ABSTRACT

Title of Dissertation: ALLOSTATIC LOAD INFLUENCES VASCULAR FUNCTION AND SYMPATHOLYSIS IN YOUNG BLACK ADULTS

Lauren E. Eagan, Candidate for Doctor of Philosophy, 2024

Dissertation directed by: Associate Professor, Sushant M. Ranadive, Ph.D., Department of Kinesiology

In the U.S., Black individuals tend to face a disproportionately higher risk for hypertension. This is largely attributed to chronic sympathetic activation induced by heightened exposure to psychosocial stressors. Allostatic load (AL), an index of cumulative physiological dysfunction from chronic stress, is associated with hypertensive risk and is also heightened in Black adults compared to those of other racial groups. Indeed, increased sympathetic activity is a hallmark characteristic of both hypertension and AL. The inability to blunt sympathetic-induced vasoconstriction during exercise (impaired functional sympatholysis) is also associated with hypertension. This dissertation aimed to investigate whether AL was associated with measures of vascular health in young Black adults, both at rest and during a sympathetic stressor. In our first study, we examined associations between AL and indices of vascular function and structure among young Black adults at rest, finding that higher AL was associated with greater macrovascular dysfunction and amplified wave-reflections. Additionally, we identified significant correlations among greater self-perceived stress with smaller brachial artery diameters and greater wave-reflections. The second aim of this dissertation focused on the associations between AL and the magnitude of functional sympatholysis among this population. Results indicated a positive association between AL and functional sympatholysis, with amplified sympatholytic responses among young Black females, as compared to their male counterparts, when forearm volume was controlled for. Overall, our findings suggest that elevated AL might predict macrovascular dysfunction at rest, with larger arterial diameters potentially compensating for chronic stress. These adaptive mechanisms, commonly observed in aging and diseased states, may also explain the positive correlations between AL and the functional sympatholytic response in young Black adults. Our consistent observations of the redundant vascular mechanisms among young Black adults allowing for adaptation to chronic stress strengthen our findings and further highlight the complex interplay between stress and cardiovascular health in Black adults.

ALLOSTATIC LOAD INFLUENCES VASCULAR FUNCTION AND SYMPATHOLYSIS IN YOUNG BLACK ADULTS

by

Lauren E. Eagan

Dissertation submitted to the Faculty of the Graduate School of the
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Advisory Committee:
Associate Professor Sushant M. Ranadive, Ph.D., Chair
Associate Professor Steven J. Prior, Ph.D.
Associate Professor Michael D. Brown, Ph.D.
Assistant Professor Fanita A. Tyrell, Ph.D.
Associate Professor Amy R. Sapkota, Ph.D.

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Dedication

This dissertation is dedicated to my nieces, Abigail, Avery, Taylor, and Rowan Eagan. Together and apart, you four have gifted me with some of my happiest memories. Hearing you laugh is the melody that has carried me through even the darkest of days. Your boundless curiosity reminds me of the magic of girlhood and inspires me to embrace wonder in each day— a very helpful tool to have as a scientist! While the idea of a Ph.D. is still a bit confusing for you, just know that you've each played a big role in helping me find my way through one. Watching you grow fills me with pride, knowing that you are destined to become strong and brave young women (and Swifties). I feel endlessly lucky to know you, to love you, and to be your Lolo.

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Table of Contents

Dedication ii
Acknowledgements iii
Table of Contents v
List of Tables viii
List of Figures ix
List of Abbreviations x
Chapter 1: Review of The Literature 1
Cardiovascular Disease and Hypertension 1
Allostatic Load 2
Autonomic Nervous System Physiology 4
Defining Stress 4
Physiological Implications of Chronic Stress 5
Salivary Assessments of Diurnal Cortisol 7
Vascular Physiology 8
Vascular Dysfunction 8
Vascular Structure 11
Inflammation 12
Vascular Health in Black Individuals 14
Chronic Stress 15
Social Determinants of Health 16
Perceived Everyday Discrimination 17
Perceived Stress 17
Chronic Stress and Vascular Dysfunction 18
Chronic Stress and Arterial Stiffness 19
Chronic Stress and Inflammation 19
Chronic Stress Adaptations Between Sexes 22
Chronic Stress and Allostatic Load in Black Adults 22
Acute Stress 23
Acute Stress and Vascular Dysfunction 24
Acute Stress and Arterial Stiffness 26
Acute Stress and Inflammation 26
Acute Stress Responses in Black Individuals 27
Functional Sympatholysis 29
Mechanisms of Functional Sympatholysis 29
Impaired Functional Sympatholysis 31
Sex Differences in Functional Sympatholysis 33
Chapter 2: Allostatic Load and Vascular Health in Young Black Adults 34
Chapter Overview 34
Methods 37
Ethical Approval 37
Participants 37
Experimental Protocol 38
Health History Questionnaire 38

Self-Perceived Scales 39
Diurnal Slope of Salivary Cortisol 42
Resting Heart Rate and Blood Pressure 43
Total Body Fat Percentage 43
Blood Collection and Analysis 44
Macrovascular Function 45
Microvascular Function 47
Vascular Structure 48
Allostatic Load Scoring 49
Data Analysis 54
Results 56
Participant Characteristics 56
Allostatic Load Biomarkers 58
Allostatic Load Scoring 60
Vascular Function and Structure 61
Self-Perceived Scales 61
Macrovascular Function 63
Allostatic Load and Macrovascular Function 64
Microvascular Function 71
Allostatic Load and Microvascular Function 71
Vascular Structure 74
Allostatic Load and Vascular Structure 74
Discussion 82
Allostatic Load and Macrovascular Function 82
Allostatic Load and Microvascular Function 84
Allostatic Load and Vascular Structure 85
Allostatic Load, Vascular Health, and Perceived Stressors 87
Limitations and Future Directions 88
Chapter 3: Allostatic Load and Functional Sympatholysis in Young Black Adults 90
Chapter Overview 90
Methods 94
Ethical Approval 94
Participants 94
Experimental Protocol 94
Diurnal Slope of Salivary Cortisol 96
Resting Heart Rate and Blood Pressure 96
Total Body Fat Percentage 96
Blood Collection and Analysis 96
Allostatic Load Scoring 97
Functional Sympatholysis 97
Assessing the Functional Sympatholytic Response 99
Data Analyses 100
Results 103
Participant Characteristics 103
Allostatic Load Biomarkers 105
Allostatic Load Scoring 107

Cardiovascular Responses to Functional Sympatholysis 109
Forearm Blood Flow Magnitude of Sympatholysis 124
Forearm Vascular Conductance Magnitude of Sympatholysis 125
Allostatic Load and Magnitude of Sympatholysis 127
Discussion 130
Allostatic Load and Functional Sympatholysis 131
Sex Differences in Functional Sympatholysis 133
Limitations and Future Directions 135
Chapter 4: Summary of Findings 136
Aim 1 136
Aim 2 138
Collective Findings 139
Future Directions 140
Bibliography 142

List of Tables

Table 1: Examples of Primary and Secondary Mediators of Allostatic Load.
Table 2: Perceived Social Determinants of Health Scale.
Table 3: Perceived Everyday Discrimination Scale.
Table 4: Perceived Stress Scale.
Table 5: Clinical Cutoff Method for Scoring Allostatic Load Biomarkers.
Table 6: Standard Deviation Method for Scoring Allostatic Load Biomarkers.
Table 7: Aim 1 - Participant Characteristics and Raw Biomarkers.
Table 8: Aim 1 - Average Allostatic Load Indices by Sex.
Table 9: Descriptive Statistics of Measures of Vascular Function and Structure and Total Scores From Self-Perceived Scales.
Table 10: Correlation Coefficients From Associations of Allostatic Load Indices With Measures of Vascular Function and Structure and Total Scores From Self-Perceived Scales.
Table 11: Aim 2 - Participant Characteristics and Raw Biomarkers.
Table 12: Aim 2 - Average Allostatic Load Indices by Sex.
Table 13: Cardiovascular Responses to the Functional Sympatholysis Protocol.

List of Figures

Figure 1: Mechanistic Pathway by Which Shear Stress Elicits Nitric Oxide-Mediated Endothelial Dependent Vasodilation.
Figure 2: Proposed Contributors Linking Chronic Stress to Vascular Dysfunction, Arterial Stiffness, and Inflammation.
Figure 3: An Overview of The Proposed Mediators Contributing to Vasoconstriction and Vasodilation During Functional Sympatholysis.
Figure 4: Confirmatory Factor Analysis Model for the Scoring of Allostatic Load.
Figure 5: Correlation Between Unscaled %Flow-Mediated Dilation and Baseline Diameter.
Figure 6: Correlations Between Allostatic Load and Measures of Macrovascular Function.
Figure 7: Correlations Between SDoH-S, EDS, and PSS Scores and Measures of Macrovascular Function.
Figure 8: Correlations Between Allostatic Load Indices and Reactive Hyperemia Area Under the Curve.
Figure 9: Correlations Between Self-Perceived Scales With Measures of Microvascular Function.
Figure 10: Correlations Between Allostatic Load and Vascular Structure.
Figure 11: Correlations Between Responses to Self-Perceived Scales and Measures of Vascular Structure.
Figure 12: Protocol for Functional Sympatholysis Assessment.
Figure 13: Correlations Between Allostatic Load, Forearm Volume, and Left Arm Lean Mass.
Figure 14: Forearm Blood Flow and Vascular Conductance Responses to the Functional Sympatholysis Protocol.
Figure 15: Forearm Blood Flow and Vascular Conductance Responses to the Functional Sympatholysis Protocol (Controlled for Forearm Volume).
Figure 16: Correlations Between Allostatic Load and %Forearm Blood Flow and % Forearm Vascular Conductance Magnitude of Sympatholysis.
Figure 17: Correlations Between Allostatic Load and %Forearm Blood Flow and % Forearm Vascular Conductance Magnitude of Sympatholysis (Controlled for Forearm Volume).

List of Abbreviations

α1 – Alpha-Adrenergic Receptor 1
α2 – Alpha-Adrenergic Receptor 2
ACE – Angiotensin Converting Enzyme
ACTH – Adrenocorticotropic Hormone
ADO – Adenosine
AIx – Augmentation Index
AIx75 – Augmentation Index Standardized to Heart Rate of 75 Beats Per Minute
AL – Allostatic Load
Ang I – Angiotensin I
Ang II – Angiotensin II
ANS – Autonomic Nervous System
ATP – Adenosine Triphosphate
BF – Blood Flow
BD – Baseline Diameter
BP – Blood Pressure
Ca2+ – Calcium
CC – Clinical Cutoff
CFI – Comparative Fit Index
CMP – Guanosine 3',5'-cyclic monophosphate
CRH – Corticotrophin-Releasing Hormone
CRP – C-Reactive Protein
CPT – Cold-Pressor Task
CVD – Cardiovascular Disease
cAMP – Cyclic Adenosine Monophosphate
DBP – Diastolic Blood Pressure
DEXA – Dual Energy X-Ray Absorptiometry
DF – Degrees of Freedom
DHEA-S – Dehydroepiandrosterone Sulfate
EC – Endothelial Cell
ECG – Echocardiograph
EDHF – Endothelium-Derived Hyperpolarization Factor
EDS – Everyday Discrimination Scale
eNOS – Endothelial Nitric Oxide Synthase
ET-1 – Endothelin-1
ETA – Endothelin-1 Receptor A
ETB – Endothelin-1 receptor B
ET-1 – Endothelin-1
FAV – Forearm Volume
FBF – Forearm Blood Flow
FIML – Full Information Maximum Likelihood
FMD – Flow-Mediated Dilation
FVC – Forearm Vascular Conductance
GTP – Guanosine-5'-triphosphate

HHQ – Health History Questionnaire
HDL-C – High-Density Lipid Cholesterol
HPA – Hypothalamic-Pituitary-Adrenal
HR – Heart Rate
ICAM-1 – Intercellular Adhesion Molecule-1
iNOS – Inducible Nitric Oxide Synthase
IL-6 – Interleukin-6
LC-NE – Locus Coeruleus-Norepinephrine
LDL-C – Low-Density Lipid Cholesterol
MAP – Mean Arterial Pressure
MAPK – Mitogen-Activated Protein Kinase
MBV – Mean Blood Velocity
MoS – Magnitude of Sympatholysis
NE – Norepinephrine
NF-κB – Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells
NO – Nitric Oxide
NOS – Nitric Oxide Synthase
NOX – Nicotinamide Adenine Dinucleotide Phosphate Oxidase
NPY – Neuropeptide Y
O2 – Oxygen
$O2^-$ – Superoxide
$ONOO^-$ – Peroxynitrite
oxLDL – Oxidized Low-Density Lipoprotein
P1 – Purinergic G-protein-Coupled Adenosine Receptor
P2X – Purinergic Ligand-Gated Ionotropic Receptor
P2Y – Purinergic G-Protein-Coupled Metabotropic Receptor
PECAM-1 – Platelet Endothelial Cell Adhesion Molecule
PGI2 – Prostacyclin
PSS – Perceived Stress Scale
Q – Cardiac Output
RAAS – Renin-Angiotensin-Aldosterone System
RBC – Red Blood Cell
RH-AUC – Reactive Hyperemia Area Under the Curve
RMSEA – Root Mean Square Error of Approximation
ROS – Reactive Oxygen Species
RHR – Resting Heart Rate
SAM – Sympatho-adrenomedullary
SD – Standard Deviation
SES – Socioeconomic Status
SDoH – Social Determinants of Health
SDoH-S – Social Determinants of Health Scale
SBP – Systolic Blood Pressure
SNA – Sympathetic Nerve Activity
SNS – Sympathetic Nervous System
SRMR – Standardized Root Mean Square Residual
SV – Stroke Volume

T1DM – Type 1 Diabetes Mellitus
TBF – Total Body Fat
TC – Total Cholesterol
TC/HDL-C – Total Cholesterol-to-High Density Lipoprotein Cholesterol Ratio
TLI – Tucker-Lewis Index
TPR – Total Peripheral Resistance
VCAM – Vascular Cell Adhesion Molecule

Chapter 1: Review of The Literature

Cardiovascular Disease and Hypertension

Cardiovascular disease (CVD) is the leading cause of death worldwide (1). The most-recent estimations of CVD prevalence among American adults 20 years of age and older is 49.2% – encompassing an estimated 127 million individuals (2). There is a disproportionately higher burden of CVD among Black adults in the U.S. with 60.1% of non-Hispanic Black males and 58.8% of non-Hispanic Black females having CVD, as compared to 53.6% and 42.1% of non-Hispanic White males and females, respectively (2). Additionally, Black individuals develop CVD earlier in life (3), and experience shorter life-expectancies due to CVD-induced mortality as compared to Americans of other racial identities (4, 5).

