism, suggests an additional contribution from vasodilator signals arising from local tissues [229].
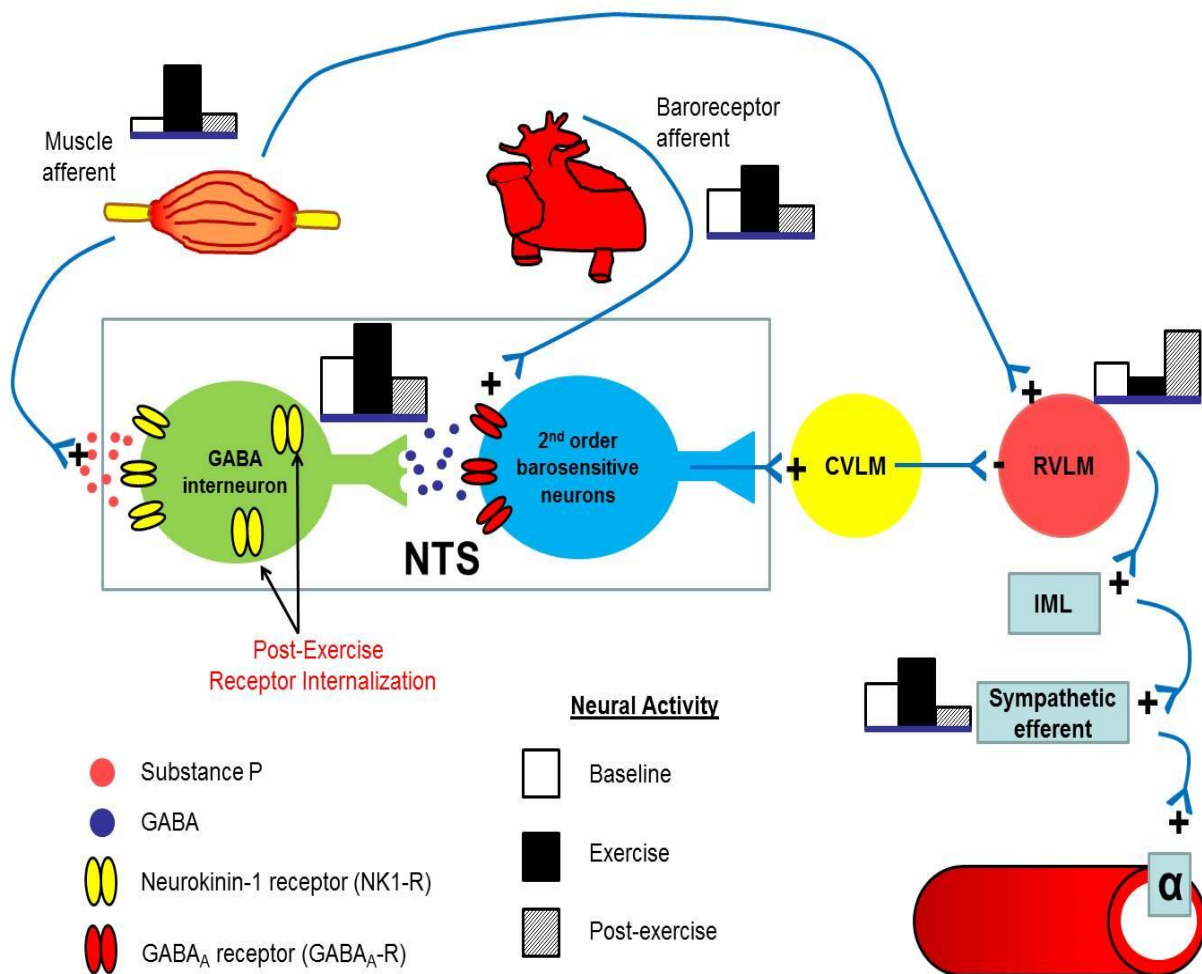
Figure 4. A Schematic Illustrating the Central Mechanisms of Post-Exercise Hypotension. During exercise, muscle afferent releases substance P to activate the GABA interneurons in the nucleus tractus solitarius (NTS), which contributes to baroreflex resetting and exercise pressor response. Activation of the NK1-R during exercise causes the receptor to internalize and become less available post-exercise. The receptor internationalization contributes the baroreflex resetting to a lower level post-exercise. Adapted from (1) Chen CY *et al*. Postexercise hypotension: central mechanisms. *Exer Sport Sci Rev.* 38(3): 122-127, 2010 and (2) Halliwill JR *et al*. Postexercise hypotension and sustained Postexercise vasodilatation: what happens after we exercise? *Exp Physiol.* 98(1): 7-18, 2013.

Local Vascular Mechanisms of Post-Exercise Hypotension

There are two succinct mechanisms of PEH: (1) immediate post-exercise hyperemia and (2) sustained post-exercise vasodilation [13]. Immediate post-exercise hyperemia describes a sustained elevation in blood flow and vasodilation in the previously exercised tissues that occurs concomitantly with the BP reduction after exercise [13, 252] . Immediate post-exercise hyperemia has been documented to last approximately 20-30 minutes and likely has multiple causes [13, 252]. Factors such as type, intensity, and duration of exercise have been shown to contribute to the magnitude and duration of immediate post-exercise hyperemia [253]. To date, few studies have sought to investigate mechanisms of immediate post-exercise hyperemia, but no specific one has been identified [252, 254-257]. For instance, prostaglandin has been identified as a potential substance that induces immediate post-exercise hyperemia in flow-restricted conditions [254] and in normal conditions [255]. When prostaglandin production is pharmacologically inhibited, blood flow measured in the calf immediately after exercise becomes partly reduced [258]. This finding indicates that prostaglandin is one of the contributors to immediate post-exercise hyperemia. While other potential contributors have not been completely identified, previous studies have confirmed that immediate post-exercise hyperemia is not related to the impairment of vascular smooth muscle cell contractility, potassium release, increased osmolality, or lactate production [252]. Moreover, excess oxygen consumption in previously exercised muscles may not contribute to immediate post-exercise hyperemia [252, 253].

Another potential contributor is histamine, which has been shown to induce immediate post-exercise hyperemia in flow-restricted conditions [254]. However, one study contradicts the potential role of histamine by demonstrating the lack of change in immediate post-exercise

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hyperemia following double blockade of both histamine 1 and 2 receptors [256]. While this study may have alluded to the fact that histamine might not even be involved in immediate post-exercise hyperemia, such inconsistency in findings may also be attributed to technical difficulties in detecting histamine [257]. Thus, while no consensus is available on which mediator is the major contributor to immediate post-exercise hyperemia, it is clear that multiple factors must be involved, whose individual and collective contributions depend largely on exercise intensity, duration, and type involved [13].

In contrast to the immediate post-exercise hyperemia, which occurs 20 minutes or less following exercise cessation, the sustained vasodilation may last at least 2 hours and may be explained by mechanisms that are different than those of immediate post-exercise hyperemia [13]. Evidence suggests that vasoactive factors released during acute exercise in response to cyclic wall stress associated with pulsatile blood flow may contribute to post-exercise vasodilation [12]. For instance, NO has been shown to modulate α_1-adrenergic receptor responsiveness to phenylephrine during recovery from exercise in rats [259]. When NO is inhibited using L-NAME, vasoconstrictor responsiveness to phenylephrine becomes enhanced in rats, suggesting that nitric oxide contributes to PEH by attenuating α_1-adrenergic receptor responsiveness [259]. In humans, acute aerobic exercise has been shown to stimulate an increase in NO formation that remains detectable post-exercise [260]. However, NO appears to contribute minimally to PEH in humans, as arterial pressure and vascular resistance remains lower post-exercise despite systemic nitric oxide synthase inhibition using L-NMMA [229].

Another potential mediator of the sustained vasodilation and PEH is prostaglandin, which is also formed within the endothelium, like NO, in response to increases in blood flow and shear wall stress [261]. While an attractive potential mediator, prostaglandin does not appear to

contribute to increased vascular conductance and PEH in humans, as inhibition of

cyclooxygenase does not attenuate increases in vascular conductance and decreases in mean

arterial pressure following aerobic exercise in normotensive men [262]. These findings suggest

that NO and prostaglandins do not appear to independently mediate PEH in humans [229, 262].

Other factors that may contribute to PEH include, but are not limited to adenosine, ATP,

potassium, hydrogen, carbon dioxide, oxygen, and osmolality, although their absolute roles in

mediating PEH remain uncertain [12, 263].

Histamine

In contrast to the aforementioned potential mediators, histamine appears to play a pivotal

role in the sustained PEH and vasodilation upon receptor activation [13]. In fact, histamine

receptor activation (both histamine 1 (H_1) and histamine 2 (H_2) receptors) brings about the

sustained post-exercise vasodilation following both small muscle-mass dynamic exercise (i.e.

single-leg dynamic knee extension exercise) [264] and whole-body aerobic exercise [224].

Following 60-min of moderate intensity single-leg exercise, the rise in leg vascular conductance

associated with exercise is entirely abolished by combined oral H_1 and H_2 receptor antagonism

with fexofenadine and ranitidine [264]. Interestingly, this histaminergic vasodilation is only

restricted to the previously exercised leg and not in the contralateral non-exercised leg and may

occur independently of the neural mechanism in the unilateral knee extension model [264, 265].

Individual contributions of H_1 and H_2 receptors [225, 266], as well as combined histamine

receptor antagonism [224], have previously been evaluated following 60 min of moderate

intensity whole body leg cycling exercise. Selective inhibition of H_1 receptors by oral

administration of fexofenadine has been shown to reduce the magnitude of increase in femoral

vascular conductance measured at 30, 60, and 60 min post-exercise [225]. However, the increase in systemic vascular conductance is blunted only at 30 min post-exercise and the reduction in mean arterial pressure at 30 and 60 min post-exercise with H_1 receptor antagonism, suggesting that H_1 receptors are responsible for the early PEH and that the residual PEH may arise from other receptor subtypes [225].

While H_1 receptors appear to mediate the early phase of PEH, a selective inhibition of H_2 receptors by oral administration of ranitidine hydrochloride blunts the increase in the vasodilation (femoral vascular conductance) and the magnitude of PEH (mean arterial pressure), particularly at 60 and 90 min post-exercise [266]. Combined H_1 and H_2 receptor antagonism has been shown to blunt an increase in femoral vascular conductance (~80%) and a reduction in mean arterial pressure (~60%) for the entire 90 min post-exercise in both sedentary and trained men and women, suggesting that both H_1 and H_2 receptors are involved in PEH produced in response to whole body exercise [224]. In contrast to aerobic exercise, the effect of resistance exercise on PEH has been inconsistent, with studies showing that resistance exercise does not decrease [264] or similarly decreases [267] BP post-exercise. It should be noted that the vasodilation in the leg vasculature is only one component of PEH, because it only accounts for ~34% of the increase in systemic vascular conductance post-exercise [268]. Moreover, skin blood flow does not contribute to PEH, as histamine receptor antagonism does not affect skin blood flow measured via forearm and thigh cutaneous vascular conductance [225, 266]. Splanchnic and renal circulations also have little or no contributions in vasodilation and PEH [268].

Sources of Histamine

Histamine concentrations have been shown to increase during [269] and after exercise [270], and the histaminergic vasodilation is only restricted to the sites of previous muscle contraction [264]. Studies have shown that mast cells, which can be found in most tissues and basophils in blood, can store and release histamine [271]. Release of histamine from mast cells results from physical stimuli, such as vibration and heat [272]. In response to exercise, reactive oxygen species are thought to stimulate the release of histamine from mast cells [273]. Sympathetic withdrawal during recovery from exercise has also been shown to induce histamine release [274].

Histamine can also be synthesized by the actions of L-histidine decarboxylase in the epidermal cells, gastric mucosal cells, neurons within the central nervous system, and cells in regenerating tissues [275]. In mice, prolonged exercise induces an increase in histidine decarboxylase mRNA expression and enzyme activities [276], which is consistent with an evidence found in human skeletal muscle following exercise [277]. The *de novo* synthesis of histamine via the induction of histidine decarboxylase is thought to help replenish the pre-existing mast histamine content stored in mast cells that become depleted with exercise [276]. Histidine decarboxylase transcription has been linked to oxidative stress [278] and hypoxia-inducible factor-1α [279]. In mice, the induction of histidine decarboxylase is thought to be mediated by skeletal muscle contraction-induced release of interleukin-1 [280].

Conversely, exercise-induced oxidative stress unlikely contributes to sustained post-exercise vasodilation in humans, because infusion of N-acetylcysteine, which scavenges reactive oxygen species, does not change post-exercise femoral vascular conductance [281]. Regardless, one human study has demonstrated that both mast cell degranulation (determined via interstitial

tryptase concentrations) and *de novo* synthesis of histamine from histidine (determined using a potent inhibitor of histidine decarboxylase, α-fluoromethylhistidine hydrochloride) contribute to the rise in interstitial histamine in skeletal muscle following unilateral dynamic knee extension exercise [282].

When histamine is released regardless of its source, it binds to its receptors and causes vasodilation via mechanisms specific to each receptor subtype, with H_1 receptors being found predominantly on vascular endothelial cells, H_2 receptors on smooth muscle cells, H_3 receptors on presynaptic nerve endings throughout tissues, and H_4 receptors on bone marrow and white blood cells [275]. H_1 receptors induce vasodilation via formation of local vasodilators such as nitric oxide and prostacyclin [275]. Although H_1 receptors have also been shown to contribute to the rise in skin blood flow during whole body heating [283], skin blood flow *per se* does not contribute to PEH [225]. H_2 receptors induce vasodilation by decreasing intracellular calcium concentrations [275]. Although both H_1 and H_2 receptors mediate PEH, the vasodilation produced by H_1 receptors has a rather rapid onset and is short-lived, whereas H_2 receptor activation produces a longer period of vasodilation after a slow onset [275].

It has been suggested that H_1 receptor stimulation may induce the early phase of PEH (~30 min), with minimal influence at 60 and 90 min post-exercise, whereas H_2 receptor stimulation is responsible for the later phase (~60-120), with minimal influence at 30 min post-exercise [225, 266]. In addition to the H_1 and H_2 receptors, H_3 receptors may induce vasodilation by inhibiting NE release [284], or by decreasing intracellular calcium concentrations in the smooth muscle cells, but its role in mediating PEH remains unclear due to the lack of an H_3 receptor antagonist for use in human subjects [275]. Additionally, H_4 receptors are another type

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of G-protein-coupled histamine receptor, like H_1-H_3 receptors, that play a major role in immune function, with no known contribution to PEH [285].

Factors That May Affect the Magnitude of Post-Exercise Hypotension

There are several factors that may influence the magnitude of PEH. Studies using 24-hour ambulatory BP measurements suggest that the magnitude of PEH appears to be greater in sedentary individuals with higher baseline BP values and in those who are not on anti-hypertensive medications [227, 286]. Specifically, BP measured in the supine position following 30-60 min of moderate intensity exercise has been shown to decline approximately by 5-10 mmHg in normotensive individuals or by nearly 20 mmHg in hypertensive individuals [12, 223]. However, the magnitude of PEH is exaggerated when BP is measured while seated or in the standing position, likely due to venous pooling as a result of gravity [223]. Conversely, factors that may minimize the magnitude of PEH include fluid replacement during exercise [287, 288] and potentially active exercise recovery [12]. Rehydration during exercise has been shown to lower both the magnitude of PEH and the magnitude of change in cardiac baroreflex sensitivity post-exercise [287]. Exercise in the heat has also been shown to mitigate the post-exercise fall in cardiac output [289].

Evidence of PEH has been documented in several studies using different aerobic exercise modalities, including leg cycling [251], single-legged dynamic knee extension [264], arm cycling [290], walking [291], and running [232]. For instance, one study sought to determine the effect of muscle mass on the magnitude and duration of PEH by comparing arm versus leg cycling ergometry performed at the same relative moderate intensity (65-70%VO_{2peak}) [290]. Interestingly, although the magnitude of PEH appears to be similar between arm versus leg

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cycling, leg cycle ergometry produces PEH for a longer duration [290] . These findings suggest that the amount of muscle mass may be an important modulator of how long PEH will last, but not of the magnitude of PEH [290]. In comparing the effect of exercise mode (aerobic versus resistance) on PEH, one study reports a similar magnitude of PEH following either 15 min of unilateral leg press (65% of one-repetition maximum (1-RM)) or leg cycling (65% VO_{2peak}) [267] . While this study concluded no influence of exercise mode on the magnitude of PEH, such a conclusion is problematic and thus caution is warranted when interpreting such findings. The conclusion is problematic because the two exercise modes might actually have activated different amounts of muscle mass even though the authors attempted to equalize the exercise intensity (65% 1-RM vs. 65% VO_{2peak}) [267]. Thus, it remains possible that exercise mode may actually influence the magnitude of PEH. Along this line, with the same amount of muscle mass activated, one study reports that single-legged dynamic knee extension exercise, but not resistance exercise, produces PEH [264]. In this study, PEH likely results from an exercise-induced increase in leg vascular conductance that is observed in the previously active leg [264]. Thus, this study suggests that exercise modality, *aerobic versus resistance exercise*, may be an important factor contributing to PEH [264].