Hypertension, or high blood pressure, is the leading cause of CVD in the U.S., affecting 47.3% of adults, or approximately 121 million people (2, 6). The prevalence of hypertension among Black individuals in the U.S. is among the highest in the world. Indeed, the prevalence of hypertension among adults 20 years and older is 58.3% among non-Hispanic Black males and 57.6% among non-Hispanic Black females, as compared to 51% and 40.5% among non-Hispanic White males and females, respectively (4). Moreover, Black individuals may have higher blood pressure beginning in childhood, as well as a higher incidence and prevalence of hypertension across the lifespan as compared to Americans of other racial identities (4, 6–8).

Allostatic Load

The physiological burden imposed by chronic stress has been termed “allostatic load” – a concept associated with allostasis, or the capacity by which the body can successfully mediate various parameters of its internal environment to accommodate for stressful challenges and/or changing demands of the external environment (9, 10). The concept of allostasis varies from the traditional "homeostatic" model, which defines health by emphasizing that physiological set-points remain within normal ranges, and that derivations from those ranges permit medical intervention. Unlike homeostasis, allostasis recognizes health as a state of optimal responsiveness, accommodates for predictive and dynamic fluctuation of physiological processes, and involves integration between the brain's feedback regulation and the whole-body’s adaptation (11). The use of allostasis has led to new ways of understanding complex, multi-system physiological activities that involve repeated allostatic responses activated during stressful situations. Indeed, chronic stress exaggerates ‘wear and tear’ on the body by curbing normal engagement of physiological allostatic load (AL) (9). Thus, chronic, or frequent challenges to allostasis beget “allostatic overload,” which is associated with various adverse health outcomes (9, 10).

AL is typically measured across four physiological systems: the 1) cardiovascular; 2) inflammatory; 3) metabolic, and 4) neuroendocrine systems (9, 10, 12–14). Traditional measures of AL include subclinical indicators of the bodily response to chronic stress, with particular focus on responses that are known to increase the risk of morbidity across the four targeted systems. The original biomarkers of AL include two classes of mediators. Primary mediators have included: concentrations of the stress hormones, norepinephrine (NE) and epinephrine, cortisol, and dehydroepiandrosterone sulfate (DHEA-S), present in the system at rest (**Table 1**) (10, 15). The second category is comprised of the physiological effects resulting from actions of the primary

mediators. Secondary mediators have included, but are not limited to: systolic and diastolic blood pressures, concentrations of inflammatory cytokines, cholesterol levels, glycated hemoglobin levels, and waist-to-hip ratio (**Table 1**) (15). AL has often been operationalized by algorithms containing measurements of primary mediators and secondary effects, with associated risks assessed on a scale, and summed to produce a total score (14, 16–19). However, there is a lack of generalized consensus on the construction of the equation for AL scores (17, 19, 20).

Table 1: Examples of Primary and Secondary Mediators of Allostatic Load.

Category	Marker	Functional Purpose
Examples of Primary Mediators	Cortisol (saliva, urine, or hair)	Integrated measure of HPA activity
	DHEA-S (serum)	Functional HPA antagonist and indicatory of adrenal gland functioning
	Epinephrine and NE (plasma or urine)	Integrated indices of SNA system activity
Examples of Secondary Mediators	Systolic Blood Pressure	Indices of cardiovascular activity and major risk factor for CVD
	Diastolic Blood Pressure	
	Waist-to-Hip Ratio	Index of long-term levels of metabolism and adipose tissue deposition
	HDL Cholesterol	Index of atherosclerotic risk protection
	Total Cholesterol	Index of long-term atherosclerotic risk
	Glycated Hemoglobin	Integrated measure of glycemia over 2-3 months

Table 1 Key: HPA, hypothalamic-pituitary-adrenal axis; DHEA-S, dehydroepiandrosterone sulfate; NE, norepinephrine; SNA, sympathetic nerve activity; CVD, cardiovascular disease; HDL, high-density lipoprotein. Adapted from: Rodriquez et al., 2019 (16).

Previous literature assessing AL suggests that higher AL scores are associated with older age (10), lower socioeconomic status (SES) (21, 22), unsupportive social relationships (23), among many other psychosocial and environmental stressors. Black race in the U.S. has also been associated with higher AL (24). Indeed, it has been well established in the literature that Black adults have higher cumulative AL as compared to their White counterparts (24–35). However, the association between AL and vascular function and/or structure has not been assessed

comprehensively in the literature, let alone in a population of young Black adults. This gap in the literature thus potentiates physiological comparisons between individuals of different racial groups without considering the unique psychosocial stressors that may be contributing to them.

Autonomic Nervous System Physiology

The central nervous system is involved in gathering sensory inputs and integrating them into motor actions. The peripheral nervous system, on the other hand, which consists of all nerves extending from the brain and spinal cord, functions to communicate impulses and signals between the central nervous system and the rest of the body. The autonomic nervous system (ANS) is a branch of the peripheral nervous system involved in involuntary bodily actions and functions such as breathing, heart rate regulation, and the control of blood pressure. The ANS is the primary regulator of cardiovascular function, consisting of two functional subdivisions – the sympathetic (SNS) and parasympathetic nervous systems.

Pre- and post-ganglionic nerves from the SNS release NE, a catecholamine that acts on α- and β-adrenergic receptors on the heart and arteries to increase heart rate (HR), cardiac contractility, and peripheral vascular resistance (36). By virtue of these three increases, augmented SNS activation inherently increases arterial pressure (when all else is equal) since, $\text{mean arterial pressure} = \text{HR} \times \text{stroke volume} \times \text{total peripherial resistance}$ (37).

Defining Stress

"Stress" is modernly understood as a bodily state of elevated homeostatic threat(s) provoked by sociological, psychological, environmental, and/or other physiological stimuli (15). The magnitude of the stress response depends on a dynamic interplay between an individual, their environmental demands, biological adaptations, and the psychosocial processes determining their

appraisal of that threat (15). Therefore, the stress response may vary between individuals possessing stress-mitigating vs. stress-amplifying psychosocial traits and/or physiological states.

Physiologically, stress can be defined as an activation of the SNS, which requires cellular adaptation to recalibrate and mitigate the threat (38). Both acute and chronic stress activate the SNS, which directly innervates vascular and perivascular tissues and spaces. While different stressors (e.g., physical or psychological, acute or chronic, etc.) engage with unique regions of the brain to be processed, all stressors converge on 1) the hypothalamic-pituitary-adrenal (HPA) axis and 2) the sympatho-adrenomedullary (SAM) axis (39). Collectively, the secretion of stress hormones stimulated by the HPA and SAM axes generate a coordinated response that, under acute or "healthy" conditions, promote energy mobilization, metabolic changes and activation and subsequent suppression of the immune system in order to restore bodily homeostasis in response to the stress (39, 40). However, with chronic activation of the stress response, the perpetual elevation in circulating stress hormones tends to desensitize or inhibit the function of the stress hormone receptors, thereby, reducing their acute ability to suppress inflammation, and alternatively, promoting an elevated basal state of inflammation (41).

Physiological Implications of Chronic Stress

Chronic stress is associated with cardiovascular dysregulation due to repeated and prolonged activation of the SNS. Mechanistically, chronic stress induced SNS activation leads to increased production of NE and cortisol, among other glucocorticoids, which are implicated in the development of excessive peripheral vasoconstriction and upregulated sodium retention, among other mediators of increased CVD risk (42). Sympathetic stimulation can be replicated in the laboratory setting through physiological induction of a sympathetic stimulus. Induction of a sympathetic stimulus increases sympathetic nerve activity (SNA), thus increasing NE release into

systemic circulation. Under healthy conditions, the body can retain allostasis to account for sudden increases in SNA. However, in unhealthy conditions, whereby homeostasis is off-balance (suggestively, among individuals with increased AL), the ability to divert the adverse downstream effects of the sympathetic stimuli are blunted, resulting in an exaggerated and/or impaired neuroendocrine, cardiovascular, metabolic, and inflammatory response. Increased AL, resulting from chronic stress and prolonged activation of stress response systems, can lead to cardiovascular, metabolic, and inflammatory dysregulation.

Indeed, chronic stress and high AL have been associated with an increase in total body fat, partially due to elevated cortisol (43). This increase in total body fat is associated with a higher risk of cardiometabolic dysregulation and disease development (44). Chronic stress and elevated AL have also been associated with dysregulation in glucose metabolism. Specifically, high cortisol levels can impair insulin action and promote gluconeogenesis, resulting in elevated blood glucose levels (48). Further, AL can affect lipid profiles, including HDL and LDL cholesterol. Chronic stress and high cortisol levels have been associated with reduced levels of HDL cholesterol, commonly referred to as "good" cholesterol (45). HDL cholesterol plays a protective role against CVD by facilitating the reverse transport of cholesterol from peripheral tissues to the liver. In contrast, chronic stress can promote higher levels of LDL cholesterol, known as "bad" cholesterol, which is associated with an increased risk of atherosclerosis and cardiovascular complications (45). Increased AL and chronic stress can also impact renal function, as reflected by alterations in serum creatinine levels (46). Creatinine is a waste product generated by muscle metabolism and excreted by the kidneys. In conditions of prolonged stress and elevated cortisol, changes in renal blood flow and impaired kidney function may occur, leading to alterations in creatinine levels (46).

The dysregulations mentioned above represent some of the metabolic and anthropometric changes associated with increased AL and chronic stress. It is worth noting that these changes are also influenced by various factors, including individual susceptibility, genetic predisposition, lifestyle factors, and the presence of pre-existing metabolic conditions. Therefore, maintaining a healthy lifestyle, including stress management techniques, regular exercise, and a balanced diet, is crucial in mitigating the adverse effects of chronic stress on metabolic health.

Salivary Assessments of Diurnal Cortisol

Cortisol concentrations follow a diurnal pattern and vary significantly across the day (i.e., high levels in the morning at waking, dramatic increase in cortisol production after waking with a general decline across the day, and lower levels in the evening hours) (47). Measuring cortisol from saliva samples instead of blood samples offers several advantages. Firstly, since it is recommended to examine cortisol levels on at least two occasions throughout the day, collecting saliva samples is considerably less invasive and more convenient for participants to do by themselves at home, as compared to asking them to visit the lab several times for blood draws. Secondly, in the blood, only about 5-10% of cortisol is in its unbound biologically active form, with the remaining 90-95% being bound to proteins in the serum (48). Salivary cortisol measures, on the other hand, provide a reliable and direct assessment of the hormone's unbound and bioavailable form, offering a more accurate representation of its functional activity in the body (48). Further, salivary cortisol levels are comparatively much more resistant to degradation by freeze-thaw cycles than blood samples, making their transportation and storage more practical (49, 50).

Vascular Physiology

Arteries are comprised of three layers that surround the vessel's lumen. The outermost later of the artery, the tunica adventitia, is composed of intertwined fibers of collagen and elastin and acts to maintain the structural integrity of the vessel. The middle layer of arteries, the tunica media, consists mostly of smooth muscle cells and elastic connective tissue. Throughout the arterial network, the tunica media is typically the thickest of the three layers, playing a key role in controlling the caliber of the arterial lumen by vasoconstricting and vasodilating in response to changes in metabolic, immunological, or nutritional needs. The inner-most layer of the arteries, the tunica intima, is made up of a monolayer of endothelial cells (ECs), which have direct contact with blood as it flows through the lumen. ECs form tight adherents and gap junctions between each other to maintain endothelial integrity. Further, ECs control vascular tone by synthesizing and releasing various vasodilating and/or vasoconstricting factors. Therefore, in its capacity to modulate the vasculature, the endothelium can also manipulate blood pressure and flow throughout the various branches of the arterial network. Collectively, the endothelium functions as a semipermeable barrier, regulating fluid, nutrient, and metabolite exchange to regulate and repair homeostasis of the vasculature.

Vascular Dysfunction

In a healthy state, the release of vasodilating mediators, such as nitric oxide (NO) (**Figure 2**) and adenosine (ADO), are balanced with the release of vasoconstricting mediators, such as endothelin-1 (ET-1) and angiotensin II (Ang II), to promote vascular homeostasis. Conversely, a state of impaired endothelial function is characterized by the reduced bioavailability of vasodilating mediators, increased bioavailability of vasoconstricting mediators, and the presence

of oxidative stress, assessed via excess production of reactive oxygen species (ROS). Since NO is the main vasodilatory mediator, its bioavailability is strongly linked to the health of the ECs. Together, lower levels of NO, and higher levels of ROS promote a pro-inflammatory and pro-thrombotic environment that impair endothelial function. As reviewed by Bonetti et al., in the presence of hypertension, reduced NO bioavailability, in particular, can promote unfavorable structural and functional alterations in the vasculature, further contributing to endothelial dysfunction (51). Thus, decreased NO bioavailability is considered an integral part of the causal pathway toward the onset of hypertension, with reduced NO bioavailability-induced endothelial dysfunction being observed prior to the manifestation of overt hypertension (51). Similarly, increased oxidative stress, as measured by an increase in ROS, is a well-documented mechanism contributing to endothelial dysfunction and subsequent onset of hypertension (52). Specifically, the ROS, Superoxide (O_2^-), reacts with bioavailable NO to form peroxynitrite (ONOO-), a potent oxidant and nitrating agent that can cause protein and lipid oxidation, DNA damage, and mitochondrial dysfunction, which contribute to EC death (23).

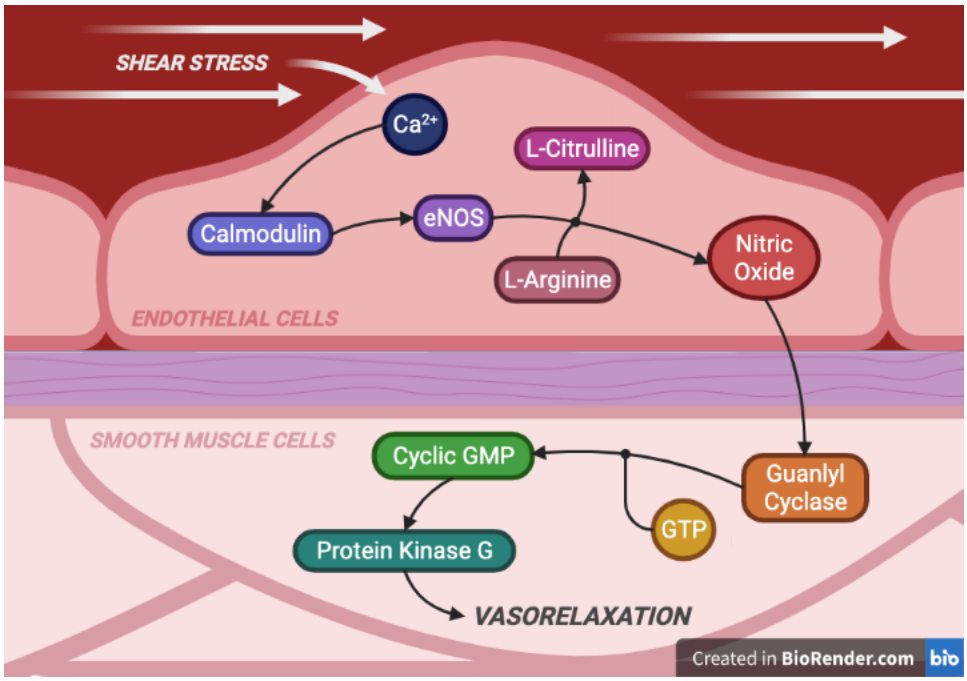


Figure 1: Mechanistic Pathway by Which Shear Stress Elicits Nitric Oxide-Mediated Endothelial Dependent Vasodilation.

Figure 1 Key: Ca^{2+}, calcium; eNOS, endothelial nitric oxide synthase; GTP, Guanosine-5'-triphosphate; CMP, Guanosine 3',5'-cyclic monophosphate. Created with BioRender.com

Evaluating Vascular Dysfunction

Flow-mediated dilation (%FMD) is a technique that assesses by the ability of the vascular endothelium to induce arterial vasodilation and is thought to be ~72% driven by NO (53). NO is well recognized as a potent inducer of endothelium-dependent vasodilation (**Figure 1**). %FMD tests the capacity for the brachial artery to vasodilate in response to reactive hyperemia following an acute occlusion of blood flow using a blood pressure cuff around the upper forearm. Generally, a larger percentage change from the artery's diameter post-occlusion compared to the diameter at baseline, serves as an indicator of healthy vascular function.

Vascular Structure

In healthy arteries, there is high distensibility of the arterial walls— meaning, that the walls can vasoconstrict and vasodilate appropriately in response to changes in blood pressure and flow. Greater distensibility indicates less stress on the vessel's walls during systole, suggesting a higher degree of vessel elasticity and compliance. Meanwhile a lower distensibility of the arterial walls (or, increased arterial stiffness) indicates higher stress on the vessels. Greater stiffness in the structural components of the arteries compromise their ability to appropriately vasoconstrict and vasodilate to accommodate for changes in pressure and flow. Arterial stiffness and endothelial dysfunction seem to occur in concert, with greater endothelial dysfunction resulting in greater arterial stiffness, and vice versa. As reviewed by Vatner et al., with normal aging, there are unfavorable shifts in the structure and function of the arterial walls, lending themselves to thicker and stiffer (less elastic) phenotypes (54). Arterial stiffness is also characteristic of increased arterial calcification, wall diameter (intima-media thickness), collagen deposition and elastin fragmentation (54). Behavioral attributes such as sedentarism (55) and high dietary salt intake (56) are known to amplify central arterial stiffness (as assessed via carotid-to-femoral pulse wave velocity). Furthermore, arterial stiffness can be a contributor to and/or consequence of various CVDs such as hypertension and diabetes, occurring independent of age-related increases in stiffness (54). Much like endothelial dysfunction, there is a bidirectional relationship between increased arterial stiffness and increased systemic inflammation. Possible mechanisms by which arterial stiffness, endothelial dysfunction, and inflammation reinforce each other's responses are outlined in **Figure 2**, adapted from Sher et al., 2020 (57).

Evaluating Vascular Structure

Carotid-to-femoral pulse wave velocity (cfPWV) is a commonly used surrogate measure to assess central arterial stiffness. Central arterial stiffness refers to the rigidity or reduced elasticity of the large arteries in the central part of the circulatory system, such as the carotid and femoral arteries. cfPWV is determined by measuring the time it takes for the arterial pressure wave to propagate from the carotid artery (located in the neck) to the femoral artery (located in the upper thigh). The principle behind this measurement is that stiffer arteries transmit the pressure wave faster than more elastic arteries. To measure cfPWV, tonometers are placed over the carotid and femoral arteries to capture the arterial pressure waveforms. The time delay between the two waveforms is then determined, along with the distance between the two measurement sites (carotid and femoral arteries). By dividing the distance by the time delay, cfPWV is calculated as the velocity of the pulse wave traveling between the two arterial sites. Higher cfPWV values indicate increased arterial stiffness, which is associated with various cardiovascular risk factors and conditions, such as hypertension (54, 58). Therefore, through its capacity to assess arterial stiffening, cfPWV serves as a valuable technique for evaluating the structure of the central arteries.

Inflammation

The immune system's main role is to provide an active defense against bacteria, viruses, toxins, and infections by eliminating foreign pathogens and promoting tissue repair and recovery. The biological response of the immune system involves processes that promote inflammation – a defense mechanism imperative to healing and restoring health. Most commonly, upon the body's recognition of a pathogenic threat, the immune response will activate leukocytes (white blood cells) that produce small signaling proteins called cytokines. Cytokines include proteins called chemokines, interferons, interleukins, lymphokines, and tumor necrosis factors. At any given time

throughout an immune response, the multitude of released cytokines can interact in pleiotropic, redundant, synergistic, antagonistic and/or cascade-inducive manners with the intention to advance the healing process. Acute cytokine activity also involves vascular responses to aid in the healing process. As reviewed by Zhang et al., during an acute inflammatory response, the microcirculation will firstly undergo vasodilation, then undergo an increase in vascular permeability, and lastly adapt to promote vascular stasis (59). In the case of an acute immune response, cellular and molecular communication (via cytokine activity) will eventually mitigate the threatening stimuli until the affected tissue/s return to their homeostatic state; whereby, acute inflammation can subside (60). However, in the presence of various social, psychological, environmental and/or biological factors, uncontrolled acute inflammation can shift into a chronic inflammation (61).

The mediators of chronic, low-grade inflammation (i.e., proinflammatory cytokines and acute phase proteins) are largely studied as surrogate biomarkers of and players in the casual pathway toward many, if not all, CVDs or events. Low-grade inflammation is commonly assessed by measuring circulating concentrations of the proinflammatory cytokine, interleukin-6 (IL-6), and the acute phase protein, C-reactive protein (CRP). Upregulated concentrations of IL-6 and/or CRP have been mechanistically linked to various adverse health outcomes such as increased ambulatory blood pressure (62, 63) arterial stiffness (64, 65), and risk for acute cardiac events (66).

The adverse health effects associated with increased IL-6 and CRP are most prominently due to their damaging effects on the endothelium (67). As recently reviewed by Sullivan et al., IL-6 is produced by and secreted from macrophages within the endothelium (68), and is involved in CRP synthesis. IL-6 can also increase secretion of other proinflammatory cytokines, making it a crucial contributor in amplification of the inflammatory response (68). Systemically elevated concentrations of IL-6 and CRP are known to interfere with the balance of endothelial substances

that regulate arterial tone, leading to vasoconstrictive predominance and consequential endothelial dysfunction (59, 69). As reviewed by Zhang et al., a potential mechanism by which elevated IL-6 and CRP promote endothelial dysfunction is via their role in inhibiting endothelial nitric oxide synthase (eNOS) and subsequentially reducing the bioavailability of NO (59). The resulting reduction in NO bioavailability hampers the anticoagulant and anti-inflammatory properties of the endothelium, thereby promoting endothelial dysfunction (59). Further, intracellular pathways such as the mitogen-activated protein kinase (MAPK) and nuclear factor kappa-B (NF-κB) pathways are initiated with continual binding of IL-6 and CRP to their receptors, as in chronic, low-grade inflammation. As reviewed by Chen et al., NF-κB expression exacerbates production of proinflammatory cytokines and inflammatory cell recruitment, further contributing to the chronic inflammatory response, suppression of vascular function, and increase in oxidative stress (70). Specifically, elevated oxidative stress begets scavenging of NO, activation of the endothelial cells, and their subsequent production of vasoconstrictive agents, such as ET-1 (71). Collectively, this process drives unfavorable vascular remodeling and perpetuation of the proinflammatory response (71). Thus, insufficient cellular protection against oxidative stress and chronic levels of proinflammatory cytokines have been implicated as major factors in the development for CVD (72).

Vascular Health in Black Individuals

Young, normotensive Black adults have greater vascular endothelial dysfunction at rest as compared to their White counterparts (73–77); however, this finding is not universal (78–80). Greater vascular dysfunction in Black adults could be mechanistically explained by results from several studies suggesting that Black individuals have impaired NO bioavailability (81, 82), elevated systemic inflammation (83–86), and/or greater oxidative stress (82, 84, 87, 88) compared

to adults of other racial identities. Similarly, arterial stiffness has been shown to be higher in young Black adults (89, 90), which could explain this group's greater propensity for endothelial dysfunction, or vice versa. Further, in Black adults with masked prehypertension (elevated blood pressure), a positive relationship has been observed between endothelial dysfunction and concentrations of CRP (91). The potential for these early adverse changes in the vascular and immune profiles of young, otherwise healthy Black individuals are of particular interest since endothelial dysfunction, arterial stiffness, and chronic inflammation have shown significantly independent and synergistic associations with greater risk for hypertension later in life (92).

While many of the aforementioned studies allude to psychosocial stress as a potential mediator for racial differences in endothelial dysfunction, arterial stiffness, and inflammation, the associations between AL and vascular function, and AL and vascular structure have yet to be explored in young Black individuals.

Chronic Stress

In the presence of prolonged periods or repeated exposures to stress, sustained arousal of the SNS impairs the plasticity of various physiological systems, thereby disrupting allostasis. Indeed, chronic stress is a well-established risk factor for various CVDs such as; atherosclerosis (93), obesity (94), metabolic syndrome (95), and hypertension (96). Taken together, overactivation of the SNS compromises the body's ability to effectively adapt to psychological stress in such a way that would otherwise be maintained by its natural drive for sustained allostasis (39).

Indeed, the physiological adaptations to chronic stress exposure have been implicated as important drivers of CVD development (97–99). Thus, a plausible explanation for the persistently higher CVD prevalence and incidence among Black individuals may be the multiple and unique sources of greater chronic stress.

In this context, it is important to acknowledge that both acute and chronic stress are multi-dimensional constructs made up of the stressor's unique characteristics and an individual's specific perceptions of, and behavioral response to, that stressful stimuli (100). Therefore, the tipping-point at which a physiological stress response shifts from being beneficial to pathological varies between and within individuals, thus complicating existing research efforts to identify the underlying mechanisms and pathological manifestations of between populations (57).

Previous studies have shown that chronic stress is a risk factor for hypertension (96, 99, 101), however, the underlying mechanisms by which this occurs in young Black adults are unclear. Insight into this specific population is warranted given the well-established vulnerability of Black individuals to experience increased chronic stress and hypertension, in conjunction with the currently limited understanding of how these conditions interact in young Black adults.

Social Determinants of Health

The Social Determinants of Health (SDoH) are the economic, social, environmental, and psychosocial domains encompassing where we are born, grow, live, work, and age that aggregate to shape overall health and well-being (102). Specifically, the SDoH include an individual's economic stability, neighborhood and built environment, access to and quality of education and health care, and social and community relationships (102). At the population level, Black individuals experience disparities in each of these domains within the U.S. (103).

SDoH domains have been identified as sources of chronic psychosocial stress to individuals suffering from low socioeconomic status (SES), unsafe housing, neighborhood violence, limited access to health care, early childhood adversity, food insecurity, and decreased sleep quality, among many others (104). These common sources of stress combine with more salient sources of stress that are particularly prevalent among Black individuals, such as perceived

racism and discrimination, to further augment risk for hypertension (96, 105–108), obesity (109), and other incident CVD events (110).