Like exercise modality, exercise intensity and duration may also modulate the magnitude of PEH, but findings are inconsistent [226, 292-294]. With regards to exercise intensity, similar BP reductions have been reported following a 30-min bout of leg cycling at 50 or 75% of VO_{2peak} in normotensive individuals, suggesting similar benefits of mild and moderate intensity exercise in reducing BP after exercise (within 60 min) [292]. Similarly, using a broader range of exercise intensity (30, 50, and 80% VO_{2peak}) for 45 min of leg cycling, no difference in the magnitude of PEH has been observed in normotensive individuals [295]. In hypertensive individuals, although

both 30-min bouts of aerobic exercise (50% and 75% of VO_{2peak}) produced PEH, the magnitude and duration of PEH was greater and longer in the 75% of VO_{2peak} bout. Thus, these studies have demonstrated that exercise intensity may become an important factor in determining the magnitude and duration of BP reductions after exercise in individuals with higher baseline BP values.

With regards to exercise duration, similar reductions in BP have been reported following 10, 15, 30, and 45 min of leg cycling at 70% VO_{2peak} in normotensive, as well as in borderline hypertensive individuals [293]. However, it was difficult to conclude in this study whether or not exercise duration has an effect on the duration of PEH, as BP was monitored only until an hour following exercise [293]. Another study suggests that the magnitude of PEH is greater with repeated exercise bouts in hypertensive individuals, but not in normotensive individuals who appear to be resistant to exercise-induced BP reductions [294]. Although inconclusive, these studies suggest that hypertensive individuals might benefit more from longer exercise duration than a shorter duration in terms of the magnitude of BP reductions and potentially the duration of PEH [293, 294].

Potential Benefits of Post-Exercise Hypotension (and Vasodilation)

PEH observed following a single exercise bout may directly contribute to long-term BP reductions when exercise is performed chronically [31]. The BP depressor effect of exercise can subside following at least 1-2 weeks of detraining, thus reinforcing the need for chronic aerobic exercise training in order to maintain BP in a healthy range [31]. The anti-hypertensive effects of aerobic exercise are proposed to result from the exercise effects on other risk factors, including glucose and lipid regulation, abdominal adiposity reduction, and possible psychological

improvements [228]. Nevertheless, the anti-hypertensive effects of aerobic exercise are likely mediated through neuro-humoral and structural adaptations that lead to a reduction in vascular resistance in the periphery [228]. Such adaptations include, but are not limited to exercise training-induced reductions in endothelin-1[296] and renin [297] concentrations, larger lumen diameter and increased compliance in conduit arteries [298], as well as an increase in baroreflex control of sympathetic nerve activity [299]. Interestingly, the magnitude of the acute BP lowering with exercise may even predict the long-term benefits of chronic aerobic exercise training interventions on BP reductions in individuals with elevated baseline BP [300]. Such information may be beneficial for personalized exercise training regime to achieve the best possible results of exercise-induced BP reductions, especially in individuals at high cardiovascular disease risks [228, 301].

In addition to the long-term benefits on BP normalization, the recovery period following exercise may also serve an important period for several important processes related to glucose and lipid metabolism [31, 302, 303]. Skeletal muscle glucose uptake is enhanced during the first 90 min after exercise, independent of insulin [302], a period at which the rate of glycogen synthesis also happens to be at its greatest [304]. In one study, combined H_1 and H_2 receptor antagonism has been demonstrated to blunt post-exercise hyperemia and lower glucose concentration within the interstitium of skeletal muscles [303]. This finding suggests that H_1 and H_2 receptors may augment capillary permeability, which can enhance glucose delivery to the interstitium, thereby allowing muscle glycogen content to be restored [303]. Along this line, H_1 H_2 receptor activation has also been demonstrated to enhance glucose uptake in cultured endothelial cells [305].

In addition to enhanced skeletal muscle glucose uptake, post-exercise whole body insulin sensitivity is reduced 25% with H_1 and H_2 receptor blockade, suggesting that post-exercise H_1 and H_2 receptor-mediated skeletal muscle vasodilation benefits glucose regulation in humans [306]. Another study also reported blunted femoral vascular conductance and leg glucose delivery with combined histaminergic receptor blockade, although leg glucose uptake is not universally affected in recreationally active individuals [307]. Additionally, a single bout of aerobic exercise has been shown to acutely reduce triglycerides and increase high-density lipoprotein concentrations [31], a mechanism that may also be partially mediated by histamine receptor activation [308, 309]. These improvements may contribute to long-term reductions in diabetes and atherosclerosis risk.

In addition to improved glucose and lipid regulation, acute aerobic exercise-induced PEH, when repeated long-term, is beneficial for cardiovascular health in various ways through positive vascular remodeling [310, 311]. For instance, chronic exercise training can lead to angiogenesis, which involves proliferation and migration of endothelial cells to form new capillaries from pre-existing vessels, thus allowing for optimal delivery of oxygen, nutrient, and vasoactive substances, as well as removal of metabolic byproducts [310]. Interestingly, histamine has been shown to promote angiogenesis by upregulating pro-angiogenetic signals and enzymes, such as vascular endothelial growth factors (VEGF) and matrix metalloproteinase 2 [312]. The exercise-induced increase in mRNA expression of VEGF is evident in gastrocnemius muscles of rats following a single bout of exercise [313]. Along this line, histamine receptor activation has been shown to up-regulate endothelial nitric oxide synthase, which is beneficial for vasodilation and endothelial health [314]. Thus, these studies have demonstrated the short-term and long-term benefits of exercise, part of which involve exercise-induced histaminergic receptor activation.

Obesity and Post-Exercise Hypotension

Obesity has been widely accepted as an important cardiovascular risk factor and has been implicated in the development of hypertension [3, 4]. When faced with sympathoexcitatory stimuli, obese individuals may also exhibit exaggerated pressor responses [5, 6], which may be carried over into the recovery period and delay the recovering hemodynamic response [12]. Surprisingly, limited numbers of studies have evaluated the influence of obesity on PEH, and the results have been controversial [24, 315-317]. One study evaluated PEH in young overweight and lean men following 20-min moderate intensity exercise (75% of heart rate reserve) versus a rest day as a control day [24]. Compared with the rest day, both overweight and lean men exhibited PEH, but PEH was driven by different mechanisms [24]. Reductions in cardiac output appear to be the primary determinant of PEH in overweight men, whereas reductions in total peripheral resistance contributes to PEH in lean men [24]. Their multiple regression analyses also concluded that BMI appears to significantly predict the magnitude of changes in cardiac output and total peripheral resistance post-exercise [24]. Despite their findings, it should be noted that exercise intensity was further adjusted in this study to account for cardiac drift, resulting in different exercise intensities between group (approximately 62% of VO_{2peak} for overweight men and 71% of VO_{2peak} for lean men). Thus, such disparities in exercise intensities may contribute to the differing determinants of PEH in both groups.

Shibata et al. [317]evaluated the influence of 45-min of moderate intensity walking exercise only in middle-age overweight and obese women. In this study, overweight and obese middle-aged obese women exhibited reductions in systolic BP by -7 mmHg and in diastolic BP by -5 mmHg from baseline values [317]. However, the lack of control participants in this study makes it difficult to compare the magnitude of PEH to determine whether obesity causes more or

less reduction in BP after exercise. In contrast, acute high-intensity exercise has been shown to elicit transient increases in both systolic BP and diastolic BP post-exercise in middle-aged overweight adults [316]. Furthermore, the magnitude of increases in BP was similar in middle-aged overweight and lean adults [316]. The inconsistency in findings from the previous studies likely stems from several factors, such as differences in participants' ages and exercise intensities. Moreover, it is noteworthy that PEH was determined from BP measured immediately after exercise in these studies, which might not completely reveal the potential group differences in the PEH responses for an extended recovery period from exercise.

In contrast to previous studies [24, 316, 317], Bunsawat et al. [318] reported that acute high-intensity exercise did not cause any change in BP measured at 15-min and 30-min post-exercise in young obese and lean adults. Despite the lack of exercise effect on BP, young obese adults had an overall higher BP compared with lean adults [318]. However, in another study, acute high-intensity exercise has been demonstrated to induce transient increases in systolic BP above baseline values up until 30 min post-exercise in young overweight women [315]. Then, systolic BP falls by -2-5 mmHg between 40 and 60 min post-exercise, whereas diastolic BP falls by 1-5 mmHg from 20-min up until 60-min post-exercise, compared with baseline values [315]. However, given the lack of lean controls in this study, the effect of obesity on the magnitude of PEH is difficult to ascertain [315]. Thus, given the inclusive results from these previous studies, a better designed study is warranted to evaluate the influence of obesity on the hemodynamic responses post-exercise compared with age-matched lean adults.

Summary

Having a tightly regulated cardiovascular system ensures appropriate cardiovascular adjustments during exercise, which allow for the matching of blood flow to meet the metabolic demands of the contracting skeletal muscles [9-11]. Functional sympatholysis is an important component of the cardiovascular systems that facilitates the normal rises in vascular conductance and blood flow to the exercising skeletal muscles without compromising BP [9-11]. Such responses are driven by an interaction between locally released vasoactive substances and the sympathetically-mediated vasoconstriction [9-11]. In contrast, when functional sympatholysis is impaired, the ability to blunt sympathetic vasoconstriction becomes attenuated, contributing to reduced blood flow delivery, exaggerated pressor response, and exercise intolerance [9-11].

The recovery period following acute exercise is also important and is a critical period for sudden cardiovascular events [31]. In healthy individuals, the rise in vascular conductance during exercise continues to persist even after exercise cessation, resulting in PEH [12-14]. PEH is driven by mechanisms (e.g. histaminergic pathways) that are distinct from functional sympatholysis and plays an important role in long-term BP normalization with exercise training [12-14]. However, the inability to reduce BP after exercise may suggest enhanced sympathetic nerve activity, impaired vasodilation, and increased vasoconstriction [12-14]. Moreover, exaggerated BP during this recovery period may transiently induce damage to the cardiovascular system [318] and increase the risk for sudden cardiovascular events [31].

Obesity is an important factor associated with increased cardiovascular risks [3, 4]. While the mechanisms for increased risks remain unclear, evidence has demonstrated greater sympathetic vasoconstriction [15, 16] and reduced local vasodilatory capacity of both small resistance and large elastic arteries in obese individuals [17-19]. Such factors may be implicated

in the impairment of functional sympatholysis, which would be consistent with findings that obese individuals often exhibit an impaired ability to sustain physical activity [20, 21] and reduced work capacity [22]. Surprisingly, the impact of obesity on functional sympatholysis has not been systematically addressed. Furthermore, in the few studies that have examined PEH in obesity, the findings have been controversial in part due to poor study design [23, 24], but it is likely that obese individuals may either not exhibit PEH or have a smaller magnitude of PEH compared with lean controls.

In this context, given the scarce information on functional sympatholysis and PEH in obesity, the overall aim of this project is to investigate whether obese adults exhibit neurovascular dysregulation in response to acute exercise.

Specific Aims

Aim 1: to determine if functional sympatholysis would differ between obese and lean adults.

We hypothesized that obese adults would have a lesser magnitude of functional sympatholysis during acute exercise compared with lean adults using lower body negative pressure with and without handgrip exercise as the excitatory tasks.

Aim 2: to determine if PEH would differ between obese and lean adults.

We hypothesized that acute exercise-induced PEH (using central and brachial BP measures) would be absent in obese adults, but would be present in lean adults following 60 min of moderate intensity cycling.

Chapter III: **The Influence of Obesity on Functional Sympatholysis**

Abstract

Sympathetic vasoconstriction is attenuated in exercising muscles to match blood flow with metabolic demand. This "functional sympatholysis" may be impaired in young obese individuals due to greater sympathetic activation and reduced local vasodilatory capacity of both small and large arteries, but this remains poorly understood. We tested the hypothesis that functional sympatholysis is impaired in obese individuals compared with lean counterparts. In thirty-six obese and lean young healthy adults (n=18/group), we measured forearm blood flow (FBF) and forearm vascular conductance (FVC) responses to reflex increases in sympathetic nerve activity induced by lower body negative pressure (LBNP) at rest and during dynamic handgrip exercise at 15% and 30% of maximal voluntary contraction (MVC). FBF and FVC were normalized to lean forearm mass. In lean individuals, LBNP evoked decreases in FBF (-13.9 ± 14.4%) and FVC (-11.8 ± 15.3%) in resting forearm, and the reductions in FBF (15%MVC: -8.9 ± 12.9%; 30%MVC: -1.6 ± 14.8%) and FVC (15%MVC: -7.2 ± 13.3%; 30%MVC: 0.9 ± 16.0%) were blunted during exercise in an intensity-dependent manner ($p<0.05$). Similarly, in obese individuals, LBNP evoked comparable decreases in FBF (-13.4 ± 17.5%) and FVC (-10.1 ± 19.9%) in resting forearm, with the reductions in FBF (15%MVC: -10.4 ± 9.0%; 30%MVC: -1.6 ± 12.5%) and FVC (15%MVC: -8.4 ± 9.5%; 30%MVC: -0.1 ± 13.2%) also blunted during exercise in an intensity-dependent manner ($p<0.05$). Our findings suggest that functional sympatholysis is not impaired in young obese individuals without overt cardiovascular diseases.

Introduction

Appropriately matching blood flow to increased metabolic demands in contracting skeletal muscle is a prerequisite for sustained exercise and requires contributions from both neural and local vascular components [9, 113]. During exercise, increased sympathetic nerve activity causes a reduction in vascular conductance in inactive tissues, whereas in active tissues, this sympathetic vasoconstrictor responsiveness is blunted or abolished [11, 319]. Locally released vasoactive substances within the active tissues are thought to attenuate the vasoconstrictor responsiveness to sympathetic stimulation [176, 197], in part, via an endothelium-dependent signaling pathway [320]. This phenomenon, termed "functional sympatholysis," is a vital component of exercise hyperemia, allowing for proper delivery of blood flow, oxygen, and nutrients to contracting skeletal muscles [166]. Impaired functional sympatholysis is attributable to factors that diminish vasodilator function and increase vasoconstriction as seen in aging [321] and hypertensive populations [205]. Importantly, impaired functional sympatholysis may contribute to reduced perfusion within contracting skeletal muscles, an exaggerated pressor response, and exercise intolerance in aging or hypertensive populations [9], but little is known about the influence of obesity on functional sympatholysis.

Obesity is a major public health concern associated with increased cardiovascular disease risks [3, 4]. Even in apparently healthy obese individuals, alterations in vascular function have been reported, including endothelial dysfunction [47, 48, 322], greater arterial stiffness [65, 66], as well as lower blood flow at rest [5] and during exercise [213]. Studies have also reported increases in resting sympathetic nerve activity [15, 16], as well as an exaggerated rise in sympathetic nerve activity and systemic vascular resistance in response to sympathoexcitation

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[5] in obese individuals. Furthermore, animal models demonstrate enhanced sympathetic vasoconstrictor responsiveness in resistance arteries [76]. Ultimately, these factors associated with obesity may contribute to increased vasoconstriction and reduced vasodilation, thereby impairing functional sympatholysis in this population.

To date, the influence of obesity on functional sympatholysis remains poorly understood. Although exercise is recommended as a lifestyle strategy to combat obesity, obesity is associated with exercise intolerance [22, 36]. Because impaired functional sympatholysis would contribute to insufficient exercise blood flow, effectively reducing work capacity and exercise tolerance, we sought to examine if functional sympatholysis was impaired in obese individuals compared with lean counterparts. We hypothesized that obese individuals would have a larger reflex sympathetic vasoconstrictor response during dynamic handgrip exercise and thus a lesser magnitude of functional sympatholysis compared with age-matched lean individuals.