Perceived Everyday Discrimination

Several studies have reported that among Black adults in the U.S., greater perceived racial discrimination is associated with significantly higher levels of chronic inflammation, a well-known contributor toward hypertension (111–113). Dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis, which controls the bodily response to stress, is another pathway through which racial discrimination is thought to lead to increased risk for CVDs (114).

In a comprehensive meta-analytic review investigating the association between discrimination and health, it was found that perceptions of discrimination were not only linked to negative psychological stress responses, but also to heightened physiological stress responses (115). The authors suggested that these stress responses, potentially mediated through AL, might contribute to adverse physical and mental health outcomes (115). However, the direct examination of these relationships was not undertaken in the study. Given that discrimination often acts as a recurring stressor and can impact individuals throughout their lives (116), it is crucial to understand the potential implications of discrimination within the framework of cumulative stress experienced by the body. Such understanding could shed light on how discrimination contributes to the existing disparities observed in AL (117).

Perceived Stress

Perceived stress is defined as individuals' perception of their ability to manage their stress and control their lives using coping resources (118, 119). Perceived stress has been shown to mediate the associations between contextual stressors and indices of individuals' wellbeing (118,

120). Specifically, experiencing contextual stressors (such as ethnic discrimination and family financial stress) diminishes the ability of young adults to manage their stress, which translates into lower life quality (120), and higher likelihood for experiencing depressive symptomology (118). Previous research has also shown that independently, higher perceptions of stress are correlated with increased levels of CRP, HDL-C, glucose, and SBP (121). While the literature is divided on whether there are (122–124) or are not (125, 126) racial differences in measures of perceived stress among individuals in the U.S. (as assessed by the 10-item perceived stress scale by Cohen et al., 1983), perceived stress may play a role in the relationship between AL and vascular health in young Black adults, and thus may help explain the higher AL and increased risk for hypertension among this population.

Chronic Stress and Vascular Dysfunction

Chronic stress is hypothesized to elicit vascular dysfunction via various independent and feed-forward mechanisms involving the neuroendocrine, cardiovascular, metabolic, and immune systems (**Figure 2**) (57). As reviewed by Lai and Kan, overactivation of the SNS, renin-angiotensin-aldosterone system (RAAS), and subsequent overproduction of stress hormones from the HPA and SAM axes in response to chronic stress, are also mechanistically linked to increased CVD risk via systemically elevated homocysteine levels (127). High levels of homocysteine induces a heightened state of vascular reactivity that is characteristic of endothelial injury due to the buildup of adhesion molecules on the ECs, themselves (127). Specifically, an increase in the adhesion molecules, platelet endothelial cell adhesion molecule (PECAM-1) and E-selectin, promote the binding, rolling, and transmigration of white blood cells into the sub-endothelial space, which is a key step in smooth muscle proliferation, as well as in the production of foam cells and ROS (128).

Chronic Stress and Arterial Stiffness

The negative effects of chronic stress on vascular function have also been observed as increases in central arterial stiffness (as measured by cfPWV). Recent data collected on Brazilian adults suggest that Black and Brown individuals 1) experience a significantly higher prevalence of racial discrimination as compared to their White counterparts and, 2) have significantly higher cfPWV as compared to their White counterparts; with cumulative data supporting a significant association between everyday discrimination and arterial stiffness (129). While a cohort of young healthy individuals have yet to be studied in the U.S., data from patients with a history of myocardial infarction show that experiences with everyday discrimination are associated with arterial stiffness among Black women, but not Black men, White women, nor White men (130). This study suggests there may be sex specific implications on the effect of chronic stress on arterial stiffness in Black adults.

Chronic Stress and Inflammation

Increased systemic inflammation (as measured by concentrations of IL-6 and CRP) are observed in individuals with various CVDs and have been associated with increased cortisol production (95, 131, 132). Thus, elevated levels of cortisol due to chronic stress have been proposed to play an important role in the process and progression of chronic inflammation and CVD onset (57, 133, 134). However, chronically elevated systemic inflammation has also been closely associated with heightened psychosocial and environmental stressors in otherwise healthy populations (104, 135). For example, concentrations of IL-6 and CRP are higher among healthy adults living in low SES environments as compared to those living in higher SES environments (136). Low SES circumstances in the early years of life (ages 2 to 3 years) predict inflated expression of inflammatory phenotypes in adolescence (137). Indeed, individuals with chronic

stress also present with consistent upregulation of the RAAS (138), an endocrine system involving kidney-derived renin regulating the production of Ang II. Specifically, under chronic stress conditions, the upregulation in SNS activation increases renin production and releases it into circulation where it acts in the formation of angiotensin I (Ang I) (42). With endothelial-derived activation of the angiotensin converting enzyme (ACE), Ang I is converted into Ang II, which, when elevated, contributes to elevated formation and release of adrenocorticotropic hormone (ACTH), cortisol, aldosterone, and NE (130). Increased levels of Ang II inhibit formation of eNOS (139) and play a role in elevating blood volume and pressure, arterial stiffness (140), and concentrations of IL-6 (141). Therefore, augmented RAAS activity throughout vascular cells seems to be an important link between chronic stress and adverse cardiovascular outcomes. Indeed, overactivation of RAAS is also evident in human and animal models of obesity and diabetes, among other chronic CVDs (139).

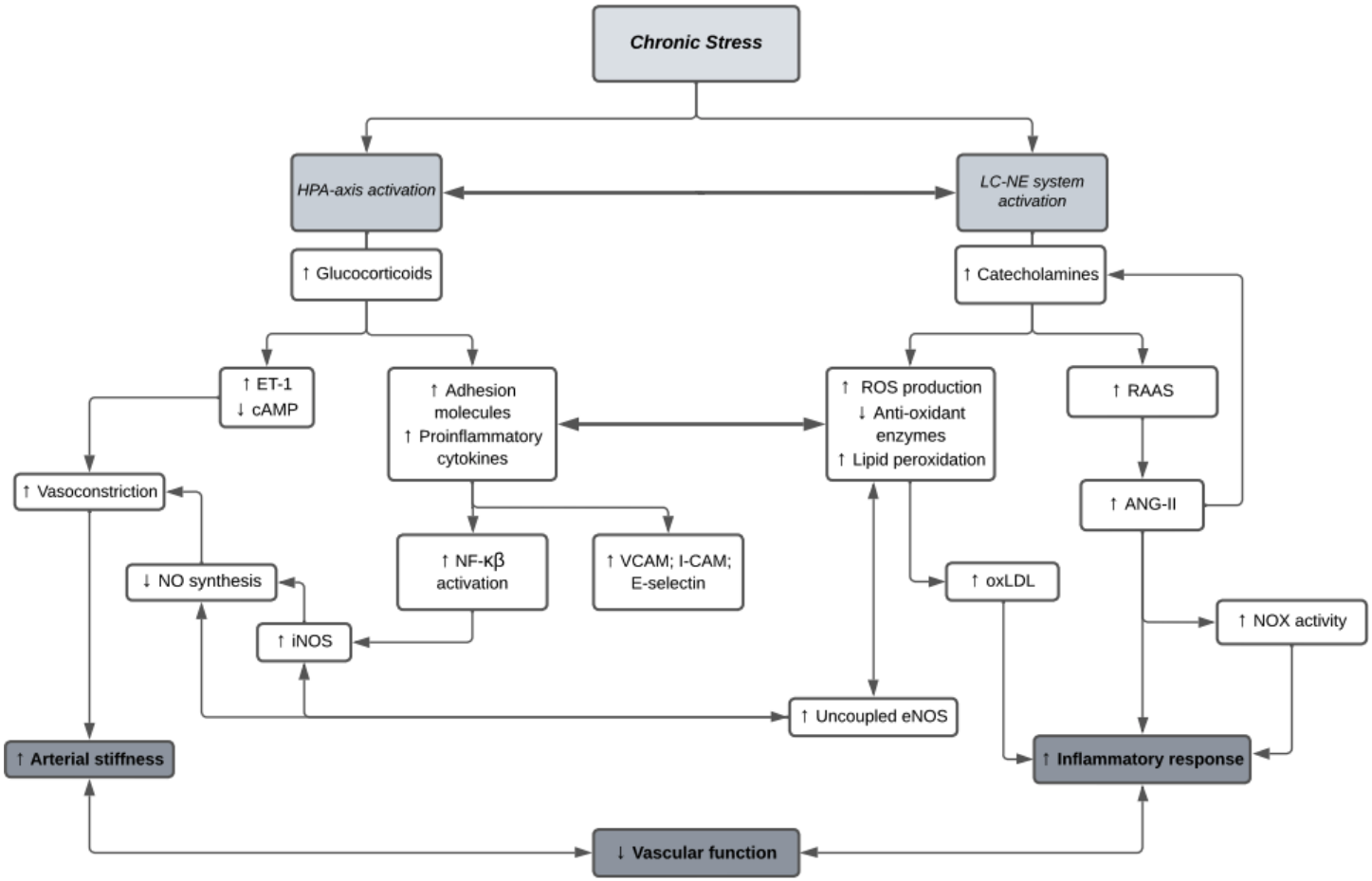


Figure 2: Proposed Contributors Linking Chronic Stress to Vascular Dysfunction, Arterial Stiffness, and Inflammation.

Figure 2 Key: Ang II, angiotensin II; cAMP, cyclic adenosine monophosphate; eNOS, endothelial nitric oxide synthase; ET-1, endothelin-1; HPA, hypothalamic-pituitary-adrenal axis; ICAM-1, intercellular adhesion molecule-1; iNOS, inducible nitric oxide synthase; LC-NE, locus coeruleus-norepinephrine; NF-κB, nuclear factor κ-light-chain-enhancer of activated B cells; NO, nitric oxide; NOX, NADPH oxidase; oxLDL, oxidized LDL; RAAS, renin-angiotensin-aldosterone system; ROS, reactive oxygen species; VCAM, vascular cell adhesion molecule. Figure adapted from Sher et al., 2020 (57).

Chronic Stress Adaptations Between Sexes

Men and women seem to have aberrant responses to acute and chronic stress mediated by sex differences in the function and regulation of the HPA axis (142). In a cohort of chronically stressed police officers, there was a significant association between higher concentrations of awakening cortisol and endothelial dysfunction (as indicated by lower %FMD) among women, of which was not apparent among men (143). Sex differences in the vascular response to acute and chronic stress may be influenced by the paradoxical effects of female vs. male sex hormones on the magnitude of the stress response from the HPA axis. Indeed, estrogen levels are known to amplify the magnitude of the stress response at the level of the HPA axis, which has been suggested as a mechanism by which females display higher levels of circulating glucocorticoids, and increased stress-related symptomology (144). Further, baseline measures of self-perceived depression and depressive symptoms significantly predicted coronary heart disease risk and AL among Black women, but not Black men (145). In a separate cohort of individuals diagnosed with major depressive disorder, women also had significantly higher concentrations of IL-6 than their male counterparts (146). Taken together, these findings suggest that there may be sex differences in the relationship between chronic stress, manifestation of AL, and incident cardiovascular-related diseases. However, whether there are sex differences in the vascular response to chronic stress (as measured by AL) among young Black adults have yet to be explored.

Chronic Stress and Allostatic Load in Black Adults

A recent review of the literature shows that Black race/ethnicity is related to higher chronic stress, inflammation, and AL as compared to White race/ethnicity (147). Higher degrees of actual and/or perceived racial discrimination have been recognized as adverse psychosocial stressors for

cardiovascular health (148). Black individuals face greater levels of actual and perceived day-to-day and cumulative lifetime discrimination as compared to individuals of other races and ethnicities in the U.S. (149). Discrimination and racism augment chronic stress to impact various health outcomes – especially in already highly at-risk populations (150). Collectively, stress-related racism and discrimination are thought to contribute to higher incidence of hypertension (96, 105–108, 151), and increased chronic inflammation (as measured by circulating concentrations of CRP) (111–113, 152). Increased perceived race-based discrimination has also been shown to adversely impact sleep quality and quantity by perpetuating rumination in Black college students (153). Along these lines, *The Weathering Hypothesis* proposes that chronic exposure to social and economic disadvantage/s promote acceleration of the normal ageing process, and thus, earlier onset of adverse health conditions (24, 154). Various researchers have described *The Weathering Hypothesis*, with AL as a metric of weathering to understand the impact of chronic mental stress on allostasis (24, 147, 154). It is possible that greater chronic stress in Black individuals beget repeated and heightened activation of the SNS, leading to downstream endothelial dysfunction. A heightened SNS response leads to vascular dysfunction via acute hemodynamic changes to vascular resistance by modulating blood pressure. However, whether increased AL and a background of elevated chronic stress exaggerate stress-hormone induced endothelial dysfunction, arterial stiffness, and inflammation in young Black adults remains unknown.

Acute Stress

Vascular responses to an acute stress test, which typically involve activation of the SNS, can provide valuable insights into how individuals may respond to chronic stress over time. Acute stress triggers sympathetic stimulation, leading to increased heart rate and blood pressure, which

can induce changes in vascular function and blood flow regulation, and initiate the immune response (155–157). Studying the relationship between acute stress-induced changes in circulating inflammation and vascular function, and how these changes relate to chronic stress-induced alterations in vascular health, can help elucidate the underlying mechanisms and pathways involved in the development of CVDs such as hypertension. Moreover, this knowledge can be used to identify individuals at increased risk for hypertension, and ideally, to develop targeted interventions to improve vascular health and reduce the risk of disease in those individuals (158).

Local cold stimulation (cold pressor task; CPT) is a robust sympathetic stimulus performed in a laboratory setting that is capable of doubling SNA within 1–2 min (156). The future development of hypertension can be predicted by the cardiovascular response to the CPT, as indicated by various studies (159–161). Studies involving both Black and White adults and children have shown that Black individuals, who are at a higher risk of developing early hypertension, exhibit greater vascular responses to the CPT as compared to their White counterparts (162). Of note, there are no differences in the vasoconstrictive response to a CPT between young women who are naturally menstruating as compared to those using oral contraceptive pills (163). However, the vasoconstrictive response to a CPT is attenuated among young women compared to young men (164), suggesting that the response to an increase in local SNA may be mediated by sex-hormones. Interestingly, certain antihypertensive drugs are not effective in blocking the exaggerated pressor response induced by cold stress, whereas alpha- and beta-blockers have shown to be the most effective (165).

Acute Stress and Vascular Dysfunction

Various studies have shown that even among young healthy adults, acute mental stress attenuates endothelial vasodilatory capacity, as measured by transient impairments in %FMD

(166–170). Several groups have elicited acute mental stress in middle-aged participants by telling them to imagine a situation in which they had been falsely accused of shoplifting, requiring them to prepare a statement in their defense for two-minutes, and having them subsequently present it in front of an audience and video camera for three-minutes (166, 170). This acute mental stress task significantly reduced %FMD in healthy participants for up to 90-minutes post-mental stress, and attenuated their %FMD response to levels that are comparable to the baseline (pre-stress) levels of diabetic participants (166). Similarly, Gottdiener et al. compared %FMD responses in otherwise healthy middle-aged adults with and without elevated cholesterol levels before and after an anger recall speech task and a mental arithmetic task (167). Despite both groups having similar %FMD before the mental stress tasks, participants with high cholesterol levels experienced significantly lower %FMD following both of the mental stress tasks as compared to participants with lower cholesterol levels (167). Further, the mental stress-induced impairments in %FMD were enhanced among participants who scored higher on a questionnaire assessing the behavioral trait of hostility (167), suggesting there exists a link between adverse behavioral traits and impaired cardiovascular responses to stress. Taken together, results of these studies suggest that various iterations of acute mental stress tasks can induce changes in endothelial function that mimic chronic CVD states, and that behavioral assessments may be able to predict this association.

Transient impairments in endothelium-dependent dilation to acute mental stress tasks have also been observed in young healthy individuals following mental-stress reactivity (168) and arithmetic tests (171). In agreement with findings from middle-aged adults, endothelial function is impaired in young adults for approximately one-hour post-acute mental stress tests (168, 171). Taken together, these studies suggest that the aforementioned findings of acute mental stress-induced endothelial dysfunction in middle-aged adults are not mediated by the effects of aging.

Impaired endothelial function in response to acute mental stress seems to be mechanistically linked to activation of ET-1 receptors (168). However, endothelium-independent dilation (in response to nitroglycerin or glyceryl trinitrate) does not change in response to acute mental stress tasks (166, 168, 171). Therefore, the temporary reduction in %FMD following acute mental stress indicates an imbalance in endothelium-derived vasodilatory NO, and vasoconstrictive ET-1 (168).

Acute Stress and Arterial Stiffness

Acute mental stress also elicits immediate increases in central artery stiffness in young healthy individuals, as indicated by significantly elevated carotid-to-femoral pulse wave velocity (cfPWV) measurements post-stress as compared to pre-stress (172–175). For example, in young healthy women, cfPWV is significantly increased immediately following a social stress task (where participants were asked to prepare a five-minute speech and present it to two interviewers and a video camera) as compared to baseline values (174). Additionally, significantly higher cfPWV values from baseline (pre-stress) have been observed up to 30-minutes following the viewing a stressful movie (172), and up to one hour following a mental arithmetic test (173), indicating that there are lingering adverse effects of an acutely stressful event on vascular structure and function, even among young healthy individuals.

Acute Stress and Inflammation

Proinflammatory responses from the immune system are not solely restricted to pathological states or conditions; an acute psychosocial stress generates a transient increase in concentrations of peripherally circulating proinflammatory cytokines among healthy adults (176). Following acute stress-induced activation of the HPA axis, corticotrophin-releasing hormone (CRH) and vasopressin are secreted from the pituitary glands. Consequentially, CRH and

vasopressin stimulate secretion of glucocorticoids from the adrenal cortex (177). The release of the glucocorticoid, cortisol, is well known to regulate multiple adaptions directed at acute stress regulation (39, 40). For example, cortisol is involved in the mobilization of glucose to promote energy utilization involved in the coping with acute stress (39, 40). As another method for coping with the acute stress, cortisol stimulates the immune system to produce monocytes and granulocytes – initiating an inflammatory response (178, 179). Concomitantly, catecholamines, such as epinephrine and NE, are also released into circulation, deriving from the SAM axis (177). Similarly, NE release from the nerve terminals of the lymphoid organs activate the release of immune cells from the bone marrow (180). Thus, there appear to be roles for both the HPA and SAM axes in promoting acute stress-induced inflammation. Following an acute stress, the most notable increases in the proinflammatory response have been observed via significantly increased concentrations of circulating IL-6 (181) and CRP (182).

Acute Stress Responses in Black Individuals

Some of the existing literature suggest that young healthy Black individuals have higher SNA in response to laboratory stressors compared to their White counterparts (183, 184). Black adults in the U.S. have a higher prevalence of hypertension than adults of other racial identities (185). Increased SNA to a laboratory stressor reflects increased systemic vascular resistance (156, 157), and is an indicator of increased risk for hypertension (157). Therefore, observations of greater SNA to a given stressor, even among young normotensive Black individuals, may partially explain this group's greater risk for developing hypertension.

However, there is also an existing body of evidence suggesting that there are no differences in arterial BP reactivity to various laboratory stressors between young healthy Black and White individuals. For example, in 1987, Light and colleagues published data supporting that young

normotensive Black and White men have similar blood pressure (BP) reactivity in response to a CPT (186). Notably, racial differences in BP reactivity did however become apparent between young Black and White men once they were stratified into groups classified as having "moderately elevated" resting BP (defined as a SBP of 135-154 mmHg and DBP ≥85 mmHg) (186). Specifically, Black men with "moderately elevated" resting BPs had significantly greater BP reactivity to the CPT as compared to White men with "moderately elevated" resting BPs (186). This finding contributes to the hypothesis that Black adults with higher-than-average AL will demonstrate impaired vascular autonomic function.

The literature is also divided regarding whether young healthy Black and White individuals have disparate (74, 157, 187–189) or similar (75, 187) peripheral vasoconstrictive/vasodilatory responses to various laboratory stressors. Young Black men have attenuated increases in forearm vascular conductance (FVC) and forearm blood flow (FBF) as compared to young White men during a high-intensity rhythmic handgrip exercise (187), during lower-body negative pressure (157), and in response to a CPT (74). However, young Black and White men seem to respond with similar FVCs and FBFs during low-to-moderate rhythmic handgrip exercise (75, 187).

There is a limited data assessing racial differences in the inflammatory response to acute mental stress with one study reporting that young healthy Black women experience a significantly heightened IL-6 response as compared to young healthy White women (190). A recent meta-analysis has attempted to explore the mediation effect of race on the inflammatory response to an acute stress but have noted there being too few studies assessing the inflammatory response in Black individuals, specifically (179).

Functional Sympatholysis

During exercise, the cardiovascular system must meet two physiological needs; firstly, to increase blood flow to match the metabolic demands of the active skeletal muscles, and secondly, to regulate blood pressure to maintain adequate perfusion pressure to the body's tissues. To accomplish these goals, the onset of exercise is accompanied by a rapid increase in cardiac output and major redistribution of the blood flow; whereby, perfusion of inactive skeletal muscles is impaired via sympathetic vasoconstriction (191–193). Meanwhile, at the site of the active/contracting skeletal muscle, increases in blood flow are maintained by the ability of local vasoactive compounds (sympatholytics) to attenuate sympathetic-mediated α-adrenergic vasoconstriction (194). When these signals are properly integrated, the active skeletal muscles achieve *functional sympatholysis* – defined as the ability of metabolites in the vascular beds of the active skeletal muscles to reduce the vasoconstrictive actions of systemic NE induced vasoconstriction (194). During contractions, sympatholysis seems to occur mostly in the smallest (third order) arterioles, while there is preserved α-adrenergic vasoconstriction in the larger, first- and second-order arterioles and feed arteries (195).

Mechanisms of Functional Sympatholysis

Despite extensive research efforts, the specific mechanisms by which functional sympatholysis occurs are incompletely understood – in part, due to the significant physiological redundancies contributing to exercise hyperemia. However, experimental evidence most prominently supports the vasodilatory-mediating role/s of the following substances: potassium ions as endothelium-derived hyperpolarization factors (EDHFs) (196, 197), the prostaglandin, prostacyclin (PGI_2) (198, 199), ADO, and NO (198–200). Interestingly, adenosine triphosphate

(ATP) can play a role in vasodilation (201–209) or vasoconstriction (209–211). While hyperemia is imperative to exercising muscles, it must also be met with restraint to defend against hypotension (212). Thus, as reviewed by Holwerda, Restaino, and Fadel (2015), non-adrenergic vasoconstrictors such as neuropeptide Y (NPY), ATP, ET-1, and Ang II, are suggested to contribute to the vasoconstrictive influence occurring during exercise (209). This delicate balance in sympathetic activity across the vascular landscape is a critical regulator in the maintenance of 1) increased blood flow through active skeletal muscle to meet metabolic demands, and 2) regulation of blood pressure to maintain adequate perfusion pressures during exercise.

NYP and ATP are neurotransmitters that are co-released with NE from the sympathetic nerves. Like NE, NPY and ATP can have vasoconstrictive effects when released into the interstitium. Specifically, interstitial NPY binds to the Y1 receptor, and ATP binds to the purinergic ligand-gated ionotropic (P2X) receptors on the smooth muscle cells. When interstitial NPY and/or ATP bind to their respective receptors, calcium (among other anions) will influx the cell, causing depolarization, and vasoconstriction (**Figure 3**) (210, 211). However, when released intravascularly, ATP blunts α-adrenergic vasoconstriction – aiding in the functional sympatholytic response to increase blood flow to the exercising muscle (**Figure 3**). Intravascular ATP is thought to be released from endothelial cells due to shear stress and/or by RBCs (red blood cells) in response to the deformation and deoxygenation they undergo during exercise (208). Mechanistically, intravascularly released ATP can promote vasodilation by binding to the purinergic G-protein-coupled metabotropic (P2Y) receptors located on the endothelial cells (212). Upon ATP's binding to the P2Y receptor, there may also be a triggered release of NO, PGI_2, and EDHFs which further induce vasodilation (**Figure 3**) (208, 209).

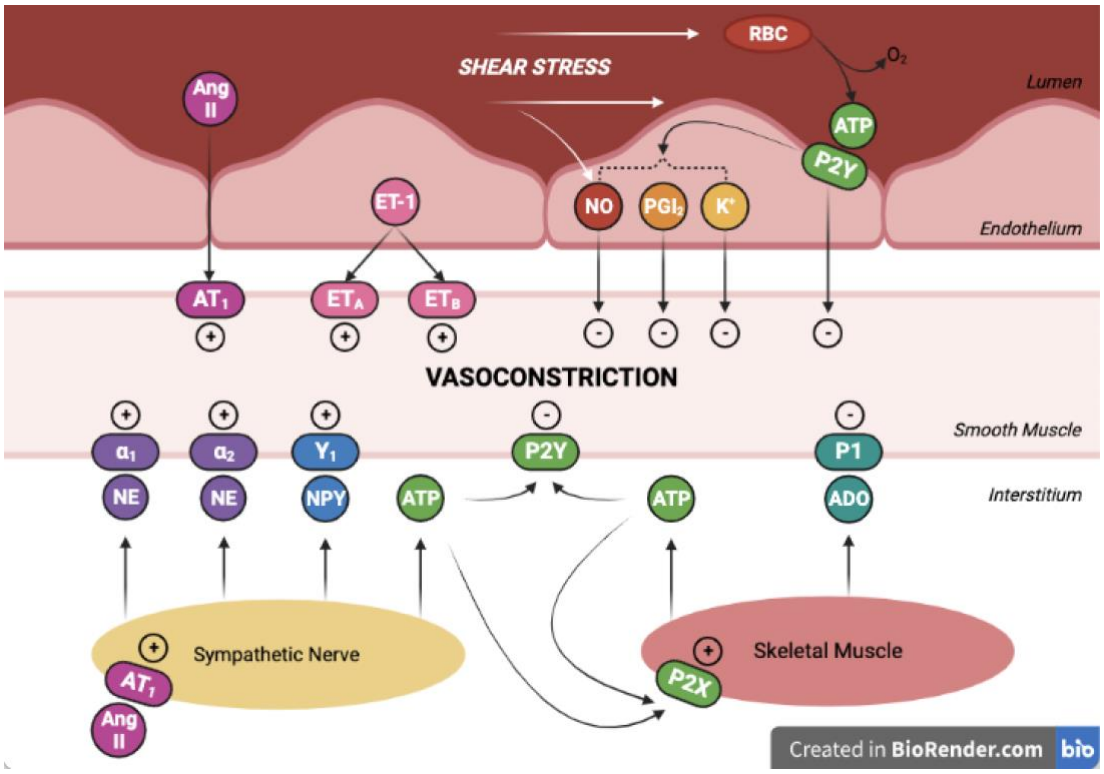


Figure 3: An Overview of The Proposed Mediators Contributing to Vasoconstriction and Vasodilation During Functional Sympatholysis.

Figure 3 Key: Vasoconstrictive pathways are indicated by (+) symbols, whereas vasodilatory mechanisms are indicated by (-) symbols. Ang II, angiotensin II; AT1, angiotensin type II receptor 1; ET-1, endothelin-1; ET_A, endothelin-1 receptor A; ET_B, endothelin-1 receptor B; RBC, red blood cell; O_2, oxygen; NO, nitric oxide; PGI_2, prostacyclin; K^+, potassium; ATP, adenosine triphosphate; P2Y, purinergic G-protein-coupled metabotropic receptor; NE, norepinephrine; α_1, alpha-adrenergic receptor 1; α_2, alpha-adrenergic receptor 2; NPY, neuropeptide Y; Y_1, neuropeptide Y receptor type 1; P2X, purinergic ligand-gated ionotropic receptor; ADO, adenosine; P1, purinergic G-protein-coupled adenosine receptor. Figure created using BioRender.com.