Methods

Subjects

Thirty-six young adults (18 lean and 18 obese) volunteered and completed a physical activity and health history questionnaire. Exclusion criteria included any known cardiovascular, metabolic, renal, or respiratory disease. None were smokers or were taking any cardiovascular medications, NSAIDS, or multivitamin/antioxidant supplements. Obese individuals had a body mass index (BMI) of 30-40 kg/m^2. Female participants had negative pregnancy tests and were studied during days 1-7 of their menstrual cycle, or during the placebo phase for those on oral contraceptives. All participants were sedentary and were not engaged in regular aerobic exercise for the past 6 mo (current physical activity level was <60 min/week). Written informed consent was obtained from all participants. All procedures were approved by the Institutional Review

Board at the University of Illinois at Chicago and conformed to the guidelines set forth by the Declaration of Helsinki.

Study Design

Using a cross-sectional design, all participants reported to the laboratory twice, at least 48 h apart, in the morning following an overnight fast (12 h) and were instructed to refrain from exercise, caffeine, and alcohol (24 h) before each visit. The first visit involved measurements of baseline descriptive characteristics, and the second visit was the experimental day. For both visits, all vascular measures were obtained in the supine position following quiet rest (10-15 min) in a temperature controlled room (~22-24°C). On the experimental day, participants underwent vascular and hemodynamic measurements at rest and during dynamic handgrip exercise. Lower body negative pressure (LBNP) was applied as an additional sympathoexcitatory stimulus to examine functional sympatholysis. LBNP was added to both resting and handgrip measures (See Figure 5 for a schematic).

Descriptive Measurements

Anthropometrics Height, weight, and waist circumference were measured to the nearest tenth decimal point, and BMI was calculated (kg/m^2). Body composition and lean forearm mass (using anatomical landmarks) were measured using whole-body dual-energy x-ray absorptiometry (DEXA) (GE Lunar iDXA, GE Healthcare, Madison, WI, USA), according to the manufacturer's guidelines.

Blood Pressure (BP) After resting quietly for 10 min, resting seated brachial BP of the non-dominant arm was obtained using an automated oscillometric cuff (HEM-907XL, Omron, Shimane, Japan) during the first visit. Measurements were made in duplicate, and the average BP value was used for analysis if the difference between the two values was ≤5 mmHg for both

systolic and diastolic BP. Otherwise, a third measurement was obtained, and the closet two of the three values were averaged. In addition, beat-to-beat BP of the non-dominant arm was measured in the supine position using finger photoplethysmography (Finometer Pro, Amsterdam, the Netherlands) during the second visit.

Brachial Artery Flow-Mediated Dilation (FMD) Brachial artery vasodilator function was measured with automated edge-detection software (Brachial Analyzer, MIA, Coralville, IA, USA), through assessment of peak dilation following cuff release after 5 min of cuff occlusion using ultrasonography with a 7.5 MHz linear array probe (Hitachi-Aloka α-7, Tokyo, Japan) according to the established guidelines [323]. The mean blood velocity (MBV) signals were corrected at an insonation angle of 60°. FMD (%) and shear rate (s^{-1}) were calculated as follows:

$$FMD(\%) = \frac{Peak\ hyperemic\ diameter\ (cm) - Baseline\ diameter(cm)}{Baseline\ diameter\ (cm)} \times 100$$

$$Shear\ rate\ (s^{-1}) = \frac{8 \times MBV\ (cm/s)}{Diameter\ (cm)} \times 100$$

Then, FMD (%) was normalized to shear stimulus using shear rate area under the curve (AUC).

Blood Lipid Profile Venous blood samples were obtained in heparinized tubes and analyzed using Cholestech LDX (Cholestech Instruments, Hayward, CA) for the following variables: fasting plasma concentrations of low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), total cholesterol, triglycerides, and glucose.

Peak Oxygen Consumption Testing (VO$_{2peak}$) VO$_{2peak}$ was measured using an open-circuit spirometry metabolic system (TrueOne 2400, Parvo Medics, Sandy, UT, USA) during an incremental graded cycling exercise test performed to exhaustion (Excaliber Sport, Lode, the Netherlands). The cycling exercise protocol was selected to support weight during locomotion, especially in obese individuals. The participants began with a 1-min warm-up with no resistance.

The first workload was set at 40 W and gradually increased by 30 W every 2 min until test termination. The participants pedaled at a cadence of 60-100 rpm. Ratings of perceived exertion were assessed once per stage. Following test termination, the recovery protocol began with 2-min light cycling (0W, 50 rpm), followed by 1 min of quiet sitting on the cycling ergometer. The criteria for test termination were described previously [318].

Experimental Protocol and Measurements

All participants were studied in the supine position. Beat-to-beat heart rate (HR) was recorded using an electrocardiogram (Biopac Systems, Santa Barbara, CA, USA). Beat-to-beat BP was continuously recorded on the non-dominant arm using finger photoplethysmography (Finometer Pro, Amsterdam, the Netherlands). Beat-to-beat HR and BP was recorded at a sampling rate of 1,000 Hz (Biopac Systems, Santa Barbara, CA, USA). Data were analyzed offline using WinCPRS (Absolute Aliens, Turku, Finland), and beat-to-beat BP was used to derive stroke volume and cardiac out that were indexed to body surface area (stroke index (SI) and cardiac index (CI), respectively).

Dynamic Handgrip Exercise Maximal voluntary contraction (MVC) for each participant was selected as the greatest of the 3 maximal squeezes of a handgrip dynamometer (TSD121C, Biopac Systems, Goleta, CA, USA). All participants performed dynamic handgrip synchronized to the rhythm of a metronome (20 handgrips per min; 50% duty cycle) at 15%, then 30% (EX15 and EX30) of MVC for 4 min, with a 10-min rest between each exercise intensity. Force production was displayed on a projector screen to provide participants with visual feedback. The selected handgrip exercise intensities have been shown not to evoke increased sympathetic nerve activity [324].

Reflex Activation of Sympathetic Nerves LBNP was used to produce reflex sympathetic

vasoconstriction in the forearm. The participant's lower body was enclosed to the level of the

iliac crest in the LBNP chamber. LBNP at -20 mmHg primarily unloads the cardiopulmonary

baroreceptors, effectively evoking reproducible reflex increases in muscle sympathetic nerve

activity [206, 324]. LBNP at -20 mmHg was applied for 2 min at rest and during the last two min

of dynamic handgrip exercise.

Forearm Blood Flow (FBF) FBF was measured for a total of 4 min at rest and during

dynamic handgrip exercise. Brachial artery diameter and MBV were measured on the dominant

arm using ultrasonography with a 7.5 MHz linear array probe (Hitachi-Aloka α-7, Tokyo, Japan)

with an insonation angle of 60°. Images were recorded using Vascular Tools (Medical Imaging

Applications, Coralville, IA, USA) during diastole and analyzed offline using automated edge-

detection software (Brachial Analyzer, MIA, Coralville, IA, USA). FBF (mL·min^{-1}) was

calculated as follows:

$$FBF\ (mL \cdot min^{-1}) = MBV(cm/s) \times \pi \times (\frac{brachial\ diameter(cm)^2}{4}) \times 60$$

Forearm vascular conductance (FVC, mL/min/mmHg) was calculated as follows:

$$FVC\ (mL \cdot min^{-1}(100\ mmHg)^{-1}) = \frac{FBF\ (mL \cdot min^{-1})}{mean\ arterial\ pressure\ (mmHg)} \times 100$$

Then, FBF and FVC were normalized to forearm lean muscle mass (measured from DEXA). As

annotated by nFBF and nFVC, respectively.

FBF, FVC, HR, and BP responses to LBNP were determined by calculating the

difference between the average over the last minute of baseline immediately preceding LBNP

and the average of the last minute during LBNP. The main dependent variables were the relative

change in FBF or FVC with LBNP (%ΔFBF and %ΔFVC) at rest and during exercise. These were calculated as follows:

$$\Delta\%FBF \text{ or } \Delta\%FVC = \frac{(FBF \text{ or } FVC \text{ during } LBNP) - (FBF \text{ or } FVC \text{ before } LBNP)}{FBF \text{ or } FVC \text{ before } LBNP} \times 100$$

Finally, the magnitude of functional sympatholysis was calculated as:

Magnitude of functional sympatholysis = nFBF (or nFVC) at rest [percent change (%Δ)] – nFBF (or nFVC) during exercise (%Δ)

This index of functional sympatholysis reflects the ability of muscle contractions to attenuate the reflex sympathetic vasoconstrictor response observed at rest [321, 325].

Statistical Analysis

Data were checked for normality of distribution using the Shapiro-Wilk tests, and non-normally distributed data were normalized using natural log transformation. Descriptive characteristics, baseline differences, and percent change variables were compared between groups using an independent *t* test or non-parametric Mann-Whitney U test. The Mann-Whitney U test was utilized for data that could not be logged transformed. To test the responses to LBNP at rest and during exercise, a 2 x 2 ANOVA with repeated measures [group (lean versus obese) by time (before and during LBNP)] was conducted. Additional analyses using a 2 x 2 ANOVA with repeated measures [sex (male versus female) by time (before and during LBNP)] were carried out to determine any potential influence of sex on functional sympatholysis in each group. Data are presented as mean ± SD. Alpha was set at *p*<0.05. All data were analyzed using SPSS (V 21.0, IBM SPSS, Inc., Armonk, NY).

Results

Descriptive Characteristics and Flow-Mediated Dilation

Obese individuals had higher weight, BMI, waist circumference, percent body fat, and absolute VO_{2peak}, as well as a lower VO_{2peak} (relative to body weight), compared with lean individuals (Table 1, $p<0.05$). Furthermore, no group differences were observed for any FMD variable, except for higher peak flow velocity in obese individuals compared with lean individuals (Table 2, $p<0.05$). Statistically controlling for shear rate AUC, %FMD also remained similar between groups (Table 2, $p>0.05$).

Forearm Blood Flow and Forearm Vascular Conductance Responses to LBNP at Rest and During Exercise

Brachial artery responses at rest, during LBNP, and with exercise are shown in Table 3, and Figures 6 and 7. At rest, lean individuals had lower FBF and FVC compared with obese individuals, with an overall lower resting FBF with or without LBNP stimulation in lean individuals (Table 3, $p<0.05$). In lean individuals, LBNP stimulation reduced FBF, nFBF, FVC, and nFVC at rest, but these reductions were attenuated during exercise in an intensity-dependent manner (Table 3 and Figure 6). Specifically, LBNP reduced nFBF by -13.9 ± 14.4% at rest, but only by -8.9 ± 12.9% and by -1.6 ± 14.8% during exercise at 15% (EX15) and 30% (EX30) of MVC, respectively (Figure 6, $p<0.05$). LBNP also reduced nFVC by -11.8 ± 15.3% at rest, but only by -7.2 ± 13.3% at EX15 and by 0.9 ± 16.0% at EX30 in lean individuals (Figure 6, $p>0.05$). Similarly, obese individuals also exhibited comparable reductions in FBF, nFBF, FVC, and nFVC in response to LBNP stimulation at rest that were attenuated during exercise in an intensity-dependent manner (Table 3 and Figure 6). Specifically, LBNP reduced nFBF by -13.4 ± 17.5% at rest, but only by -10.4 ± 9.0% at EX15 and by -1.6 ± 12.5% at EX30 in obese

individuals (Figure 6, *p*<0.05). Similarly, LBNP also reduced nFVC by -10.1 ± 19.9% at rest,

but only by -8.4 ± 9.5% at EX15 and by -0.1 ± 13.2% at EX30 in obese individuals (Figure 6,

p>0.05). No group differences in nFBF or nFVC responses to LBNP were observed at rest or

during exercise at both intensities (Table 3 and Figure 6). In addition, no group differences at rest

or in response to LBNP were found for brachial artery diameter (*p*>0.05, Table 3), but LBNP

reduced brachial artery diameter slightly, but significantly during both exercise intensities

similarly in both groups (*p*<0.05, Table 3).

Magnitude of Sympatholysis

In both groups, the percent reduction in FBF, nFBF, FVC, and nFVC in response to

LBNP stimulation became more attenuated to a greater extent with increasing exercise intensity

(Figure 6, *p*<0.05). To further quantify these changes, we calculated the magnitude of

sympatholysis, i.e. the difference in FBF and FVC in absolute and normalized values between

rest and exercise (Figure 7) [321, 325]. The magnitude of sympatholysis was similar between

groups and was intensity-dependent, with a greater sympatholysis at EX30 versus EX15 (Figure

7, *p*<0.05).

Hemodynamic Responses to LBNP at Rest and During Exercise

Compared with lean individuals, obese individuals had an overall lower SI and CI at rest,

EX15, and EX30 (Table 4, *p*<0.05). LBNP stimulation reduced MAP slightly, but significantly

at rest and during handgrip exercise at EX15 and EX30similarly in both groups (Table 4,

p<0.05). LBNP stimulation also reduced SI and CI, as expected, at rest and during handgrip

exercise at both exercise intensities similarly in both groups (Table 4, *p*<0.05).

Discussion

The main findings of the present study are two-fold. First, sympathetic vasoconstriction was attenuated in the exercising forearm similarly in obese and lean healthy individuals. Second, the ability to attenuate sympathetic vasoconstriction in an exercise intensity-dependent manner was similar between groups. To the best of our knowledge, this study is the first to evaluate functional sympatholysis in young, otherwise healthy obese individuals. These findings are in contrast to our hypotheses and suggest that young, otherwise healthy obese individuals have preserved functional sympatholysis.

In the present study, sedentary lean individuals maintained their ability to blunt reflex sympathetic vasoconstriction in response to LBNP stimulation during dynamic handgrip exercise at both exercise intensities, with the magnitude of sympatholysis being greater at 30% of MVC than at 15% of MVC. Our findings are in agreement with previous work on preserved functional sympatholysis during reflex sympathetic activation, evoked by either LBNP or infusion of α-adrenergic agonists in young lean individuals [325-327], in an exercise intensity dependent manner [11, 328]. Surprisingly, we also report that the ability to blunt reflex sympathetic vasoconstriction during muscular contraction at both 15% and 30% of MVC in an exercise intensity-dependent manner was comparable between young obese and lean individuals. Our findings of preserved functional sympatholysis in young obese individuals may suggest an intact ability to attenuate α1- and α2-adrenergic receptors, but whether an individual contribution of α1- versus α2-adrenergic receptors to induce vasoconstriction was not studied in the present study. In young obese individuals with metabolic syndrome (~31-35 yrs of age), intra-arterial infusions of α1- and α2-adrenergic agonists evoked reductions in forearm vascular conductance at rest, and this sympathetic vasoconstriction was attenuated during low intensity dynamic

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handgrip exercise (15% of MVC) in both groups [218]. Interestingly, despite comparable responsiveness to α1-adrenergic receptor activation, obese individuals with metabolic syndrome exhibited greater vasoconstrictor responsiveness to α2-adrenergic receptor activation at rest and during exercise [218], suggesting increased sensitivity to α2-adrenergic receptor activation, increased α2-adrenergic receptor density, or both [329, 330].

Work from Thaning et al. [207] further supports preserved functional sympatholysis in obesity, whereby exercise-induced increases in leg blood flow and leg vascular conductance were maintained in middle-aged overweight individuals with type 2 diabetes (~55 yrs of age, BMI 29.1 kg/m^2), even during increased sympathetic vasoconstriction induced by tyramine infusion. Moreover, the vasodilatory response to acetylcholine was also intact in overweight individuals with type 2 diabetes compared with age-match healthy controls (~55 yrs of age, BMI 26.5 kg/m^2), suggesting preserved endothelial function occurs concomitantly with "normal" functional sympatholysis [207]. Indeed, recent work from Hearon et al. [320] supports the role of endothelium-dependent signaling during muscle contraction in enhancing the ability to blunt sympathetic vasoconstriction in humans, particularly via stimulation of endothelium-derived hyperpolarization-like vasodilation. In the present study, we also assessed brachial artery FMD and report no differences in endothelial function between our lean and obese individuals. As such, our data and that of Thaning et al. [207] indicate that our participants were relatively healthy with normal endothelial function and that impaired functional sympatholysis may need to occur in the presence of endothelial dysfunction, likely due to an imbalance of vasodilators and vasoconstrictors [331]. However, given inherent differences in vasodilator responses between the arms and the legs [222], as well as differences between populations (young otherwise healthy

obese versus middle-aged overweight type 2 diabetes), we could not make direct comparisons between these findings [207].