Impaired Functional Sympatholysis

Aging contributes to declines in cardiovascular responses to exercise by way of decreased cardiac output, reduced skeletal muscle vasodilatory capacity and augmented SNA (213). Independent of the limitations in central cardiac function with maximal exercise, aging also induces peripheral impairments in vascular function observed during submaximal or even small muscle mass exercises (214, 215). For example, healthy White men have been shown to experience blunted α-adrenergic vasoconstriction in response to low- and moderate-intensity exercise (216–

218). During a cycling exercise, Koch and colleagues used a CPT to increase SNA in a group of healthy younger (aged 20-30) and older (aged 62-74) men (216). The researchers reported a greater decrease in leg blood flow in the older men as compared to younger men, suggesting an impaired ability for the older men to block sympathetic stimulation on the arterioles feeding the contracting muscles (216) – also described as *impaired functional sympatholysis*. These results have been replicated in both older men and women (219), using absolute and relative exercise workloads, measuring responses among different vascular beds, and via the utilization of various laboratory methods to invoke sympathetic α-adrenergic stimulation.

Impaired functional sympatholysis has been observed in middle-aged adults with hypertension (220). However, after a four-week supplementation with an Ang II receptor blockade (an antihypertensive medication), functional sympatholysis was restored in the hypertensive middle-aged adults, lending to the conclusion that Ang II acts as a key mediator of the excessive sympathetic vasoconstriction in the exercising muscles of hypertensive individuals (220). The conclusions from this study were further supported by a lack of restoration in functional sympatholysis among hypertensive adults after a four-week supplementation with a thiazide-type diuretic (also an antihypertensive medication), despite it decreasing resting and exercising blood pressure to the same degree as the Ang II receptor blockade (220). While Ang II does mediate the release of NE, its impairment in functional sympatholysis in the human hypertensive model is proposed to occur via reduction in α_2-adrenergic mediated vasoconstriction. Further, Ang II may be impairing functional sympatholysis in hypertensive adults due to its role in the production of ROS (220–222).

As previously mentioned, it is likely that individuals with increased AL (especially due to their increased SNA, levels of Ang II, and potential hypertensive status), would also have impaired

functional sympatholysis. Given that young Black adults may have divergent contributors to vascular health as compared to White adults, it is imperative to understand how functional sympatholysis is affected across a spectrum of AL scores in this population.

Sex Differences in Functional Sympatholysis

There may be sex-related differences in the response of Black men vs. Black women to functional sympatholysis. Sudhir et. al have reported an inhibitory effect of estrogen in the binding of α-adrenoreceptors on the vasculature of contracting muscles (223). These findings suggest that estrogen may play a role in the facilitation and/or augmentation of functional sympatholysis by perpetuating vasodilation. To further elucidate this conclusion, Sudhir et al. studied the functional sympatholytic responses following exogenous estrogen exposure among perimenopausal women (224). Findings of this study showed that following exposure with exogenous estrogen, perimenopausal women displayed blunted NE-induced vasoconstriction (improved functional sympatholysis) (224). These findings are supported by report of impaired functional sympatholysis in the contracting muscles of ovariectomized rats and postmenopausal women (219). Similarly, ovariectomized rats and postmenopausal women experience attenuated impairment in functional sympatholysis following administration of exogenous estrogen (219). Taken together, in models of impaired functional sympatholysis, estrogen may aid in the restoration of blood flow regulation through the active skeletal muscles, as exhibited by reduced α-adrenergic (NE-induced) vasoconstriction during exercise. Despite the suggestive effect of estrogen on improved functional sympatholysis, there have been no studies assessing the moderation effect of sex on the associations between AL and functional sympatholysis in young Black adults.

Chapter 2: Allostatic Load and Vascular Health in Young Black Adults

Chapter Overview

In the United States, Black individuals are disproportionately affected by hypertension (4, 6), and tend to have heightened risk-factors for hypertension in earlier stages of life as compared to individuals belonging to other racial groups (4, 7, 8). Racial disparities in the prevalence and incidence of hypertension are thought to be influenced, at least in part, by the chronic physiological burden from the stress response (25), resulting from heightened exposure to psychosocial stressors such as increased perceptions of discrimination (149), adverse perceptions of one's social determinants of health (104, 225); and greater perceptions of stress (96). The physiological burden imposed by chronic stress has been termed *allostatic load* (AL), a concept associated with allostasis, or the capacity by which the body can successfully mediate various parameters of its internal environment to accommodate for stressful challenges and/or changing demands of the external environment (9). The use of allostasis as a tool to assess health has led to new ways of understanding complex, multi-system physiological activities that involve repeated allostatic responses activated during stressful situations. Indeed, chronic stress exaggerates 'wear and tear' on the body by curbing normal engagement of physiological AL (9). Thus, chronic or frequent challenges to allostasis beget "allostatic overload," which is associated with various adverse health outcomes (9, 10, 26, 34, 45, 46, 180, 226–228), including hypertension (180, 228).

Prolonged periods of and/or repeated exposures to stress sustain arousal of sympathetic nerve activity (SNA), a primary driver of AL (9). Indeed, increased SNA has been implicated in the progression of hypertension (229), partially due to its adverse roles in the modulation of

vascular function (230), and structure (231). Specifically, sustained SNA can reduce the production and release of nitric oxide (NO) (232), a key vasodilatory substance that is largely implicated in vascular health and function. Typical consequences of vascular dysfunction include perpetuation of a proinflammatory, pro-oxidative, and prothrombotic state, which impaired vascular reactivity (233). Likewise, arterial stiffness is characteristic of increased arterial calcification, collagen deposition, and elastin fragmentation, which impair the ability for effective vasodilation and vasoconstriction to accommodate for changes in blood pressure and flow (54). Human and animal research have implicated vascular dysfunction and arterial stiffness as mechanisms by which chronic psychological stressors lead to downstream cardiovascular complications (57, 234, 235). Additionally, estrogen has known cardioprotective effects due to its role in augmenting the bioavailability of NO, thus suggesting female sex in the promotion of vascular function and mitigation of arterial stiffness, at least while women are of premenopausal age (236–238). However, associations between chronic physiological stress (i.e., AL), sex, and these measures of vascular health are currently unknown. Additionally, it is unknown whether perceptions of psychological stress are intertwined in the potential associations between AL and vascular health.

Young, normotensive Black adults have greater vascular dysfunction at rest as compared to their White counterparts (73–77); however, this finding is not universal (78–80). Greater vascular dysfunction in Black adults is explained by several studies suggesting that Black individuals have impaired NO bioavailability (81, 82), elevated systemic inflammation (83–86), and/or greater oxidative stress (82, 84, 87, 88) as compared to adults of other racial identities. Similarly, arterial stiffness is higher in young Black adults (89, 90). Among Black adults with masked prehypertension (elevated blood pressure), a positive relationship has been observed

between vascular dysfunction and concentrations of the inflammatory marker, C-Reactive Protein (CRP) (91). The potential for these early adverse changes in the vascular and immune profiles of young, otherwise healthy Black individuals is of particular interest given: i) vascular dysfunction and arterial stiffness have shown both independent and synergistic associations with greater risk for hypertension (92); and ii) due to the increased prevalence of hypertension among this demographic (4, 6). Further, it has been well established in the literature that Black adults have higher cumulative AL as compared to their White counterparts (24–34). However, understanding the associations between AL and vascular function and structure have not been assessed comprehensively in the literature, let alone in a population of young Black adults.

Therefore, the first purpose of the study was to evaluate the associations between indices of AL and vascular function and structure (i.e., vascular health) in young Black adults. Vascular health was assessed via measures of macrovascular function (%flow-mediated dilation; %FMD and brachial artery diameter; BD), microvascular function (reactive hyperemic response following forearm ischemia; RH-AUC), arterial stiffness (carotid-to-femoral pulse wave velocity; cf-PWV), and wave reflection (augmentation index; AIx, and AIx standardized to a heart rate of 75 bpm; AIx75). We hypothesized that higher indices of AL would be negatively correlated with %FMD and RH-AUC in young Black adults, and positively correlated with cf-PWV, AIx and AIx75 in young Black adults.

Along with this goal, we also developed two exploratory aims. Firstly, we sought to determine whether biological sex moderated any associations between AL and vascular health in young Black adults. Based on evidence of sex-differences in vascular function between young Black men and women (239), we hypothesized that sex would moderate the association between AL and measures of vascular health, especially in combination with evidence of the protective

role of estrogen on the maintenance of vascular health, even in conditions of amplified behavioral and physiological stress (144). Secondly, to capture the interactive effects of physiological and psychosocial stress on vascular health, we investigated whether AL mediated any associations between A) perceived social determinants of health (SDoH) and vascular health; B) perceived everyday discrimination and vascular health; and/or C) perceived stress and vascular health in young Black adults. Perceived SDoH, everyday discrimination, and stress were quantified as summary scores of participants' responses to the SDoH Scale (SDoH-S), Everyday Discrimination Scale (EDS), and Perceived Stress Scale (PSS), respectively. We hypothesized that AL would mediate associations between A) SDoH-S and measures of vascular health, B) EDS and measures of vascular health, and C) PSS and measures of vascular health in young Black adults.

Methods

Ethical Approval

All experimental procedures were approved by the Institutional Review Board at the University of Maryland, College Park (reference number: 1853726). Experimental procedures complied to the most recent provisions of the Declaration of Helsinki (except for registration in a database). All participants provided their written informed consent prior to taking part in the study.

Participants

To recruit participants for this study, we sought out young Black adults in and around the University of Maryland, College Park who self-reported as conforming to the following initial inclusion criteria: 1) identifying as Black (e.g., African-American, Afro-Caribbean, Afro-Latinx, African); 2) being between the ages of 18-25 years old; 3) speaking and understanding English;

and 4) being willing for researchers to collect blood and saliva samples. Initial eligibility was confirmed using an online screening questionnaire (Qualtrics, Seattle, WA, U.S.A.), of which interested participants completed prior to visiting the laboratory for testing. Eligible participants were contacted by the research team and requested to report to the Human Integrative Physiology Laboratory at The University of Maryland, College Park for one visit. Prior to the agreed-upon time and date of the study visit, participants were requested to: fast from all food and drink other than water for at least six hours, refrain from exercising or consuming alcohol or caffeine for at least 12 hours; abstain from NSAIDs and other non-regular medications for at least 24 hours; and to abstain from supplements (described as "non-physician recommended/prescribed medications or substances, such as multivitamins or creatinine") for at least 72 hours. Lastly, prior to the visit, participants were requested not to wear, or to be able to easily remove, any metal items such as jewelry or jackets with zippers to avoid interference with images obtained from dual energy x-ray absorptiometry (DEXA) body composition assessments.

Experimental Protocol

Upon arrival to the laboratory, potential participants were familiarized with the entirety of the study's procedures, including any potential risks and discomforts involved with the measures carried out in the study. Experimentation was then carried out in a dimly lit, noise- and temperature-controlled room in The School of Public Health at The University of Maryland, College Park.

Health History Questionnaire

While seated, participants completed a health-history questionnaire (HHQ) which requested participants to provide responses to questions regarding their past and current health

conditions, cardiovascular health status (including medication use), and historic and/or current usage of nicotine and/or tobacco products. If female, participants were also asked whether they were currently using hormonal contraception or were naturally menstruating, if they were currently pregnant, and/or if they were currently breastfeeding. Female participants who indicated current pregnancy or breastfeeding were excluded from participating in the remainder of the study's procedures. The questionnaire was administered through Qualtrics (Seattle, WA, U.S.A.) on an Apple iPad (Cupertino, CA, U.S.A.).

Self-Perceived Scales

Participants were asked to answer questions to a Perceived Social Determinants of Health Scale (SDoH-S; **Table 2**), Perceived Everyday Discrimination Scale (EDS; **Table 3**), and Perceived Stress Scale (PSS; **Table 4**). The SDoH-S is a seven-item questionnaire created by our laboratory with the intention to succinctly assess participants' self-perceived access to and/or the quality of various conditions among their environment which affect health, functioning, and quality of life in the past year. The SDoH-S addresses the five key domains recognized by The U.S. Department of Health and Human Services as areas that contribute to health outcomes: 1) economic stability; 2) education access and quality; 3) health care access and quality; 4) neighborhood and built environment; and 5) social and community context (102). The EDS is a nine-item questionnaire adapted from Williams (240) and is validated tool to assess perceived discrimination among a cohort of Black adults in the U.S. (111). The 10-item PSS was utilized to measure participants' subjective appraisal of particular life events/situations as being unpredictable, uncontrollable, and/or evoking feelings of being "overloaded" within the past month – with higher PSS scores being associated with higher levels of chronic stress (119, 241).

Prior to answering the questionnaires, participants were informed that the questionnaires included questions that could be perceived as distressing and/or sensitive, and that they (the participant) had the right to skip responding to any questions that might have made them feel uncomfortable or that they did not want to answer.

Table 2: Perceived Social Determinants of Health Scale.

Please select the option that best indicates how you identify with the following statements based on your experiences *in the past 12 months.* * Note: you do not have to identify with all of the examples provided in order to select "Agree" or "Strongly Agree" – These examples are offered simply to help you better understand the statement.					
Questions:	**Responses:**				
1. I feel as though I have good access to health care. *Examples: I have a primary care physician; I have or know how to find a health specialist if I need(ed) one; I would be able to book an emergency appointment at a doctor's office if I needed to; etc.*	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree
2. I feel as though I have access to complete/adequate nutrition. *Examples: I am able to find fresh fruits, vegetables, protein sources, etc. without going far out of my way, I can find or prepare balanced meals, I have enough of the kinds of food that I want to eat.*	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree
3. I feel as though I have access to a good education. *Examples: I have helpful and intelligent professors; I have a supportive learning environment; I have access to tutoring/ study sessions if I chose to utilize them, etc.*	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree
4. I feel as though I have access to good housing conditions. *Examples: I have a safe space to rest, heating/cooling, quiet space, etc.; I feel safe in/around my home.*	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree
5. I feel as though I live in a high stress environment. *Examples: I have constant sources of worry; I feel like I am behind everyone else; I feel like I don't have control over the important things in my life; I don't feel like I can cope with all the things I have to do; I am treated with less respect or courtesy as other people are; others direct racial or ethnic slurs at me.*	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree

6. I feel socially accepted at my college/university or community (I belong).	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree
7. My family and I are in a financially stable position. *Examples: Debt is relatively low and/or income is relatively high.*	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree

Table 3: Perceived Everyday Discrimination Scale.

In your *day-to-day life*, how often do any of the following things happen to you?						
Questions:	**Responses:**					
1. You are treated with less courtesy than other people are.	Never	Less than once a year	A few times a year	A few times a month	At least once a week	Almost every day
2. You are treated with less respect than other people are.	Never	Less than once a year	A few times a year	A few times a month	At least once a week	Almost every day
3. You receive poorer service than other people at restaurants or stores.	Never	Less than once a year	A few times a year	A few times a month	At least once a week	Almost every day
4. People act as if they think you are not smart.	Never	Less than once a year	A few times a year	A few times a month	At least once a week	Almost every day
5. People act as if they are afraid of you.	Never	Less than once a year	A few times a year	A few times a month	At least once a week	Almost every day
6. People act as if they think you are dishonest.	Never	Less than once a year	A few times a year	A few times a month	At least once a week	Almost every day
7. People act as if they're better than you are.	Never	Less than once a year	A few times a year	A few times a month	At least once a week	Almost every day
8. You are called names or insulted.	Never	Less than once a year	A few times a year	A few times a month	At least once a week	Almost every day
9. You are threatened or harassed.	Never	Less than once a year	A few times a year	A few times a month	At least once a week	Almost every day

Table 4: Perceived Stress Scale.

The following questions ask you about your feelings and thoughts *during the past month*. In each case, you will be asked to indicate how often you felt or thought a certain way.	
Questions:	**Responses:**

1. In the past month, how often have you been upset because of something that happened unexpectedly?	Never	Almost Never	Sometimes	Fairly Often	Very Often
2. In the past month, how often have you felt that you were unable to control the important things in your life?	Never	Almost Never	Sometimes	Fairly Often	Very Often
3. In the past month, how often have you felt nervous and "stressed"?	Never	Almost Never	Sometimes	Fairly Often	Very Often
4. In the past month, how often have you felt confident about your ability to handle your personal problems?	Never	Almost Never	Sometimes	Fairly Often	Very Often
5. In the past month, how often have you felt that things were going your way?	Never	Almost Never	Sometimes	Fairly Often	Very Often
6. In the past month, how often have you found that you could not cope with all the things that you had to do?	Never	Almost Never	Sometimes	Fairly Often	Very Often
7. In the past month, how often have you been able to control irritations in your life?	Never	Almost Never	Sometimes	Fairly Often	Very Often
8. In the past month, how often have you felt that you were on top of things?	Never	Almost Never	Sometimes	Fairly Often	Very Often
9. In the past month, how often have you been angered because of things that were outside of your control?	Never	Almost Never	Sometimes	Fairly Often	Very Often
10. How often have you felt difficulties were piling up so high that you could not overcome them?	Never	Almost Never	Sometimes	Fairly Often	Very Often

Diurnal Slope of Salivary Cortisol

Participants were provided with four cryovials and requested to collect their waking and bedtime saliva samples on two subsequent days. Prior to each of the saliva collections, participants were asked to rinse their mouth with water to remove food residue, and to wait at least 10-minutes after rinsing and prior to collecting saliva to avoid sample dilution. Participants were then instructed to passively drool into the cryovial until 1-1.5 mL of saliva was collected. Following collection of each sample, the participant was instructed to tightly attach the cap to the cryovial and store it in their refrigerator until they were able to hand-deliver all samples (two days of morning and evening collections) back to the research team. Upon receipt of the samples,

researchers stored them in a -80°C freezer. All samples were mailed to Salimetrics® (State College, PA, U.S.A.), where they were assessed for concentrations of cortisol using the Salimetrics® Expanded Range High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit.

Reported concentrations of cortisol from the two awakening samples were averaged to provide a single morning concentration for each participant. The base 10 logarithms of the average values from each participant's morning samples were then calculated (mean morning cortisol). Similarly, reported concentrations of cortisol from the two bedtime samples were averaged to provide a single evening concentration for each participant (mean evening cortisol), and the base 10 logarithms of the average values from evening samples were calculated. Log transformations were applied to normalize the values. The diurnal slope of salivary cortisol for each participant was calculated by subtracting the log10(mean morning cortisol) from the log10(mean evening cortisol). The values were then standardized so that they could be compared on a common scale.

Resting Heart Rate and Blood Pressure

Following at least 10 minutes of seated rest, participants were instructed to sit upright in a chair while resting systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) were measured on the right brachial artery with an automated oscillometric device (Omron Corporation, Kyoto, Japan). SBP, DBP, and HR measures were collected in duplicate with a 60 second break between readings. The average values from the two BP and HR measurements were calculated and used for analysis.

Total Body Fat Percentage

A DEXA scan was performed on participants using a Prodigy Primo DEXA machine (GE Healthcare Lunar Radiation Corp., Madison, WI, U.S.A.) to attain measures of the participants'

total body fat percentages (%TBF). The DEXA scan utilizes two low-dose x-ray beams to measure body density and subsequently quantify the mass and distribution of total body fat, lean muscle, and bone (242). DEXA scans were performed and analyzed with enCORE software. To perform the DEXA scan, participants were asked to lie flat on their back in the center of the DEXA's bed with their arms at their sides for 6-11 minutes while the movable x-ray arm scanned their body. Because the test involves a low-level of radiation, all female participants were asked to confirm they were not pregnant at the time of the study. No current pregnancy of participants was established via self-report from the participants during the HHQ. All participants were offered a pregnancy test to confirm that they were not pregnant. Because requiring participants to take a pregnancy test may induce psychological distress, the test was made optional; however, participants who waived the test were asked to sign a statement affirming that they were not pregnant, and that they waived the option to take a pregnancy test.

Blood Collection and Analysis

Participants were requested to lie supine on a hospital bed. Twenty mL of blood was drawn from an antecubital vein and collected into serum separator tubes. After sitting at room temperature for 45 minutes, the two serum separator tubes were spun at 1,500 g for 15 minutes at 4°C. Serum from one of the 10 mL serum separator tubes was then pipetted into 1 mL aliquots and stored in a -80°C freezer on-site until the conclusion of all data collection.

C-Reactive Protein

The serum samples that were aliquoted and frozen on-site were analyzed for circulating concentrations of C-Reactive Protein (CRP). Concentrations of CRP were quantified using commercially available enzyme-linked immunosorbent assay kits ([ELISA] CRP: QK1707; R&D

Systems, Minneapolis, MN, U.S.A.). Sandwich ELISAs were performed on study samples and standards with known concentrations, in accordance with manufacturer instructions. All samples were assayed in duplicate, and their absorbance was measured with a spectrophotometer (Synergy H1 Hybrid Reader; BioTek, Winooski, VT, U.S.A.) at 450 nm. The known relationship between absorbances and concentrations of the provided standards was used to create a standard curve, which was then utilized to quantify the concentrations of CRP in each of the samples. Concentrations of CRP in the duplicated samples are reported as a single averaged value.

Glucose, Creatinine, and Total Cholesterol-to-High-Density-Lipid Cholesterol Ratio

After being spun, the remaining 10 mL of blood was labeled with the participants' unique study ID numbers and sent to a commercial blood chemistry laboratory (Quest Diagnostics - East Region; Quanum Lab Services, U.S.A.) for characterization of blood lipids and cardiometabolic markers. Specific to the aims of the present study, Quest Diagnostics provided fasting serum concentrations of glucose, creatinine, and the total cholesterol-to-high-density-lipid cholesterol ratio (TC/HDL-C) for each participant.

Macrovascular Function

Macrovascular function was evaluated via measures of percent flow-mediated dilation (%FMD). To obtain measures of %FMD, participants laid supine with their left arm extended onto a table so that it was abducted from the torso and situated around the level of the heart, per current FMD guidelines (243). A blood pressure cuff was placed around the left forearm, immediately proximal to the antecubital fossa (Hokanson, Bellevue, WA, U.S.A.). The diameter of the brachial artery was imaged with a high-resolution ultrasound probe equipped with a 5-18 MHz linear transducer (Hitachi-Aloka Arietta 70, Tokyo, Japan). The brachial artery was imaged

longitudinally and continuously throughout the 8.5-minute test while the probe was stabilized with a stereotactic adjustable probe-holding device. Image capture was attained with Quipu Cardiovascular Suite FMD Studio (Pisa, Italy). Brachial artery diameter and hemodynamics were measured via Doppler ultrasound at baseline for 1-minute prior to rapid forearm cuff inflation to 220 mmHg. The cuff remained inflated for 5 minutes while retaining image capture with the probe. After which, the forearm cuff deflated, and the diameter and hemodynamics were continually measured for an additional 2.5 minutes to observe the reactive hyperemic response. The arterial diameter was calculated by averaging the second-by-second diameter from the first minute of measurement, the baseline diameter (BD). The peak diameter (PeakD) was calculated as the greatest diameter achieved during the last 2.5 minutes of measurement. %FMD was calculated, as in **Equation 1**, in accordance with current FMD guidelines (243).

Equation 1.

$$FMD\ (\%) = \frac{PeakD\ (mm) - BD\ (mm)}{BD\ (mm)} \times 100$$

Because of the tendency of %FMD measures to have a linear relationship with the BD (244), we investigated whether potential differences based on sex existed in the allometric scaling of the PeakD compared with the BD. This was achieved via linear regression analysis of the natural log of the BD (lnBD) and PeakD (lnPeakD). In the linear mixed model, difference in diameter (lnD_{diff} = lnPeakD – lnBD) was the dependent variable, sex was a fixed factor, and lnBD was the covariate. Scaled %FMD for individual participants were estimated using the logged diameters and sex-specific unstandardized β-coefficients from the linear regressions, as in **Equation 2**.

Equation 2.

$$Scaled\ \%FMD = \left[\frac{(lnPeakD)}{(lnBD)^{\beta}} - 1\right] \times 100$$

Microvascular Function

To assess microvascular function, the reactive hyperemic area under the curve (RH-AUC) of blood flow (BF) during the baseline and post-occlusion portions of the FMD test was calculated. The baseline portion of the FMD encompasses the first 60 seconds of the test (pre-occlusion), and the post-occlusion portion encompasses the 150 seconds following deflation of the forearm cuff. Positive and negative blood velocity signals and brachial artery diameter (BD) were recorded on a second-by-second basis throughout the FMD test via commercially available software (Quipu Cardiovascular Suite FMD Studio; Pisa, Italy). Specifically, the second-by-second positive and negative blood velocity values were summed to calculate mean blood velocity (MBV). During data collection, the sample volume was centered within the vessel, maximized according to the vessel's size, and situated with an insonation angle of 60° (Hitachi-Aloka Arietta 70, Tokyo, Japan). Blood flow (BF) was calculated, as in **Equation 3**.

Equation 3.

$$BF\ \left(\frac{mL}{min}\right) = MBV\ \times\ \pi\ \times \left(\frac{BD\ (cm)}{2}\right)^{2} \times\ 60$$

The mean BF at baseline was determined by averaging the calculated BFs from the initial 60 seconds of the FMD test, resulting in a single baseline BF value. The mean baseline BF, along with the second-by-second calculated BFs from the 150-second post-occlusion phase of the FMD test, were utilized to compute the RH-AUC for each participant. These analyses were performed using GraphPad Prism Version 10.1.1 (San Diego, CA, U.S.A.).

Vascular Structure

Structure of the central arteries were assessed as indices of arterial stiffness and wave reflection. The gold standard for measures of central arterial stiffness is carotid-to-femoral pulse-wave-velocity (cfPWV) (245, 246). To capture measures of cfPWV two different pulse waves were recorded sequentially by applanation tonometry; the first was measured at the pulse of the right common carotid artery and the second was measured at the pulse of the femoral artery. The distance travelled by the pressure waves between the two sensors was estimated using a tape measure; distances (m) between the participants' suprasternal notch to the pulse of their femoral artery, the pulse of their carotid artery to their suprasternal notch, and from the site of the femoral pulse to the placement of the thigh cuff, were obtained and inputted into the SphygmoCor XCEL software. Transit time of the pressure wave from the carotid artery to the femoral artery was determined via automatic detection of the diastolic foot at each pressure wave. cfPWV was calculated by the SphygmoCor XCEL equipment, as in **Equation 4**.