In contrast to our findings and to those of previous studies [207, 218], Vongpatanasin et al. [205] has shown that obese individuals with hypertension (~47 yrs of age; BMI ~30 kg/m^2) exhibit impaired functional sympatholysis during dynamic handgrip exercise (30% of MVC), compared with age-matched overweight normotensive individuals (BMI ~29 kg/m^2). Specifically, LBNP (-20 mmHg) evoked decreases in forearm blood flow and forearm vascular conductance during rhythmic handgrip exercise in obese hypertensive individuals, but not in overweight normotensive individuals [205]. Differences in sympathetic outflow are not responsible for the differences in forearm blood flow and forearm vascular conductance, since both groups exhibited similar muscle sympathetic nerve activity increases at rest and during handgrip exercise [205]. The finding that functional sympatholysis was restored in a subset of obese hypertensive individuals following short-term treatment with angiotensin receptor subtype 1 blockade *irbesartan* suggests that an angiotensin-dependent mechanism may, in part, impair functional sympatholysis in obese hypertensive individuals [205]. Angiotensin II may augment sympathetic vasoconstriction presynaptically by facilitating noradrenaline release [332] and post-junctionally by potentiating α2-adrenergic receptor-induced vasoconstrictor responsiveness to noradrenaline [333].

Mechanisms underlying preserved functional sympatholysis have been extensively investigated, although no specific "sympatholytic" factor has been identified [9]. Locally released vasoactive substances, such as nitric oxide (NO) and adenosine triphosphate (ATP) [9, 119], have been implicated in the attenuation of sympathetic vasoconstriction. NO has been shown to blunt sympathetic vasoconstriction via ATP-sensitive potassium channels [179] and

may act synergistically with other vasoactive substances, such as prostaglandins [189, 190]. Restoration of functional sympatholysis following aerobic exercise training has been shown to occur via an NO-dependent mechanism [184]. ATP is another putative sympatholytic factor, which has been shown to attenuate sympathetic vasoconstriction evoked by intra-arterial infusion during both handgrip [192] and leg exercise [182] in humans. ATP can bind to G-protein-coupled purinergic 2Y receptors within the vascular endothelium and induce subsequent release of NO, prostaglandins, and endothelium-derived hyperpolarizing factors [195, 196]. ATP can also induce vasodilation independently on the endothelium by activating sodium-potassium ATPase pumps and inwardly rectifying potassium channels in humans [201], but whether these activation sites contribute to the sympatholytic effect of ATP remains poorly understood [202].

There are limitations to this study. First, our exercise protocol engaged a small muscle mass in the arm, and it remains unclear if obesity would impact the response during exercise involving a larger muscle mass. However, one study previously reported that functional sympatholysis is mediated via blunting of α1- and α2-adrenergic receptor activation similarly in the forearm and in the leg [326], suggesting muscle mass may not alter our findings of preserved functional sympatholysis in obesity. Second, there could potentially be sex differences in the responses to our protocol [334-336]; however, follow-up analyses controlling for sex did not change our findings. Finally, we did not measure MSNA and thus were unable to directly demonstrate if MSNA increased more or less in obese individuals during handgrip exercise with and without LBNP stimulation. Inclusion of MSNA would elucidate whether an exaggerated MSNA response to LBNP was present in young obese individuals and whether this translates into greater sympathetic vasoconstriction and impaired skeletal muscle perfusion during exercise.

Conclusions

In conclusion, young, otherwise healthy obese individuals maintained the ability to attenuate reflex sympathetic vasoconstriction during dynamic handgrip exercise compared with age-matched lean counterparts.

Table 1. Descriptive Characteristics in Lean (n=18) and Obese (n=18) Participants.

	Lean	Obese
Age (years)	26±5	27±4
Sex (m/f)	9/9	9/9
Height (cm)	169.9±5.7	172.2±7.7
Weight (kg)	65.2±6.9	96.5±12.7 [#]
BMI (kg/m^2)	22.6±1.8	32.4±2.2 [#]
Waist circumference (cm)	83.3±5.8	109.4±7.6 [#]
Body fat (%)	29.9±5.7	41.2±5.7 [#]
Seated SBP (mmHg)	111±6	110±12
Seated DBP (mmHg)	71±6	74±6
Total cholesterol (mg/dL)	177±40	167±35
HDL cholesterol (mg/dL)	59±13	50±15
LDL cholesterol (mg/dL)	103±35	95±32
Triglycerides (mg/dL)	94±55	100±52
Glucose (mg/dL)	95±10	96±14
VO_{2peak} (L/min)	2.07±0.35	2.54±0.53 [#]
VO_{2peak} (mL/kg/min)	31.8±4.3	26.2±4.0 [#]
VO_{2peak} / FFM (mL/kg/min)	45.2±7.0	44.3±7.2

Data are mean ± SD.

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high density lipoprotein; LDL, low-density lipoprotein; VO_{2peak}, peak aerobic capacity.

[#] Different from lean participants ($p<0.05$)

Table 2. Brachial Artery Flow-Mediated Dilation Variables in Lean (n=18) and Obese (n=18) Participants.

	Lean	Obese
Baseline brachial diameter (mm)	3.47±0.39	3.68±0.45
Baseline flow velocity (cm/s)	14.2±4.4	16.2±4.3
Peak brachial diameter (mm)	3.78±0.41	4.03±0.48
%FMD	9.21±1.23	9.66±1.58
Flow velocity at peak diameter (cm/s)	36.9±16.7	37.6±8.9
Shear rate at peak flow velocity (s^{-1})	786±361	744±143
Peak flow velocity (cm/s)	80.7±16.0	91.3±15.4 [#]
Peak shear rate (s^{-1})	1843±460	1984±443
Shear rate AUC (AU)	46949±18319	56930±18641
%FMD/Shear rate AUC (AU)	$2.34 \times 10^{-4} \pm 1.17 \times 10^{-4}$	$1.89 \times 10^{-4} \pm 0.71 \times 10^{-4}$

Data are mean ± SD.

FMD, flow-mediated dilation; AUC, area under the curve; AU, arbitrary units.

[#] Different from lean participants ($p<0.05$)

Table 3. Brachial Artery Diameter and Blood Flow Responses to Exercise With and Without Lower Body Negative Pressure Stimulation.

	Group	Rest	Rest +LBNP	EX15	EX15 +LBNP	EX30	EX30 +LBNP
Diameter (mm)	Lean	3.52±0.46	3.49±0.43	3.55±0.44	3.53±0.43 [a]	3.65±0.41	3.62±0.41 [a]
	Obese	3.73±0.57	3.69±0.56	3.77±0.56	3.73±0.53 [a]	3.85±0.55	3.82±0.53 [a]
FBF (mL/min)	Lean	90±32	76±24 [a]	195±83	181±100 [a]	284±135	284±156
	Obese	117±39 [#]	103±48 [a,b]	228±78	205±73 [a]	335±107	329±115
nFBF (AU)	Lean	9.6±3.5	8.0±2.3 [a]	20.2±5.4	18.4±6.1 [a]	29.5±9.0	28.8±9.1
	Obese	10.3±3.3	9.1±4.0 [a]	19.8±5.0	17.6±4.5 [a]	29.0±6.5	28.6±7.8
FVC (mL/min/mmHg)	Lean	91±35	78±27 [a]	192±73	182±93 [a]	276±106	284±136
	Obese	118±41 [#]	108±55 [a]	229±79	210±78 [a]	320±98	319±108
nFVC (AU)	Lean	9.8±3.8	8.3±2.6 [a]	20.0±5.6	18.7±6.5 [a]	28.8±7.4	29.2±8.9
	Obese	10.4±3.6	9.5±4.7 [a]	19.9±5.3	18.1±4.9 [a]	27.8±6.0	27.9±7.8

Data are mean ± SD.

LBNP, lower body negative pressure; EX15, exercise at 15% of maximal voluntary contraction; EX30, exercise at 30% of maximal voluntary contraction; FBF, forearm blood flow; nFBF, forearm blood flow normalized to lean forearm mass; FVC, forearm vascular conductance; nFVC, forearm vascular conductance normalized to lean forearm mass; AU, arbitrary units.

[a] Different than before LBNP stimulation (Main LBNP Effect) ($p<0.05$).

[b] Overall group difference at rest, EX15, or EX30 with and without LBNP (Main Group Effect) ($p<0.05$).

[#] Different from lean participants at this time point ($p<0.05$).

Table 4. Hemodynamic Responses to Exercise With and Without Lower Body Negative Pressure Stimulation.

	Group	Rest	Rest +LBNP	EX15	EX15 +LBNP	EX30	EX30 +LBNP
MAP (mmHg)	Lean	100±10	98±8 [a]	102±11	100±10 [a]	102±12	100±12 [a]
	Obese	100±6	97±7 [a]	100±6	98±6 [a]	104±7	103±8 [a]
HR (bpm)	Lean	60±6	61±7	65±7	66±9 [a]	66±8	69±9 [a]
	Obese	61±9	61±9	62±9	65±10 [a]	66±8	70±8 [a]
SI (mL/m^2)	Lean	51±10	46±10 [a]	52±11	46±10 [a]	52±10	47±10 [a]
	Obese	43±6 [#]	38±6 [a,b]	42±6 [#]	37±6 [a,b]	43±7 [#]	38±6 [a,b]
CI (L/min/m^2)	Lean	3.1±0.7	2.8±0.6 [a]	3.3±0.8	3.0±0.6 [a]	3.4±0.8	3.2±0.6 [a]
	Obese	2.7±0.6	2.3±0.5 [a,b]	2.7±0.7 [#]	2.4±0.5 [a,b]	2.9±0.7	2.7±0.6 [a,b]

Data are mean ± SD.

MAP, mean arterial pressure; HR, heart rate; SI, stroke index or stroke volume normalized to body surface area; CI, cardiac index or cardiac output normalized to body surface area.

[a] Different than before LBNP stimulation (Main LBNP Effect) ($p<0.05$).

[b] Overall group difference at rest, EX15, or EX30 with and without LBNP (Main Group Effect) ($p<0.05$).

[#] Different from lean participants at this time point ($p<0.05$).
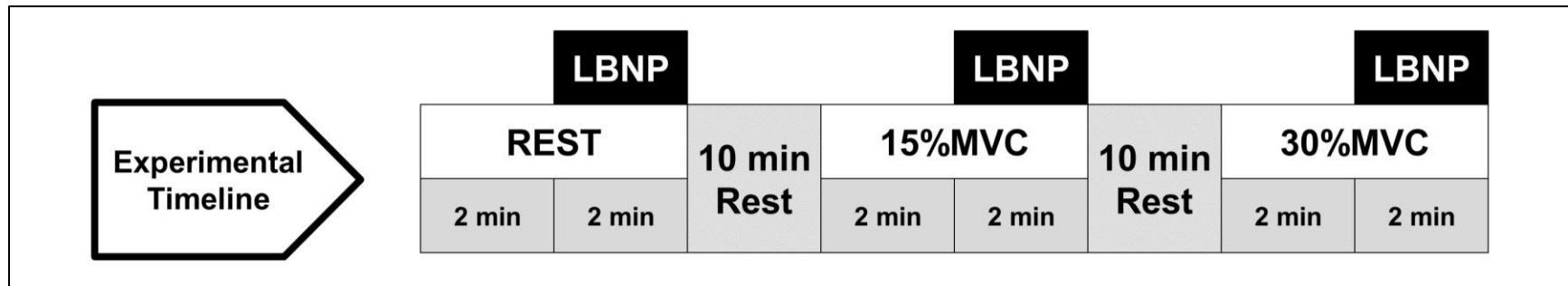
Figure 5. Schematic of the Experimental Design.
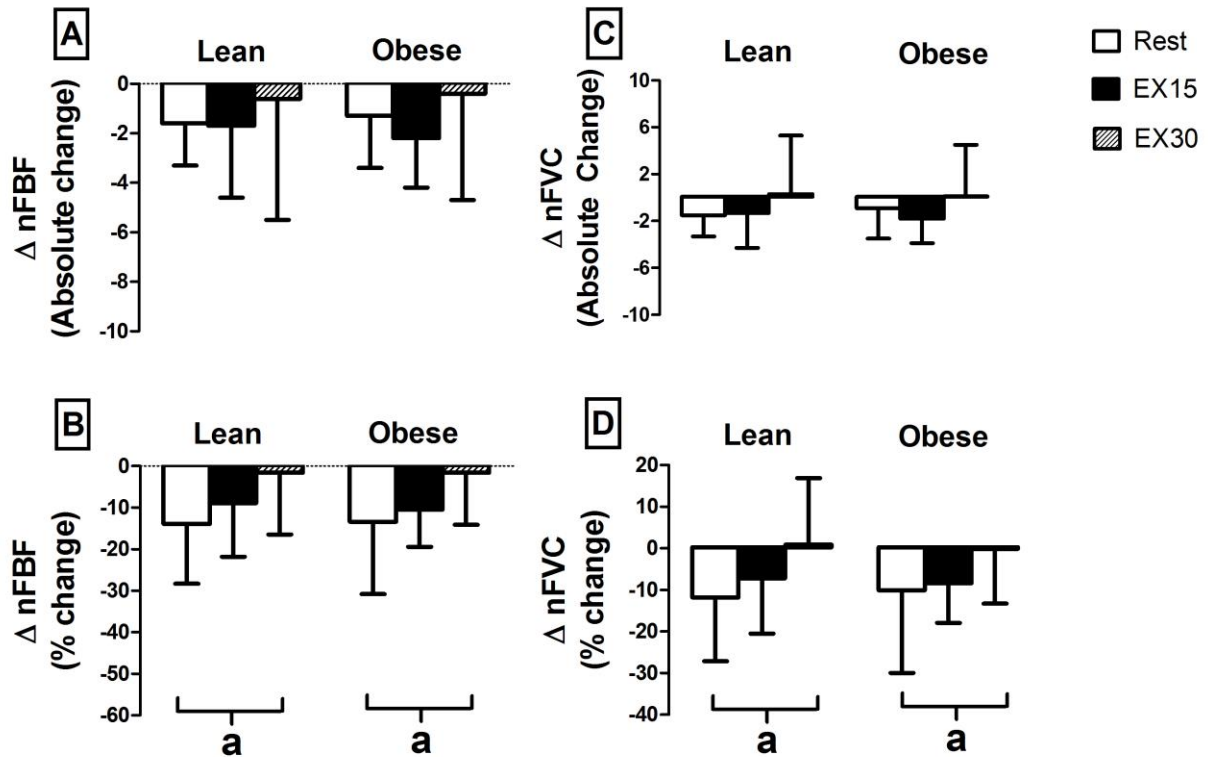
Figure 6. Changes in Forearm Blood Flow and Forearm Vascular Conductance in Response to Sympathetic Stimulation.

Absolute and percent (%) changes in normalized forearm blood flow (Panels A-B) and calculated forearm vascular conductance (Panels C-D) at rest and during dynamic handgrip exercise at 15% (EX15) and 30% (EX30) of maximal voluntary contraction in response to lower body negative pressure stimulation were similar between groups.

Data are mean ± SD.

nFBF, forearm blood flow normalized to lean forearm mass; nFVC, forearm vascular conductance normalized to lean forearm mass.

[a] Time effect ($p<0.05$).

Figure 7. Magnitude of Sympatholysis.

Magnitude of sympatholysis or the calculated difference in normalized forearm blood flow (Panel A) and forearm vascular conductance (Panel B) between rest and exercise at 15% (EX15) and 30% (EX30) of maximal voluntary contraction to lower body negative pressure stimulation was similar between groups.
Data are mean ± SD.
nFBF, forearm blood flow normalized to lean forearm mass; nFVC, forearm vascular conductance normalized to lean forearm mass.
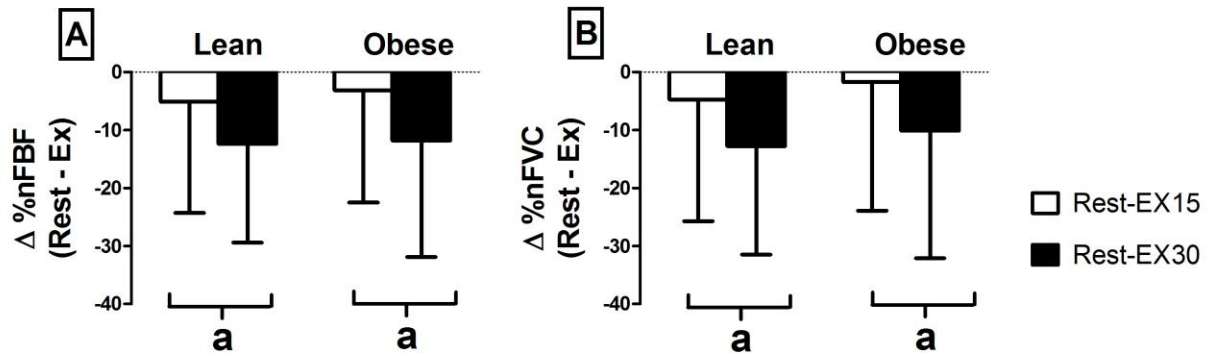[a] Time effect; Rest-EX30 had greater changes than Rest-EX15 ($p<0.05$).

Chapter IV: **The Influence of Obesity on Post-Exercise Hypotension**

Abstract

Obesity is associated with increased risk of incident hypertension. Obese individuals often have elevated brachial and central blood pressure (BP) and wave reflection, as well as abnormal cardiovascular reactivity to sympathoexcitation. Aerobic exercise is often recommended as a lifestyle strategy for the prevention of obesity-induced hypertension, but its acute hypotensive effects on brachial and central BP post-exercise remain unclear in obese individuals. This study sought to determine the effects of acute aerobic exercise on post-exercise hypotension (PEH) in lean and obese individuals. Thirty-six individuals (18 lean and 18 obese, 50%male) underwent measurements of peripheral and central hemodynamics and wave reflection at rest and at 30 min, 60 min, and 90 min after acute moderate-intensity cycling exercise (60% of peak aerobic capacity for 60 min). Obese individuals exhibited similar reductions in central BP (systolic and mean arterial pressures) and wave reflection (augmentation pressure and augmentation index), as well as increases in leg blood flow and vascular conductance following exercise, compared with lean counterparts. Brachial BP was unchanged after exercise in either group. We concluded that acute moderate-intensity aerobic exercise evoked central PEH similarly in lean and obese individuals who lack overt cardiovascular diseases. Importantly, despite lack of brachial BP reductions, both groups had reductions in central BP, which confirms the beneficial effects of acute aerobic exercise on central BP regulation. In addition, our findings of differential BP responses post-exercise highlight an important notion that brachial BP is a poor surrogate of central BP due to BP amplification, as BP waves travel from elastic central arteries to stiffer brachial arteries.

Introduction

Obesity escalates the risk for cardiovascular diseases (CVD), such as hypertension [4, 337, 338]. Furthermore, mortality from CVD is also elevated with obesity, with each 5-unit increment in body mass index (BMI) conferring up to 34% greater CVD risk [32]. Factors associated with obesity that contribute to increased CVD risk include, but are not limited to endothelial dysfunction [47, 48], central arterial stiffening [65, 66], sympathetic overactivity [15, 16], dyslipidemia [83], activated renin-angiotensin-aldosterone system [84, 99], and reduced baroreflex sensitivity [89]. In addition to these risk factors, altered cardiovascular responses have been documented in obese individuals [5, 6, 322]. Indeed, when faced with sympathoexcitatory tasks, such as mental stress, cold pressor test, or static handgrip exercise, obese individuals exhibit exaggerated blood pressure (BP) reactivity [5, 6]. Furthermore, impaired ability to vasodilate during dynamic exercise has also been demonstrated in obese individuals [322], which may contribute to malperfusion and exaggerated BP response during sympathoexcitatory tasks [113, 143], as well as reduced aerobic capacity often observed in obesity [22, 36].

Aerobic exercise is an effective lifestyle strategy to improve BP regulation and reduce CVD risk [339]. Long-term benefits of aerobic exercise stem, in part, from an accumulation of acute exercise's hypotensive effects, which can be observed post-exercise [13]. This "post-exercise hypotension (PEH)" generally results from increased peripheral vascular conductance that is incompletely offset by elevations in cardiac output [12]. Although the magnitude and duration of PEH may be affected by factors such as baseline BP, exercise intensity, and duration [12, 294, 340], moderate-intensity exercise has been shown to be reliably effective in producing PEH and peripheral vasodilation [248, 266, 290, 306, 307, 341]. Additionally, although most studies investigated PEH using brachial BP, recent studies in lean individuals have demonstrated

differential PEH responses between central BP and brachial BP [342, 343], but this remains unclear in obese individuals. This is important, because central BP is superior to brachial BP in predicting incident hypertension and mortality from CVD [344]. Assessment of BP at both sites may offer additional insight into BP regulation post-exercise.

To date, the role of obesity on PEH following moderate-intensity aerobic exercise remains unclear, and existing studies have yielded conflicting results, likely due to different exercise modalities, study populations (age and overweight vs. obese), or flaws in study design [24, 316-318]. Understanding how obesity impacts post-exercise hemodynamics is important, as it may not only provide physiologic insight, but also unravel potential therapeutic targets for lifestyle/pharmacologic interventions. As such, the purpose of this study was to evaluate the acute effect of moderate-intensity aerobic exercise on post-exercise hemodynamics in young obese individuals compared with age-matched lean counterparts. We hypothesized that young obese individuals would exhibit reduced or absent PEH for both central and brachial BPs compared with lean individuals.

Methods

Participants

Thirty-six young adults (18 lean and 18 obese) between the ages of 18 and 40 years participated in this study. All participants had normal BP (systolic BP <140 mmHg and diastolic BP <90 mmHg), and were sedentary for the past 6 mo (weekly physical activity level <60 min). Exclusion criteria included known cardiovascular, metabolic, renal, or respiratory disease, as well as smoking. None were taking medication known to affect BP or heart rate (HR) (including anti-inflammatories or allergies). Obesity was classified based on a BMI of 30-40 kg/m^2. Female

participants were tested during days 1-7 of their menstrual cycle, or during the placebo phase of oral contraceptives. All participants provided written informed consent prior to participation.

Study Design

Using a cross-sectional design, all participants reported to the laboratory twice in the morning, separated by at least 48 h. All participants fasted overnight (12 h) and refrained from exercise, caffeine, and alcohol (24 h for all) prior to each visit. Visit 1 involved measurements of baseline descriptive characteristics including a peak aerobic capacity (VO_{2peak}) test, and Visit 2 was the experimental day. Following quiet rest (10-15 min), all vascular measures were collected in the supine position in a temperature controlled room (~22-24°C) for both visits. On the experimental day, all participants underwent vascular and hemodynamic measurements at baseline pre-exercise (Pre) and at 30-min (P30), 60-min (P60), and 90-min (P90) post-exercise. The Institutional Review Board at the University of Illinois at Chicago approved all procedures, which conformed to the guidelines set forth by the Declaration of Helsinki.

Descriptive Measurements

Anthropometrics Height, weight, and waist circumference were collected to the nearest tenth decimal point, and BMI was calculated (kg/m^2). Body composition and leg mass were determined using whole-body dual-energy x-ray absorptiometry scan (DEXA) (GE Lunar iDXA, GE Healthcare, Madison, WI, USA) in accordance with the manufacturer's guidelines.

Ankle-Brachial Index (ABI) ABI was determined based upon the systolic BP in both upper (brachial arteries) and lower (tibial arteries) BP (VaSera VS 1500 AU, Fukuda Denshi, Japan) using a previously described method [345]. ABI was calculated as a ratio of the ankle systolic BP and the brachial systolic BP. Given that ABI is an index of arterial stiffness [346],

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inclusion of this measure would provide additional information on obesity-related arterial function.

Blood Lipid Profile Venous blood samples were collected in heparinized tubes and analyzed by Cholestech LDX (Cholestech Instruments, Hayward, CA) for fasting plasma levels of low-density (LDL) and high-density (HDL) lipoprotein cholesterols, total cholesterol, triglycerides, and glucose.

Peak Oxygen Consumption Testing VO_{2peak} was collected by an open-circuit spirometry metabolic system (TrueOne 2400, Parvo Medics, Sandy, UT, USA) during a graded cycling exercise test until exhaustion (Excaliber Sport, Lode, the Netherlands). All participants pedaled for 1 min with no resistance (warm-up), after which the initial workload began at 40 W and then increased by 30 W every 2 min until test termination. The participants pedaled at a cadence ≥ 60 rpm. Following test termination, the recovery protocol began with a 2 min of light cycling (0W, 50 rpm), followed by 1 min of quiet sitting on the cycling ergometer. The test was terminated when the participants met 3 of the following 4 criteria: (1) ratings of perceived exertion (RPE) ≥ 17 on the Borg scale (scale 6-20), (2) respiratory exchange ratio (RER) ≥ 1.1, (3) HR change within ± 10 bpm of age-predicted maximum HR, and (4) volitional fatigue.

Seated Brachial Blood Pressure Resting seated brachial BP was measured on the non-dominant arm by an automated oscillometric cuff (HEM-907XL, Omron Corporation, Japan) during Visit 1 according to established guidelines [347]. Two measurements were obtained and averaged for analysis if both systolic and diastolic BP differed ≤ 5 mmHg. Otherwise, an additional measurement was obtained, and the closest two of the three measures were averaged.

Experimental Protocol and Measurements

On Visit 2, all participants were studied in the supine position, and all measurements were obtained before and following exercise cessation. All participants exercised on the cycle ergometer (Excaliber Sport, Lode, the Netherlands) for 60 min at 60% of VO_{2peak} determined from the VO_{2peak} test during Visit 1, consistent with previous published protocol [248, 266, 290, 306, 307, 341].

Hemodynamics Beat-to-beat HR was collected using electrocardiogram (Biopac Systems, Santa Barbara, CA, USA). Brachial systolic BP (bSBP) and diastolic BP (bDBP) was collected on the dominant arm by an automated oscillometric cuff (HEM-907XL, Omron Corporation, Japan) as previously described. Mean brachial pressure (bMAP) was calculated from the obtained BP. Additionally, beat-to-beat BP was continuously recorded on the non-dominant arm using finger photoplethysmography (Finometer Pro, Amsterdam, the Netherlands). Beat-to-beat HR and BP data were analyzed offline (WinCPRS, Absolute Aliens, Turku, Finland), and the BP waveforms were used to derive hemodynamic parameters (stroke volume (SV), cardiac output (CO), and total peripheral resistance (TPR)) that were indexed to body surface area (stroke index (SI), cardiac index (CI), and total peripheral resistance index (TPRi)).

Central Blood Pressure and Wave Separation Analysis Radial pressure waveforms were collected using applanation tonometry with a high-fidelity strain-gauge transducer (SphygmoCor, AtCor Medical, Sydney, NSW, Australia) and calibrated using bSBP and bDBP obtained from an automated oscillometric cuff (HEM-907XL, Omron Corporation, Japan). Using a generalized validated transfer function [348], central systolic BP (cSBP), central diastolic BP (cDBP), and central MAP (cMAP) were derived from integrating the aortic BP waveforms. Contour analysis of the radial pressure waveforms was also utilized to determine augmentation

index (AIx), AIx normalized to HR of 75 bpm (AIx@75), subendocardial viability ratio (SEVR), and left ventricular end systolic pressure (ESP). The characteristics of aortic BP waveforms were reconstructed using a simulated flow wave to investigate their magnitudes of impact on aortic BP [349]. The derived variables included forward wave pressure (Pf), reflected wave pressure (Pb), reflection index (RI), as well as the time to wave reflection (Pb-T). Importantly, Pf is the pressure generated during left ventricular contraction, whereas Pb arises from sites of impedance mismatch, due to differences in arterial stiffness at bifurcations [350]. The time to wave reflection is the time from the foot of the Pf to the foot (point of inflection) of the Pb. RI was determined as Pf/Pb. Augmented pressure (AP) was defined as the difference between cSBP and the inflection point at the end of the first systolic shoulder.

AIx@75 was calculated as the ratio of the amplitude of the pressure wave above its systolic shoulder (i.e. the difference between the early and late systolic peaks of the arterial waveform, or AP) to the aortic pulse pressure, and then normalized to a HR of 75 bpm to minimize an influence from HR [351]. AIx@75 is affected by the intensity of Pf and Pb, Pb-T, or a combination of both [352-354]. SEVR was calculated as the ratio of the area under the diastolic pressure-time waveform integral to the systolic pressure-time waveform integral [355]. SEVR is an index of the arterial system's ability to perfuse myocardial tissue to meet the heart's metabolic demand [355] and is related to HR, left ventricular (LV) ejection duration, and arterial load [356]. Decreased SEVR indicates reduced energy reserve of the heart, effectively lowering tolerance for strenuous physical activities such as moderate-to-high intensity exercise [357]. ESP is the pressure at the end of LV contraction [358] and provides a relatively load-independent measure of LV systolic performance [359]. The utility of the generalized transfer function has also been validated against invasive measures in response to exercise [360].

Leg Blood Flow (LBF) LBF was measured for 1 min at each time point (i.e. Pre, P30, P60, and P90). Common femoral artery diameter and mean blood velocity (MBV) were measured on the right leg using ultrasonography with a 7.5 MHz linear array probe (Hitachi-Aloka α-7, Tokyo, Japan) and an insonation angle of 60°, approximately 2-3 cm proximal to the bifurcation. Images during diastole were recorded by Vascular Tools (Medical Imaging Applications, Coralville, IA, USA) and analyzed offline by automated edge-detection software (Brachial Analyzer, MIA, Coralville, IA, USA). LBF (mL·min^{-1}) was calculated as follows:

$$LBF\ (mL \cdot min^{-1}) = MBV(cm/s) \times \pi \times (\frac{femoral\ artery\ diameter(cm)^2}{4}) \times 60$$

Leg vascular conductance (LVC, mL/min/mmHg) was calculated as follows:

$$LVC\ (mL \cdot min^{-1}(100\ mmHg)^{-1}) = \frac{LBF\ (mL \cdot min^{-1})}{mean\ arterial\ pressure\ (mmHg)} \times 100$$

Then, LBF and LVC were normalized to leg lean muscle mass (measured from DEXA) (nLBF and nLVC, respectively).

Statistical Analysis

Data were checked for normal distribution by the Shapiro-Wilk tests, and non-normally distributed data were normalized by natural log transformation. Descriptive characteristics and baseline differences were compared between groups using an independent *t*-test or non-parametric Mann-Whitney U test. The Mann-Whitney U test was used for data that could not be logged transformed. A 2 x 4 ANOVA with repeated measures [group (lean versus obese) by time (pre, P30, P60, P90)] was conducted on all dependent variables to compare group differences in response to exercise. Data were visually and statistically inspected for extreme outliers and three obese and one lean individuals were removed from analysis for hemodynamic variables (SV, SI,

CO, CI, TPR, and TPRi), as they were outliers. Data are presented as mean ± SD. Alpha was set at $p<0.05$. All data were analyzed using SPSS (V 21.0, IBM SPSS, Inc., Armonk, NY).

Results

Baseline descriptive characteristics are depicted in Table 5. Compared with lean counterparts, obese individuals had higher weight, BMI, waist circumferences, body fat, absolute VO_{2peak}, and RER_{peak}, as well as a lower VO_{2peak} (relative to body weight) ($p<0.05$). No group difference was found for VO_{2peak}/FFM (Table 5, $p>0.05$). At baseline (pre), obese individuals had higher bDBP, cDBP, LBF, and LVC compared with lean counterparts (Table 6, Figures 8-10, $p<0.05$).

Following acute exercise, PEH was observed; cSBP was lower throughout the 90-min post-exercise period compared with baseline (Figure 8D, $p<0.05$). cMAP was lower through 60-min post-exercise period and returned to baseline at 90-min post-exercise (Figures 8F, $p<0.05$). Other BP variables did not change with exercise for either group, but obese individuals exhibited an overall higher bDBP than lean counterparts throughout recovery from exercise (Figures 8A-8C and 8E, $p<0.05$). Acute exercise increased LBF, nLBF, LVC, and nLVC above baseline values similarly in both groups (Figure 9, $p<0.05$). In addition, both groups exhibited similar increases in diameter, HR, CO, and CI, as well as decreases in SV and SI following acute exercise compared with baseline (Table 6, $p<0.05$), with no exercise effect on TPR or TPRi (Table 6, $p>0.05$).

Central hemodynamic and wave separation analyses variables are depicted in Table 7 and in Figure 10. Acute exercise induced similar reductions in AIx, SEVR, ESP, Pb, Pf, RI, AP, and AIx@75 in both groups ($p<0.05$). These variables were lower than pre-exercise values throughout 90-min post-exercise.

Discussion

The main findings of the present study were that young obese individuals exhibited comparable reductions in central BP (cSBP and cMAP) as did lean counterparts, without changes in brachial BP post-exercise. The reductions in central BP were accompanied by similar changes in central hemodynamics and wave separation analysis variables in both groups. In addition, there was increased local vasodilation (LBF and LVC) throughout the recovery period. Our findings suggest a lack of impaired central PEH and vasodilatory function post-exercise in young, otherwise healthy obese individuals. To the best of our knowledge, this study is the first to evaluate central hemodynamics, in addition to brachial BP assessment, following moderate-intensity exercise in young, otherwise obese individuals.

Baseline Comparisons of Hemodynamic Variables and Local Vasodilation

At rest, we observed no group differences in resting systolic BP and MAP (both central and brachial) in the present study, whereas previous findings reported elevations at both central [318] and brachial [65, 318] sites. However, brachial and central diastolic BP at rest were slightly higher in obese individuals compared with lean individuals, which was consistent with our previous reports of obesity-related BP elevations at rest [65, 318]. Additional analyses of the central BP waveforms revealed that central hemodynamic and wave separation analysis variables were similar between groups (Table 7 and Figure 10). In agreement with our findings, previous studies have reported LBF (normalized) to be either lower [213] or similar [215, 220] between lean and obese individuals.

Obesity and Systemic Hemodynamics Following Exercise

Acute moderate-intensity exercise has been demonstrated to produce PEH in healthy [224, 248, 266] and in hypertensive individuals [361, 362]. Little information is available

regarding PEH in obese individuals with normal BP, but whom are at increased risk for incident hypertension [337, 338]. In the present study, moderate-intensity exercise did not reduce brachial BP significantly from baseline in either group, but brachial diastolic BP was overall higher in obese individuals than in lean individuals, which is consistent with the notion of obesity-related BP elevation [318]. In contrast to our findings, other studies have consistently demonstrated that reductions in brachial BP (i.e. MAP reduces by ~2-6 mmHg) following moderate-intensity exercise using the same exercise protocol in similarly aged lean individuals [224, 266, 289]. Shibata et al. [317] reported reductions in brachial systolic BP by ~7 mmHg and diastolic BP by ~5 mmHg in overweight and obese middle-aged women following 45-min of moderate intensity walking. However, the lack of control group and a combination of overweight and obese individuals make it difficult to definitively determine whether obesity *per se* causes more or less reductions in brachial BP following exercise [317]. Following high-intensity exercise, we have previously shown no reductions in brachial BP for obese individuals compared with lean counterparts [318]. As such, our previous work [318] and the present study may suggest that lower exercise intensity (such as light walking) may be more effective for reducing brachial BP in young obese individuals.

In the general population, PEH stems from a sustained decrease in systemic vascular resistance (or a sustained increase in systemic vascular conductance) that is not completely offset by increases in cardiac output (generally due to increased HR and maintained stroke volume) following exercise [12]. In the present study, although stroke volume decreased slightly from pre-exercise, HR increased, thus augmenting cardiac output as expected [12]. However, a decrease in systemic vascular resistance (TPR and TPRi) post-exercise approached statistical significance in both groups and may explain why we did not observe significant reductions in

brachial BP in the present study (for instance, bSBP reduced by -1-2 mmHg, whereas cSBP reduced by -4 mmHg post-exercise). It should be noted, however, that baseline brachial BP was already normal in both groups, which might also contribute to the lack of further brachial BP reductions post-exercise. In contrast to our findings, Hamer et al. [24] reported that overweight men exhibit brachial BP reductions following 20-min moderate-intensity cycling exercise (75% of HR reserve), primarily due to reductions in cardiac output, whereas reductions in total peripheral resistance drive brachial BP reductions in lean individuals. SV also decreased following exercise in our study, which may explain why we observed a reduction in central BP. However, we could not directly compare our findings to theirs, due to differences in study population (overweight versus obese) and exercise protocol (75% of HR reserve for 20 min versus 60% of VO_{2peak} for 60 min). Furthermore, their exercise intensities were further adjusted due to cardiac drift, resulting in overweight individuals exercising at ~62% of VO_{2peak} and lean individuals at ~71% of VO_{2peak}, which may contribute to differences in the determinants of PEH in this study [24].

Obesity and Central Hemodynamics Following Exercise

Central (aortic) BP is the pressure to which the heart, kidneys, and major arteries are exposed [350] and is more strongly related to incident CVD than brachial BP [344]. Interestingly, previous work from our group has demonstrated that central BP may respond differently than brachial BP following acute moderate-intensity aerobic exercise, with an exaggerated central BP being reported in individuals at increased CVD risk, such as African-Americans [342, 343]. Surprisingly, little data exist regarding changes in central BP in obese individuals following exercise. We observed similar reductions in central BP (cSBP and cMAP) in both groups following exercise, without any change in cDBP. The reductions in central BP

were accounted for by an attenuation of both forward (Pf) and reflected wave pressures (Pb and RI) in both groups. Furthermore, both groups exhibited similar reductions in central systolic loading, including ESP, AP, AIx, and AIx@75, which suggest reduced wave reflection following moderate-intensity exercise, likely attributable to muscular artery vasodilation or reduced SV [363]. Importantly, the differential changes in central and brachial BPs highlight the notion that brachial BP is not always reflective of the pressure load at the heart [350, 364].

Similarly, Millen et al. [365] reported that young-to-middle aged individuals (average age ~46 yrs) with pre-hypertension and hypertension, who are either overweight or obese (average BMI ~30.9 kg/m^2), reduced central BP, but not brachial BP following 50-min of moderate intensity exercise (60-75% of VO$_{2peak}$). The reduction in central BP was accompanied by declines in reflected wave pressure, reflection index, time to wave reflection, augmentation pressure, and augmentation index without any change in forward wave pressure [365]. Their findings showed similar benefits of exercise on central BP in overweight and obese individuals, despite differences in study population and exercise protocol. Our study included young, otherwise healthy obese individuals and prescribed cycling exercise intensity to match 60% of VO$_{2peak}$ for all participants. Conversely, their study included individuals between ages of 30-50 yrs, with 35% of their participants being overweight and 65% being obese, who had pre-hypertension or hypertension [365]. Furthermore, we used cycle ergometry only for determining VO$_{2peak}$ and for the exercise session, whereas Millen et al. [365] determined VO$_{2peak}$ on a treadmill, but their participants exercised using either walking or cycling to achieve the prescribed target HR determined from the treadmill test. Most importantly, the lack of age-matched lean and obese individuals without high BP made it difficult to conclude whether obesity *per se* had any effect of PEH in this study.

Additionally, we observed that reductions in central BP were accompanied by post-exercise declines in SEVR in both groups. SEVR represents a balance between cardiac oxygen and demand [356], in which a reduction indicates a lower supply to demand ratio of oxygen in the coronary arteries, hence impaired perfusion to the heart (or reduced energy reserve of the heart) [357]. While our findings of reduced SEVR suggests lower myocardial perfusion post-exercise, SEVR must reach at least 50% before it can lead to actual myocardial ischemia [366]. The post-exercise reduction in SEVR is most likely due to the decrease in diastolic time (when the myocardium is perfused) because of the higher HR post-exercise. Our findings are consistent with other studies, which also report post-exercise reductions in SEVR compared with pre-exercise values [364, 367, 368]. Furthermore, SEVR has been shown to increase with exercise training [369], contributing to better exercise tolerance [357]. In patients with coronary artery disease, higher SEVR has been associated with greater improvement in fitness following cardiac rehabilitation [370]. Taken together, our findings of similar resting SEVR and post-exercise responses in both groups suggest a lack of effect of obesity on myocardial perfusion dynamics in young, otherwise healthy individuals.

Obesity and Local Vasodilation Post-Exercise

Previous studies demonstrated higher LBF and LVC following 60-min of moderate-intensity exercise (60% of VO_{2peak}) in lean individuals [224, 289]. In the present study, overall lower leg perfusion and local vasodilation (in normalized values) nearly approached significant differences between obese and lean individuals, suggesting perhaps more power was needed to achieve such group differences. Furthermore, we observed similar increases in LBF and LVC (both absolute and normalized values) from pre-exercise in both lean and obese individuals following exercise, suggesting post-exercise hyperemia is not impaired in obese individuals. This

finding is clinically relevant, especially because post-exercise hyperemia helps to facilitate skeletal muscle glucose uptake for increasing skeletal muscle glycogen storage and to improve lipid metabolism [31, 302-304, 306]. Repeated exposure to hyperemia, as occurs with regular bouts of exercise, may contribute to long-term reductions in diabetes and atherosclerosis risk with chronic aerobic exercise training [31].

Study Limitations

There are several limitations to the study: (1) central BP was measured using non-invasive techniques, although this technique has been validated against more invasive techniques [360]; (2) we did not evaluate whether histaminergic mechanisms of PEH were similar between groups, as it was not the main focus of the current study; (3) there could potentially be sex differences in the responses to exercise [367]; however, we evenly matched our participants (50% females in each group) to minimize any potential sex influence on our data. While our study was not originally powered to detect sex differences, this important area warrants future examination.

Conclusion

In conclusion, young, otherwise healthy obese individuals exhibited reductions in central BP, but not brachial BP post-exercise, similar to lean counterparts. Both groups also had a comparable increase in local hyperemia post-exercise, suggesting lack of impaired post-exercise responses in young obese individuals.

Table 5. Baseline Descriptive Characteristics in Lean (n=18) and Obese (n=18) Participants.

	Lean	Obese
Age (years)	26±5	27±4
Sex (females, %)	50	50
Height (cm)	169.9±5.7	172.2±7.7
Weight (kg)	65.2±6.9	96.5±12.7 [#]
BMI (kg/m^2)	22.6±1.8	32.4±2.2 [#]
Waist circumference (cm)	83.3±5.8	109.4±7.6 [#]
Body fat (%)	29.9±5.7	41.2±5.7 [#]
Seated SBP (mmHg)	111±6	110±12
Seated DBP (mmHg)	71±6	74±6
Left ABI	1.06±0.06	1.08±0.10
Right ABI	1.07±0.06	1.07±0.11
Blood Lipid Profile		
Total cholesterol (mg/dL)	177±40	167±35
HDL cholesterol (mg/dL)	59±13	50±15
LDL cholesterol (mg/dL)	103±35	95±32
Triglycerides (mg/dL)	94±55	100±52
Glucose (mg/dL)	95±10	96±14
Peak Exercise Testing		
VO$_{2peak}$ (L/min)	2.07±0.35	2.54±0.53 [#]
VO$_{2peak}$ (mL/kg/min)	31.8±4.3	26.2±4.0 [#]
VO$_{2peak}$ (mL/FFM-kg/min)	45.2±7.0	44.3±7.2
HR$_{peak}$ (bpm)	187±10	182±13
RER$_{peak}$	1.15±0.04	1.11±0.06 [#]
RPE$_{peak}$	19±1	19±1
Peak work rate (watts)	163±29	170±33
Time to exhaustion (sec)	557±171	646±128

Data are mean ± SD.

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; ABI, ankle-brachial index; HDL cholesterol, high-density lipoprotein cholesterol; LDL cholesterol, low-density lipoprotein cholesterol; VO$_{2peak}$, peak aerobic capacity; HR$_{peak}$, peak heart rate; RER$_{peak}$, peak respiratory exchange ratio; RPE$_{peak}$, peak ratings of perceived exertion.
[#] Different than lean individuals ($p<0.05$)

Table 6. Beat-to-Beat Hemodynamic Variables at Baseline (Pre) and at 30-min (P30), 60-min (P60), and 90-min (P90) Post-Exercise.

	Group	Pre	P30	P60	P90
Femoral Artery Diameter (mm) [a]	Lean	7.99±0.95	8.43±0.94	8.43±0.97	8.37±0.92
	Obese	8.49±0.75	8.83±0.72	8.73±0.69	8.70±0.69
HR (bpm) [a]	Lean	61±6	76±10	73±10	72±12
	Obese	62±9	79±14	76±14	75±13
SV (mL) [a]	Lean	89±11	78±15	81±16	84±13
	Obese	89±9	70±15	73±13	79±10
SI (mL/m^2) [a]	Lean	47±9	41±10	43±11	44±11
	Obese	46±7	36±8	38±8	41±8
CO (L/min) [a]	Lean	5.4±0.8	5.9±0.8	5.9±1.1	5.9±0.8
	Obese	5.5±1.0	5.4±1.0	5.5±1.0	5.8±0.8
CI (L/min/m^2) [a]	Lean	2.8±0.7	3.1±0.6	3.1±0.8	3.1±0.7
	Obese	2.8±0.5	2.8±0.5	2.9±0.6	3.0±0.6
TPR (mmHg*min/L)	Lean	16.2±2.3	15.1±1.5	15.3±1.7	15.6±1.8
	Obese	16.5±3.2	16.4±3.3	17.0±3.8	16.3±2.3
TPRi (mmHg*min/L/m^2)	Lean	31.6±6.9	29.3±5.1	29.7±5.4	30.4±5.9
	Obese	32.3±8.3	31.9±7.1	33.2±8.9	31.9±6.3

Data are mean ± SD.

HR, heart rate; SV, stroke volume; SI, stroke index or stroke volume normalized to body surface area; CO, cardiac output; CI, cardiac index or cardiac output normalized to body surface area; TPR, total peripheral resistance; TPRi, total peripheral resistance normalized to body surface area.

[a] Time effect ($p<0.05$).

Table 7. Central Hemodynamic and Wave Separation Analysis Variables at Baseline (Pre) and at 30-min (P30), 60-min (P60), and 90-min (P90) Post-Exercise.

	Group	Pre	P30	P60	P90
AIx (%) [a]	Lean	8±11	-4±11	-4±8	-2±10
	Obese	10±11	-1±11	-2±11	-1±13
SEVR (%) [a]	Lean	166±22	125±30	143±55	137±27
	Obese	181±41	129±21	133±25	145±37
ESP (mmHg) [a]	Lean	83±8	77±10	80±9	81±7
	Obese	88±8	81±8	82±7	85±7
Pb (mmHg) [a]	Lean	25±5	24±4	24±4	25±4
	Obese	23±3	22±3	22±4	22±4
Pf (mmHg) [a]	Lean	42±18	31±14	34±14	35±17
	Obese	45±15	35±13	35±12	37±14
RI (%) [a]	Lean	16.5±11.0	13.4±12.7	14.4±12.4	15.0±12.2
	Obese	16.9±15.6	11.5±8.7	12.1±11.3	12.9±11.5
Time to wave reflection (ms)	Lean	269±16	258±31	255±18	264±27
	Obese	271±12	260±17	267±29	270±48

Data are mean ± SD.

AIx, augmentation index; SEVR, (Buckberg) subendocardial viability ratio; ESP, end systolic pressure; Pf, forward wave pressure; Pb, reflected wave pressure; RI, reflection index.
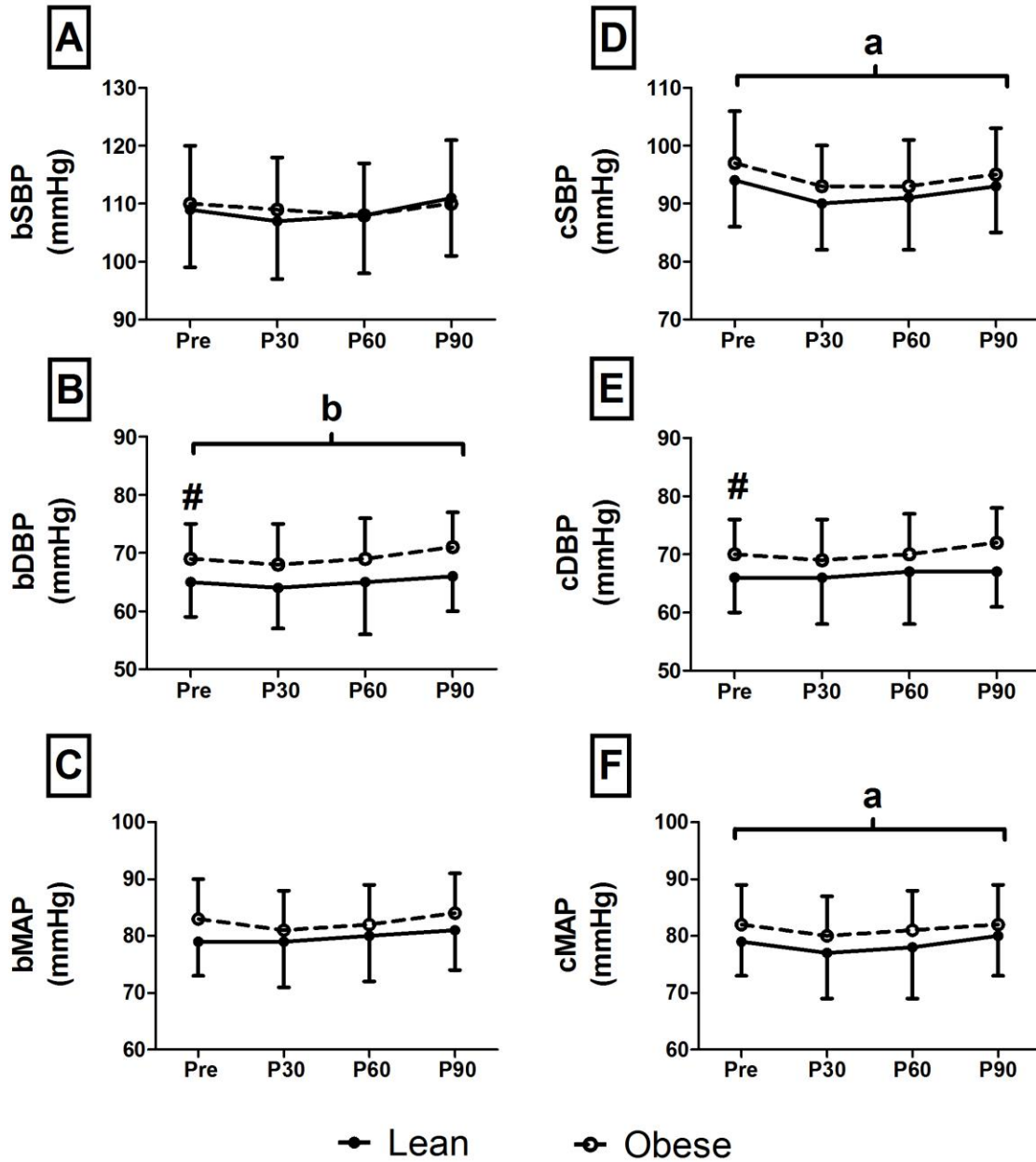
[a] Time effect ($p<0.05$).

Figure 8. Post-Exercise Brachial and Central Blood Pressure Responses.

Brachial (Panels A-C) and central (Panels D-F) blood pressure (BP) at baseline (Pre) and at 30-min (P30), 60-min (P60), and 90-min (P90) post-exercise.
Data are mean ± SD.
bSBP, brachial systolic BP; bDBP, brachial diastolic BP; bMAP, brachial mean arterial pressure; cSBP, central systolic BP; cDBP, central diastolic BP; cMAP, central mean arterial pressure.
[a] Time effect ($p<0.05$).
[b] Group effect; obese group had an overall higher bDBP than lean group ($p<0.05$).
[#] Different than lean individuals at baseline (Pre) ($p<0.05$).
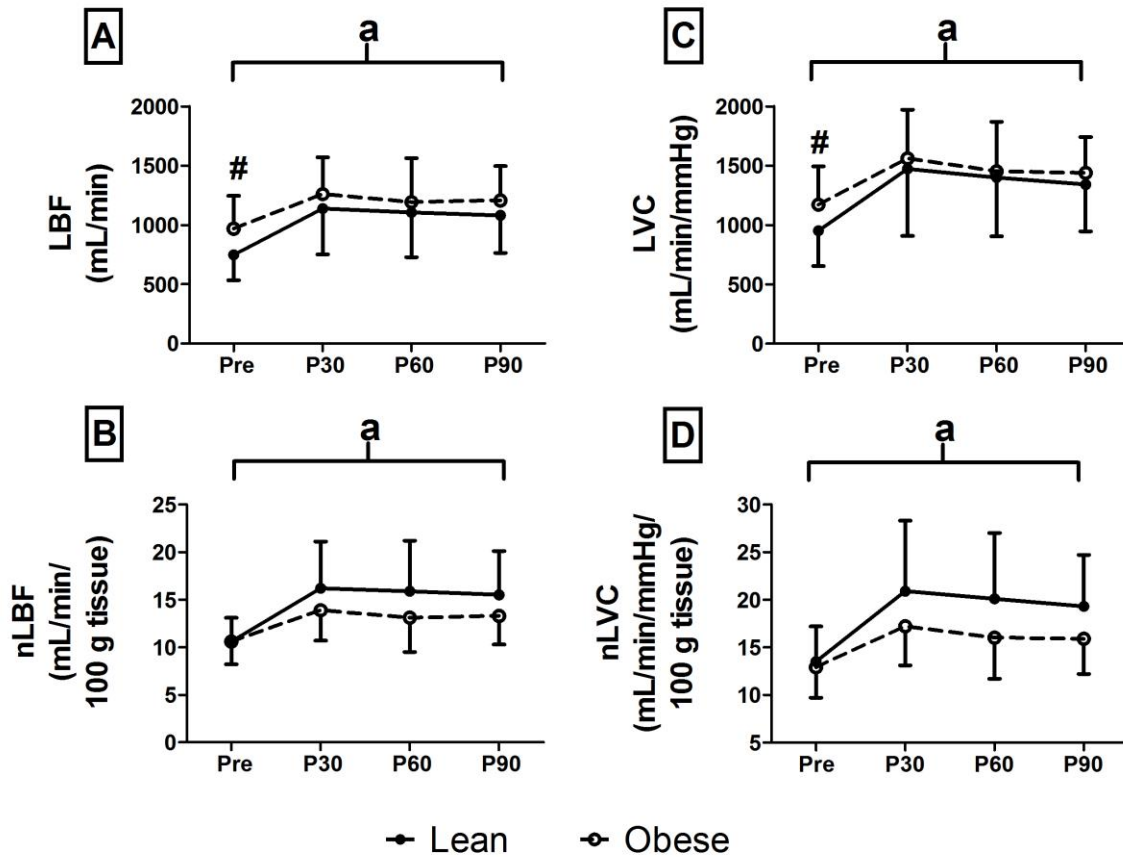
Figure 9. Post-Exercise Leg Blood Flow and Leg Vascular Conductance Responses.

Absolute and relative leg blood flow (Panels A-B) and calculated leg vascular conductance (Panels C-D) at baseline (Pre) and at 30-min (P30), 60-min (P60), and 90-min (P90) post-exercise.

Data are mean ± SD.

LBF, leg blood flow; nLBF, leg blood flow normalized to lean leg mass; LVC, leg vascular conductance; nLVC, leg vascular conductance normalized to lean leg mass.

[a] Time effect ($p<0.05$).

[#] Different than lean individuals at baseline (Pre) ($p<0.05$).

Figure 10. Post-Exercise Augmentation Pressure and Augmentation Index Responses.

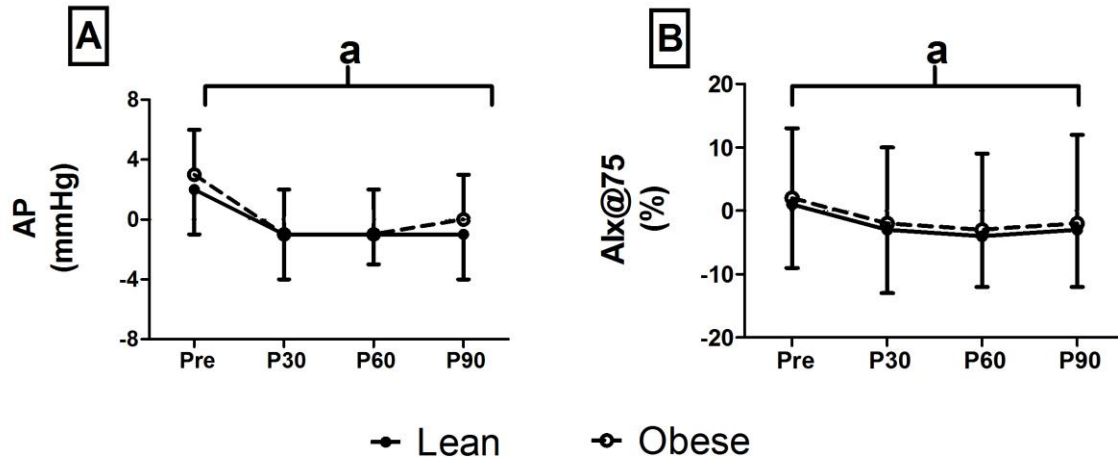Augmentation pressure (Panel A) and normalized augmentation index (Panel B) at baseline (Pre) and at 30-min (P30), 60-min (P60), and 90-min (P90) post-exercise.
Data are mean ± SD.
AP, augmentation pressure; AIx@75, augmentation index normalized to heart rate of 75 bpm.
[a] Time effect ($p < 0.05$).

Chapter V: Summary of Results and Future Directions

Young, otherwise healthy obese adults did not exhibit impaired ability to attenuate sympathetic vasoconstriction during dynamic exercise at low-to-moderate intensity in an intensity-dependent manner, suggesting the lack of impaired functional sympatholysis during low-to-moderate intensity, small muscle mass contraction. This finding is clinically important and suggests that young obese individuals were able to match blood flow delivery to meet the increased metabolic demand of working muscles at low-to-moderate exercise intensity. Nevertheless, since impaired functional sympatholysis is likely the mechanism explaining exercise intolerance, it is possible that young obese individuals might exhibit impaired functional sympatholysis during large muscle mass contraction at higher or maximal exercise intensity, which would explain why we observed reduced peak aerobic capacity obtained during an incremental cycling exercise test in this cohort.

Furthermore, findings from the post-exercise hypotension study demonstrated that young obese individuals, indeed, experienced comparable BP reductions following acute moderate-intensity exercise compared with lean counterparts. The post-exercise BP reductions were observed only in central BP, driven mainly by driven by reductions in systolic and mean BP, without any change in brachial BP. The lack of brachial BP reductions was not surprising, since baseline brachial BP was normal in this young healthy obese cohort. However, the exercise-induced post-exercise central BP reductions are of clinical significance, given that strong relation between central BP and incident hypertension, highlighting the potential benefits of acute aerobic exercise on central BP reduction. As such, our finding reinforces incorporation of moderate-intensity exercise regime for BP regulation and thus prevention of incident hypertension in young obese individuals.

The lack of obesity-related impairment of neurovascular responses to exercise was in contrast to our study hypotheses, but our obese cohort was very healthy. However, our findings do not suggest that obesity does not have negative impacts on cardiovascular function by any means. It is possible that the negative consequences of obesity become more apparent and detectable following longer exposure to excess body weight, with these negative outcomes likely being magnified in the presence of aging, other CVD risk factors, or established CVD. Given the increased global prevalence of obesity, it is important that future studies examine these topics further, so that our understanding of obesity-related pathophysiology may become better understood, which would allow more improved patient-tailored medical/personal care. In particular, future studies should incorporate pharmacologic interventions to better understand individual contributions of α1- and α2-adrenergic receptors to exercise blood flow at different exercise intensities, as well as the contribution of ATP, NO, and other EDHFs in functional sympatholysis in obese individuals in different age groups with or without CVD risk factors. With regards to PEH, future studies should evaluate the influences of exercise intensities, durations, and modes on post-exercise brachial and central BP and vasodilation, as well as evaluate mechanisms of PEH (i.e. histaminergic or neural pathways) in these individuals. Ultimately, a detection of impaired functional sympatholysis or PEH should be followed by short-term vs. long-term lifestyle strategy or pharmacologic interventions.

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Major: Exercise Science (Cum Laude; Periclean Honors Society)
Thesis Title: The Effects of Whey Protein Intake With and Without Exercise Training on Total Muscle and Fat Mass, Abdominal Fat Mass, Blood Lipids, Glucose, Insulin, Mood State, Hunger and Muscular Strength
Advisor: Paul Arciero, PhD

TEACHING EXPERIENCE

Invited Lecturer
1. KN152 - *Introduction to Exercise Science and Health (3 credits)* – Lecture title: "Temperature Regulation," Fall 2016 (122 students), Date: 03-10-2016 – University of Illinois at Chicago

Laboratory Instructor (Undergraduate Level)
1. KN152 - *Introduction to Exercise Science and Health (3 credits)* - Fall 2014 (34 students), Spring 2015 (47 students) – University of Illinois at Chicago
2. KN352 – *Physiology of Exercise (4 credits)* – Fall 2013 (40 students), Spring 2014 (20 students) – University of Illinois at Chicago
3. KIN150 - *Bioscience of Human Movement (3 credits)* – Fall 2011 (29 students) – University of Illinois at Urbana-Champaign

PUBLISHED MANUSCRIPTS

1. Effect of Oxidative Stress on Racial Differences in Vascular Function at Rest and During Hand Grip Exercise. Kappus RM, **Bunsawat K**, Brown MD, Phillips S, Haus JM, Baynard, Fernhall B. *J Hypertens*. 2017. [Epub].
2. The Effect of Acute Maximal Exercise on Postexercise Hemodynamics and Central Arterial Stiffness in Obese and Normal-Weight Individuals. **Bunsawat K**, Ranadive SM, Lane-Cordova AD, Yan H, Kappus RM, Fernhall B, Baynard T. *Physiol Rep*. 5(6): e13226, 2017.
3. No Evidence of Racial Differences in Endothelial Function and Exercise Blood Flow in Young, Healthy Males Following Antioxidant Supplementation. Kappus R, **Bunsawat K**, Rosenberg A, Fernhall B. *Int J Sports Med.* 38(3): 193-200, 2017.
4. How Does High Fitness Attenuate the Risk of All-Cause Mortality in Individuals with Increased Coronary Artery Calcification? Jae SY, **Bunsawat K**, Park JB. *Atherosclerosis*. (Correspondence). 254: 309, 2016.

5. Cardiac Autonomic Modulation and Blood Pressure Responses to Isometric Handgrip and Submaximal Cycling Exercise in Individuals with Down Syndrome. **Bunsawat K**, Baynard T. *Clin Auto Res*. 26(4): 253-260, 2016.
6. Protein-Pacing from Food or Supplementation Improves Physical Performance in Overweight Men and Women: The PRISE 2 Study. Arciero PJ, Edmonds R, **Bunsawat K**, Gentile CL, Ketcham K, Darin C, Renna M, Zheng Q, Zhang J, Ormsbee MJ. *Nutrients*. 8(5): E288, 2016.
7. Attenuated Heart Rate Recovery After Exercise Testing and Risk of Incident Hypertension in Men. Jae SY, **Bunsawat K**, Fadel PJ, Fernhall B, Choi-YH, Park JB, Franklin BA. *Am J Hypertens*. 29(9): 1103-8, 2016.
8. Autonomic Recovery is Delayed in Chinese Compared with Caucasian Following Treadmill Exercise. Sun P, Ranadive SM, Lane AD, Kappus RM, **Bunsawat K,** Baynard T, Hu M, Li S, Fernhall B. *Plos One*. 11(1): e0177104, 2016.
9. Effect of Aspirin Supplementation on Hemodynamics in Older Firefighters. Lane-Cordova AD, Ranadive SM, Kappus RM, Sun P, **Bunsawat K**, Smith DL, Horn GP, Ploutz-Snyder R, Fernhall B. *Med Sci Sports Exerc*. 47(12): 2653-9, 2015.
10. Blood Pressure Changes Following Aerobic Exercise in Caucasian and Chinese Descendants. Peng S, Yan H, Ranadive S, Lane A, Kappus R, **Bunsawat K**, Baynard T, Li S, Fernhall B. *Int J Sports Med* 36(3): 189-96, 2015.
11. Caffeine Delays Autonomic Recovery Following Acute Exercise. **Bunsawat K**, White DW, Kappus RM, Baynard T. *Eur J Prev Cardiol*. 22(11): 1473-9, 2015.
12. Effects of Acute Active Video Games on Endothelial Function Following a High-Fat Meal in Overweight Adolescents. Park SH, Yoon ES, Lee YH, Kim CH, **Bunsawat K**, Heffernan KS, Fernhall B, Jae SY. *J Phys Act Health*. 12(6): 869-874, 2015.
13. Age-Related Ventricular-Vascular Coupling During Acute Inflammation in Humans: Effect of Physical Activity. Lane AD, Kappus RM, **Bunsawat K**, Ranadive SM, Yan H, Phillips S, Baynard T, Woods J, Motl R, Fernhall B. *Eur J Prev Cardiol* 22(7): 904-11, 2015.
14. Normal Heart Rate with Tilt, Yet Autonomic Dysfunction in Persons with Down Syndrome. **Bunsawat K**, Goulopoulou S, Collier SR, Figueroa A, Pitetti KH, Baynard T. *Med Sci Sports Exerc*. 47(2): 250-6, 2015.
15. Usefulness of Blood Pressure Rises Prior to Exercise Stress Testing to Predict the Risk of Future Hypertension in Normotensive Korean Men. Jae SY, Heffernan KS, Kurl S, Laukkanen JA, Choi YH, Park WH, **Bunsawat K**, Fernhall B, Kang SM, Park JB. *Am J Cardiol* 114(8): 1238-42, 2014.

PUBLISHED ABSTRACTS

1. Muscle Blood Flow Responses to Dynamic Handgrip Exercise in Young Obese Adults. **Bunsawat K**, Grigoriadis G, Griffith G, Wee SO, Schroeder E, Fernhall B, Baynard T. *Med Sci Sports Exerc*. 49(5S): 827, May 2017.
2. Exercise Cardiac Power and Coronary Artery Calcification in Men. Jae SY, **Bunsawat K**, Yoon ES, Kim HJ, Park WH, Fernhall B. *Med Sci Sports Exerc*. 49(5S): 786, May 2017.
3. Sex Differences in the Influence of Leg Strength on Arterial Stiffness. Grigoriadis G, Rosenberg AJ, Wee SO, Schroeder EC, **Bunsawat K**, Fernhall B, Baynard T. *Med Sci Sports Exerc*. 49(5S): 341-342, May 2017.
4. Effect of Sex on Systemic Arterial Hemodynamics and Cerebral Blood Flow Dynamics Following an Acute Isokinetic Resistance Exercise. Rosenberg AJ, Wee SO, Schroeder EC, **Bunsawat K**, Grigoriadis G, Fernhall B, Baynard T. *Med Sci Sports Exerc*. 49(5S): 823-824, May 2017.
5. Arterial Distensibility and Compliance Following Hypovolemic Challenge in Individuals with and without Down Syndrome. Wee SO, Rosenberg AJ, **Bunsawat K**, Griffith G, Baynard T, Fernhall B. *Med Sci Sports Exerc*. 49(5S): 813-814, May 2017.
6. Treadmill vs. Cycle Ergometry Graded Exercise Test Responses in Multiple Sclerosis Patients. Griffith G, Hilgenkamp T, Rosenberg A, Grigoriadis G, **Bunsawat K**, Wee SO, Schroeder E, Saed B, Baynard T. *Med Sci Sports Exerc*. 49(5S): 596, May 2017.
7. No Evidence of Impaired Functional Sympatholysis in Young Obese Adults. **Bunsawat K**, Grigoriadis G, Wee SO, Griffith G, Brown MD, Phillips SA, Fadel PJ, Clifford PS, Fernhall B, Baynard T. *FASEB J.* April 2017; 31: 836.24.
8. The Effects of Acute Exercise on Cerebral Blood Flow and Cognition in Persons with Multiple Sclerosis. Rosenberg AJ, Wee SO, Schroeder EC, Grigoriadis G, **Bunsawat K**, Hilgenkamp TIM, Griffith GJ, Baynard T. *FASEB J.* April 2017; 31: 842.4.
9. Carotid Vascular Blood Flow in Individuals with Down Syndrome Following Lower Body Negative Pressure Challenge. Wee SO, Rosenberg AJ, **Bunsawat K**, Griffith GJ, Baynard T, Fernhall B. *FASEB J.* April 2017; 31: 840.21.
10. Sympathetic Vasoconstrictor Response to Lower Body Negative Pressure in Young Obese Adults: the Preliminary Finding. **Bunsawat K**, Grigoriadis G, Fernhall B, Baynard T. *Artery Research* 16: p67, December 2016.

11. Higher Carotid Strain in Individuals with Down Syndrome at Rest and During Hypovolemic Sympathoexcitation. Wee SO, Rosenberg AJ, **Bunsawat K**, Griffith G, Baynard T, Fernhall B. *Artery Research* 16: p63, December 2016.

12. The Detrimental Effects of Live Firefighting on Arterial Function in Firefighters. Rosenberg A, **Bunsawat K**, Wee T, Baynard T, Horn G, Smith D, Fernhall B. *Artery Research* 16: p62, December 2016.

13. Central Hemodynamics and Arterial Stiffness in Young Obese Adults: the Preliminary Finding. **Bunsawat K**, Grigoriadis G, Fernhall B, Baynard T. *Artery Research* 16: p91, December 2016.

14. Blood Pressure Variability and Baroreceptor Sensitivity in Normotensive Obese in Response to Aerobic Exercise. Grigoriadis G, **Bunsawat K**, Fernhall B, Baynard T. *Artery Research* 16: p93-94, December 2016.

15. Effect of Acute Isokinetic Resistance Exercise on Systemic Arterial Hemodynamics and Cerebral Blood Flow Dynamics: Is There A Mismatch? Rosenberg A, Wee SO, Schroeder E, **Bunsawat K**, Grigoriadis G, Fernhall B, Baynard T. *Artery Research* 16: p101-102, December 2016.

16. Vascular Function in Individuals with Down Syndrome: Central and Systemic Vascular Function. Wee SO, Rosenberg AJ, **Bunsawat K**, Griffith G, Baynard T, Fernhall B. *Artery Research* 16: p91-92, December 2016.

17. Sex Differences in Vascular Function Following Antioxidant Supplementation. Kappus RM, **Bunsawat K**, Haus JM, Baynard T, Phillips SA, Brown MD, Fernhall B. *Artery Research* 16: p93, December 2016.

18. Parasympathetic Modulation in Aging Adults: the Influence of Body Composition. **Bunsawat K**, Kappus RM, Rosenberg AJ, Wee SO, Fernhall B, Baynard T. *Med Sci Sports Exerc.* 48(5 Suppl 1): 50, May 2016.

19. Cardiorespiratory Fitness Attenuates the Risk of Subclinical Atherosclerosis in Men with Cardiometabolic Syndrome. Jae SY, Franklin B, **Bunsawat K**, Park WH, Fernhall B. *Med Sci Sports Exerc.* 48(5 Suppl 1): 226, May 2016.

20. Hemodynamic and Peripheral Vascular Conductance in Individuals with Down Syndrome Following Hypovolemic Pressure Challenge. Wee SO, Rosenberg AJ, **Bunsawat K**, Griffith G, Baynard T, Fernhall B. *Med Sci Sports Exerc.* 48(5 Suppl 1): 840, May 2016.

21. Blood Pressure and Wave Separation Analysis: Lower Body Negative Pressure in Individuals with Down Syndrome. Grigoriadis G, Wee SO, Griffith G, Rosenberg AJ, **Bunsawat K**, Fernhall B. *Med Sci Sports Exerc.* 48(5 Suppl 1): 840, May 2016.

22. Effects of Acute Induced Inflammation on Arterial Stiffness and Compliance in Overweight Adults. **Bunsawat K**, Lane-Cordova AD, Fernhall B, Baynard T. *FASEB J.* April 2016; 30(1): 737.5.

23. Vascular Responses to Hypovolemic Pressure Challenge in Individuals with Down Syndrome. Wee SO, Rosenberg AJ, **Bunsawat K**, Griffith G, Baynard T, Fernhall B. *FASEB J.* April 2016; 30(1): 763.9.

24. Females Have Altered Hemodynamics During Exercise Recovery with Caffeine Consumption. Grigoriadis G, **Bunsawat K**, Hilgenkamp TI, Baynard T. *FASEB J.* April 2016; 30(1): LB744.

25. The Influence of Sex and Age on Arterial Function in Response to an Acute Inflammatory Stimulus. Rosenberg A, Lane-Cordova A, **Bunsawat K**, Wee SO, Baynard T, Fernhall B. *Artery Research* 12: 46, December 2015.

26. The Influence of Resting Heart Rate on Low- and Very Low-Frequency Blood Pressure Variability. **Bunsawat K**, White DW, Shafer BM, Marqui PA, Wang Y, Fernhall B, Baynard T. *Artery Research* 8(4): 166-167, December 2015.

27. Carotid Arterial Circumferential Strain Has a Greater Association to Vascular Aging than Conventional Carotid Arterial Stiffness. Rosenberg A, Lane-Cordova AD, Kappus RM, **Bunsawat K**, Wee SO, Baynard T, Fernhall B. *Artery Research* 8(4): 166-167, December 2015.

28. Influence of Age on Blood Pressure Variability and Baroreceptor Sensitivity in Response to Acute Exercise. Rosenberg AJ, Kappus RM, **Bunsawat K**, Shafer B, Wee SO, Baynard T, Fernhall B. *FASEB J. April 2015; 29(1): LB672.*

29. Autonomic Function during Exercise in Older Adults: the Influence of Antioxidant Supplementation. **Bunsawat K**, Kappus RM, Rosenberg AJ, Shafer B, Wee SO, Baynard T, Brown MD, Haus JM, Phillips SA, Fernhall B. *Med Sci Sports Exerc.* 47(5S): 744, May 2015.

30. Blood Pressure during Submaximal Exercise as a Predictor of Future Hypertension in Normotensive Men. Jae SY, Franklin B, **Bunsawat K**, Park WH, Fernhall B. *Med Sci Sports Exerc.* 47(5S): 173, May 2015.

31. The Effect of Acute Antioxidant Supplementation on Vascular Dysfunction with Aging. Kappus RM, **Bunsawat K**, Rosenberg AJ, Baynard T, Haus JM, Phillips SA, Brown MD, Fernhall B. *Med Sci Sports Exerc.* 47(5S): 851, May 2015.

32. Racial Differences in HRV in Response to an Acute Bout of Forearm Exercise. Rosenberg AJ, **Bunsawat K**, Kappus RM, Shafer B, Wee SO, Baynard T, Brown MD, Haus JM, Phillips SA, Fernhall B. *Med Sci Sports Exerc.* 47(5S): 161, May 2015.

33. Home-Based Exercise in Persons with Multiple Sclerosis: Fitness and Walking Mobility Preliminary Results. Griffith G, Wee SO, Klaren RE, Thur L, Kappus RM, Shafer B, **Bunsawat K**, Molt R, Baynard T, Fernhall B. *Med Sci Sports Exerc*. 47(5S): 361, May 2015.
34. Effects of Acute Induced Inflammation on Pressure Waveforms: Does Age Matter? **Bunsawat K**, Lane AD, Kappus RM, Ranadive SM, Yan H, Wee SO, Phillips SA, Baynard T, Woods JA, Molt R, Fernhall B. *Artery Research* 8(4): 166-167, December 2014.
35. Sex Differences in Hemodynamic Responses Following Acute Inflammation: Wave Separation Analysis. Wee SO, Lane AD, **Bunsawat K**, Rosenberg A, Fernhall B. *Artery Research* 8(4): 174-175, December 2014.
36. Effects of Caffeine on Cardiac Autonomic Function During Exercise Recovery. **Bunsawat K**, Rosenberg AJ, Wee SO, Kappus R, Hultgren K, Fernhall B, Baynard T. *Med Sci Sports Exerc*. 46(5S): 327, May 2014.
37. Altered Autonomic Response to Upright Tilt in Individuals with Down Syndrome. **Bunsawat K**, Goulopoulou S, Collier S, Figueroa A, Pitetti K, Fernhall B, Baynard. *Med Sci Sports Exerc*. 45(5S): 31, May 2013.
38. Effects of Acute, Induced Inflammation on Cardiovascular Function**.** Lane AD, Ranadive SM, Yan H, Kappus RM, Baynard T, Phillips S, **Bunsawat K**, Woods JA FACSM & Fernhall B FACSM. *Med Sci Sports Exerc*. 45(5S): 34, May 2013.
39. Acute, Induced Inflammation Affects Arterial Load. Lane AD, Kappus R, **Bunsawat K**, Phillips S, Woods J, Motl R, Baynard T, Fernhall B. *Artery* 6(4): 195, December 2012.
40. Sex Affects Autonomic Recovery Following a 45-minute Bout of Aerobic Exercise. **Bunsawat K**, Sun P, Yan H, Ranadive S, Kappus R**,** Lane A, Baynard T, Fernhall B, *Med Sci Sports Exerc*. 44(5S): 661, May 2012.
41. Whey Protein, Resistance and Sprint Interval Training and Healthy Lifestyle Strategies: Effects on Cardiometabolic Risk. Arciero PJ, Darin C, Zhen Q, **Bunsawat K**, Zhang J, Steward N. *FASEB J.* April 2011; 25:223.7.

SUBMITTED ABSTRACTS

1. Effect of Acute Resistance Exercise on Arterial Hemodynamics and Cerebral Blood Flow Dynamics: Does Sex Matter? Rosenberg AJ, Wee SO, Schroeder EC, **Bunsawat K**, Grigoriadis G, Griffith G, Fernhall B, Baynard T. Artery, Pisa, Italy, 2017.
2. Effect of Age on Systemic Hemodynamics and Carotid Artery Circumferential Strain Following Acute Resistance Exercise. Rosenberg AJ, Wee SO, Schroeder EC, Grigoriadis G, **Bunsawat K**, Griffith G, Baynard T. NAA, Chicago, 2017.
3. Sex Differences in Carotid Strain Following Resistance Exercise. Grigoriadis G, Rosenberg AJ, Wee SO, Schroeder EC, **Bunsawat K**, Griffith G, Baynard T. NAA, Chicago, 2017.
4. Effect of Age on Cerebral Blood Flow Dynamics Following Acute Resistance Exercise. Rosenberg AJ, Wee SO, Grigoriadis G, Schroeder EC, **Bunsawat K**, Griffith G, Baynard T. American Physiological Society, Cardiovascular Aging: New Frontiers and Old Friends, Westminster, CO, 2017.

PRESENTATIONS

1. Hemodynamics and Vascular Conductance Following Hypovolemic Pressure Challenge in Individuals with Down Syndrome. Wee SO, Rosenberg AJ, **Bunsawat K**, Griffith G, Fernhall B. Midwest ACSM 2015 Regional Chapter Annual Meeting, Ft. Wayne, IN, November 6-7, 2015 (Oral Presentation).
2. Effects of Caffeine on Cardiac Autonomic Function during Exercise Recovery. **Bunsawat K**, Rosenberg AJ, Wee SO, Kappus RM, Hultgren K, Fernhall B, Baynard T. Midwest ACSM 2013 Regional Chapter Annual Meeting, Radisson – Star Plaza, Merrillville, IN, November 8-9, 2013 (Oral Presentation).
3. Effects of Caffeine Supplementation on the Relationship between Total Arterial Compliance and Hemodynamic Parameters during Exercise Recovery. **Bunsawat K**, Rosenberg AJ, Wee SO, Kappus RM, Hultgren K, Fernhall B, Baynard T. The 3rd Annual Meeting of the North American Artery Society, September 7, 2013 (Poster Presentation).
4. Altered Autonomic Response to Upright Tilt in Individuals with Down Syndrome. **Bunsawat K**, Goulopoulou S, Collier S, Figueroa A, Pitetti K, Fernhall B, Baynard T. Midwest ACSM 2012 Regional Chapter Annual Meeting, Maumee Bay Lodge and Conference Center, Oregon, OH, November 1-3, 2012 (Oral Presentation).
5. Comparison of whey protein and resistance training versus healthy lifestyle strategies on total and abdominal body fat distribution and blood lipids in overweight/obese adults. Arciero PJ, Darin C, Zheng Q, **Bunsawat K**, Zhang J, Steward N. Distinguished Professional Free Communication Session, Mid-Atlantic Regional Chapter of ACSM, 33rd Annual Meeting, Harrisburg, PA November 5-6, 2010 (Oral Presentation).

RESEARCH GRANT

External Grant Funded

Predoctoral Fellowship—American Heart Association, December 2015. "The Role of Obesity on Neurovascular Responses to Exercise." (01-01-16 to 12-31-17; 16PRE26430096). $52,000 USD. PI-Bunsawat; Sponsor-Baynard

RECOGNITION/AWARDS

2017	- The American College of Sports Medicine – 2017 GSSI-ACSM Young Scholar Travel Award (American Colleges of Sports Medicine 2017 Conference; $1,000 USD cash prize)
	- The American Physiology Society – 2017 Caroline tum Suden / Frances Hellebrandt Professional Opportunity Award (Experimental Biology 2017 Conference; $500 USD cash prize)
2016	- University of Illinois at Chicago – Health Professional Student Council Travel Grant
	- University of Illinois at Chicago – Graduate Student Council's Travel Award
2015	- University of Illinois at Chicago – Graduate Student Council's Travel Award
	- University of Illinois at Chicago – Graduate College Student Presenter's Travel Award
2014	- University of Illinois at Chicago – Graduate College Student Presenter's Travel Award
	- University of Illinois at Chicago – Discretionary Funds
2013	- University of Illinois at Chicago – Graduate Student Council's Travel Award
2012	- University of Illinois at Chicago – Graduate College Student Presenter's Travel Award

AD HOC REVIEWER

2014-	Journal of Physical Activity and Health

CERTIFICATIONS AND MEMBERSHIPS

2015-	American Physiological Society (APS) member
2015-	Graduate Women in Science – Eta member
2014-	American Heart Association (AHA) member
2012-	Midwest American College of Sports Medicine (MWACSM) member
2010-	American College of Sports Medicine (ACSM) member