Equation 4.

$$cfPWV\ (^{m}/_{s}) = \frac{Distance\ (m)}{Transit\ time\ (s)}$$

Central pressure waves generated by the SphygmoCor® XCEL were used by the software's pulse wave analysis (PWA) function to generate the augmentation index (AIx). AIx is a composite index reflecting the influence of arterial stiffening in the large arteries, peripheral resistance in the medium and small arteries, and the reflected waveform to the heart on the central BP (247). The AIx is calculated as in **Equation 5**, where P_1 and P_2 are the first and second peaks of the central arterial waveform, respectively, and the SBP and DBP are the central systolic and diastolic blood pressures, respectively. Since an individual's HR has an impact on measures of AIx (248, 249),

values are normalized to a HR of 75 beats-per-minute (AIx75) to minimize inter-subject variability.

Equation 5.

$$AIx\ (\%) = \frac{(P_2 - P_1)}{(SBP - DBP)} \times 100$$

Allostatic Load Scoring

Most commonly, the "scoring" of a total AL (AL index) has been assessed using dichotomous scoring methods. General dichotomous scoring techniques utilize ranges of standard deviations (SD) from the mean to identify a high-risk category for each marker (10, 24, 25, 27, 29). With this technique, for most variables (i.e., %TBF, SBP, DBP, RHR, glucose, and CRP), one SD above the mean was identified as the high-risk category; whereas, for some other variables (i.e., diurnal slope of salivary cortisol, and creatinine), one SD above or below the mean was identified as high-risk categories. Variables identified in the high-risk category were assigned a score of 1, and a summary score was calculated to represent overall AL index.

A major limitation of this approach is that the division of data into ranges of SD from the mean of the sample can be arbitrary and may not be clinically or scientifically meaningful for all variables. Therefore, another utilized approach to dichotomous scoring is to identify clinical cutoffs (CC) for variables (34, 226, 250–253). For example, according to the American Heart Association and American College of Cardiology, an individual's blood pressure is classified as "normal" when SBP is less than 120 mmHg and DBP is less than 80 mmHg (254). Therefore, participants with "normal" resting blood pressure were assigned a score of 0 for this variable. Alternatively, "elevated blood pressure" is defined by a SBP between 120-129 mmHg and a DBP <80 mmHg (254), which constituted a score of 1 for these variables. The utilization of CC methods

for calculating AL index may also be advantageous over the use of SD from the mean for calculating AL index as the CC method facilitates a more straightforward approach to comparing data across different studies. However, there are not nationally recognized CCs for all variables (i.e., diurnal slope of salivary cortisol, TC/HDL-C, etc.). Therefore, a combination of two dichotomous scoring methods were deployed, whereby, CCs were used for the scoring of variables which have normative ranges, and one SD from the mean were used for the scoring of variables without established normative ranges.

In factor analyses, latent factors are identified by examining the patterns of correlation among the observed variables and are assumed to represent unobserved constructs that influence those correlations. Latent factors are often used to explain complex phenomena and to simplify the analysis of larger datasets by reducing the number of variables needed to represent a given phenomenon (255). Therefore, while factor analyses can capture the complex relationships among multiple variables and provide a more nuanced understanding of the underlying constructs, one limitation of this approach is that it assumes that the variables are linearly related to each other.

Due to the strengths and limitations of the CFA method and the two dichotomous scoring methods (CC and SD), all three models were calculated and assessed to provide a quantitative measure of each participant's AL index, allowing for a more precise assessment of the relationships between chronic stress and physiological health outcomes in young Black adults.

Confirmatory Factor Analysis Method

A confirmatory factor analyses (CFA) was used to assess a measurement model using the Lavaan package (256) in R Statistics (IBSN, Vienna, Austria). Several CFA models were tested to comprehensively assess the latent construct of AL. Progressively, models were simplified to reduce the degrees of freedom by removing variables that were 1) not significantly contributing to

the variability in the data and 2) had low-weighted impact on the prediction of AL. The model with the best fit parameters was chosen to compute the AL index (CFA method). In the best-fit model of the CFA generation of AL index, log transformations were applied to the following raw AL biomarkers: diurnal slope of cortisol, CRP, creatinine, and TC/HDL. Any missing values were missing completely at random. Full Information Maximum Likelihood (FIML) was employed as a robust statistical technique to address missing data in the CFA. FIML analysis was conducted using the Lavaan package in R (256). Given the missing data were identified as missing completely at random, FIML was chosen due to its ability to utilize all available information in estimating model parameters, thus enhancing the validity of our findings by effectively handling missing data within the model's framework (257).

In the CFA, the following correlations were covaried for: 1) SBP with DBP; 2) TBF with TC/HDL-C; and 3) TBF with CRP. For factor analyses, non-significant χ^2 values indicate good fit. However, because χ^2 is sensitive to sample size and parameter ratios, the root mean square error of approximation (RMSEA) and Tucker-Lewis Index (TLI) were also included to assess model fit. RMSEA values < 0.08 and <0.05 indicate acceptable and good model fit, respectively (258). TLI values > 0.90 indicate acceptable fit (259) and values > 0.95 indicate good model fit (258).

Dichotomous Scoring Methods

Clinical Cutoff Method:

The first method to calculate AL index utilized dichotomous scoring for each variable by determining if the values were within normative clinical ranges (score =0) or outside of the normative clinical ranges (score =1). For variables that did not have normative clinical ranges (i.e., diurnal slope of salivary cortisol), the mean ±1 standard deviation (SD) was used as a cutoff. Scores that fell within ±1 SD of the mean were given a score = 0. Scores that fell outside ±1 SD of the

mean were given a score = 1. The score for each variable was then summed, creating a total AL score for each participant. The lowest possible score was = 0, indicating the lowest possible AL index (lower risk). Whereas the highest possible AL index was = 9, indicating the highest possible AL index (higher risk). Because there are no established CC to score %TBF, we separated men and women and calculated within-sex SDs. The choice to separate by sex for measures of %TBF was to account for the well-established differences in %TBF between men and women (260). Sexes were also separated in the scoring of creatinine, as there are sex-specific CC for this biomarker (261). This is consistent with previous research suggesting that sex-specific values should be used to refine the concept of AL (30). For this model, cutoffs were determined as shown in **Table 5.**

Table 5: Clinical Cutoff Method for Scoring Allostatic Load Biomarkers.

Biomarker:	**Normative Range (score=0)**	**Abnormal Range (score=1)**
Salivary Cortisol (Diurnal Slope)	Outside ±1 SD of mean of all participants	Within ±1 SD of mean for all participants
Total Body Fat, %	Men: < +1 SD above mean of all men Women: < +1 SD above mean of all women	Men: > +1 SD above mean of all men Women: > +1 SD above mean of all women
Resting SBP, mmHg	< 120 mmHg	≥ 120 mmHg
Resting DBP, mmHg	< 80 mmHg	≥ 80 mmHg
Resting HR, bpm	< 100 bpm	≥ 100 bpm
Fasting Glucose, mg/dL	≤ 99 mg/dL	> 99 mg/dL
Fasting Creatinine, mg/dL	Men: 0.74-1.35 mg/dL Women: 0.59-1.04 mg/dL	Men: < 0.74 or > 1.35 Women: < 0.59 or > 1.04
Fasting TC/HDL-C, mg/dL	< +1 SD above mean of all participants	≥ +1 SD above mean of all participants
Fasting CRP, mg/dL	< 0.3 mg/dL	≥ 0.30 mg/dL

Table 5 Key: SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; bpm, beats per minute; TC/HDL, total-cholesterol-to-high-density-lipoprotein-cholesterol ratio; CRP, C-Reactive Protein. Ranges for resting SBP, DBP, and HR are from the American Heart Association (254). The range for fasting glucose is from the CDC (262). The range for Creatinine is from The Mayo Clinic (261). The range for CRP is from the NIH (263).

Standard Deviation Method:

Post-hoc analysis revealed that only N=2 participants fell within the abnormal range for resting HR and N=1 fell within the abnormal range for measures of fasting glucose. Because there was low variability within the scores for these markers, we conducted another dichotomous scoring method for total AL, which utilized the mean + and/or -1 standard deviation (SD) as a cutoff for all nine variables, as in **Table 6**. Mean + and/or - 1 SD score determination was separated by sex for %TBF and creatinine. This is consistent with previous research suggesting that sex-specific values should be used to refine the concept of AL (16, 30).

Table 6: Standard Deviation Method for Scoring Allostatic Load Biomarkers.

Biomarker:	**Normative Range (score=0)**	**Abnormal Range (score=1)**
Salivary Cortisol (Diurnal Slope)	Outside ±1 SD of mean of all participants	Within ±1 SD of mean for all participants
Total Body Fat, %	Men: < +1 SD above mean of all men Women: < +1 SD above mean of all women	Men: ≥ +1 SD above mean of all men Women: ≥ +1 SD above mean of all women
Resting SBP, mmHg	< +1 SD above mean of all participants	≥ +1 SD above mean of all participants
Resting DBP, mmHg	< +1 SD above mean of all participants	≥ +1 SD above mean of all participants
Resting HR, bpm	< +1 SD above mean of all participants	≥ +1 SD above mean of all participants
Fasting Glucose, mg/dL	< +1 SD above mean of all participants	≥ +1 SD above mean of all participants
Fasting Creatinine, mg/dL	Men: within ±1 SD of mean of all men Women: within ±1 SD of mean of all women	Men: outside ±1 SD of mean of all men Women: within ±1 SD of mean of all women
Fasting TC/HDL-C, mg/dL	< +1 SD above mean of all participants	≥ +1 SD above mean of all participants
Fasting CRP, mg/dL	< +1 SD above mean of all participants	≥ +1 SD above mean of all participants

Table 6 Key: SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; bpm, beats per minute; TC/HDL, total-cholesterol-to-high-density-lipoprotein-cholesterol ratio; CRP, C-Reactive Protein.

Data Analysis

Analyses were performed using SPSS Version 29.0.0 (SPSS Inc., Chicago, IL, U.S.A.), GraphPad Prism Version 10.1.1 (San Diego, CA, U.S.A.), and R (Vienna, Austria). Data from all variables are reported as means (±SD). Univariate normality was assessed using Shapiro-Wilk tests and outliers were identified using the ROUT method (Q=1%). The significance level for all analyses was set at a P-value of <0.05.

Among the total sample, dichotomous scoring methods for AL index (CC vs. SD methods) were compared using a Wilcoxon matched pairs signed rank test. Baseline descriptive characteristics, raw-biomarker values, AL indices, measures of vascular function (unscaled %FMD, scaled %FMD, BD, and RH-AUC) and structure (cfPWV, AIx, and AIx75), and total scores from the self-perceived scales (SDoH-S, EDS, and PSS) were compared between sexes using unpaired t-tests when data from both sexes were normally distributed, or using Mann-Whitney tests when data from one or both sexes were non-normally distributed.

The associations between the AL index (CFA, CC, and SD methods) and measures of macrovascular function (via unscaled and scaled %FMD, and BD), microvascular function (via RH-AUC), and vascular structure (via cfPWV, AIx, and AIx75), were independently calculated using Pearson's r correlation analyses when both variables were normally distributed, or Spearman's correlation analyses when one or both variables were not normally distributed.

Sex-dependent Pearson's product-moment or Spearman's rank-order correlations were performed to assess the associations between the various methods for scoring AL index (CFA, CC, and SD methods) and the measures of vascular function (unscaled %FMD, scaled %FMD, BD, and RH-AUC), and vascular structure (cfPWV, AIx, and AIx75). When correlations were

significant, simple linear regression analyses were performed to further establish the magnitude of the associations between AL indices and measures of vascular function and/or structure.

To determine whether biological sex was a moderator of significant associations between AL index and measures of vascular health, multivariable regression analyses were first conducted to determine the overall association/s between AL index and measures of vascular function and structure with sex as a moderating variable.

To generate aggregate scores for the SDoH, a five-point Likert scale was used to numerically code responses to each question and responses were summed with a potential of 35 total points, with higher aggregate scores indicating more desirable SDoH conditions. For questions numbered 1-4 and 6-7, a response of "strongly disagree" was scored as = 1; "disagree" was scored as = 2; "neutral" was scored as = 3; "agree" was scored as = 4; and "strongly agree" was scored as = 5. For question number 5, responses were reverse scored, whereby a response of "strongly agree" was scored as = 1; "agree" was scored as = 2; "neutral" was scored as = 3; "disagree" was scored as = 4; and "strongly disagree" was scored as = 5.

Similarly, responses to the perceived Everyday Discrimination Scale (EDS) were numerically coded and summed to generate an aggregate score for each participant. For all questions on the perceived EDS, a response of "never" was scored as = 0; "less than once a year" was scored as = 1; "a few times a year" was scored as = 2; "a few times a month" was scored as = 3; "at least once a week" was scored as = 4; and "almost every day" was scored as = 5. By using this scoring method, higher aggregate scores were intended to be associated with greater perceived everyday discrimination.

To assess perceived stress, participants' responses to the PSS were numerically coded and summed to generate an aggregate score for each participant (total possible score = 40). The stem

for the items was “In the last month, how often have you...” Two sample items follow: “Found that you could not cope with all the things that you had to do?” and “Felt confident about your ability to handle your personal problems?” (reverse coded). Response choices ranged from 0 = “never” to 4 = “very often”.

To determine whether AL index (CFA, CC, and/or SD methods) was mediating any associations between perceived SDoH, everyday discrimination, and/or stress, and measures of vascular health, mediation analyses were conducted via the PROCESS macro package (264) in SPSS.

Results

Data from all variables are reported as means ± standard deviation (SD). Data are presented with and without the identified outliers.

Participant Characteristics

Eighty-five (N=85) young Black adults met screening eligibility and came to the laboratory for testing. Of the N=85 participants, N=1 arrived at the laboratory without following pre-visit instructions, so was not permitted to undergo testing, and N=7 participants were removed from the study because researchers were unable to perform a blood draw. Therefore, a total of N=78 young Black adults who participated in and completed this study are included in the analysis (Females, N=45; Males, N=33). Demographic characteristics of the participants and descriptive statistics of their AL biomarkers are provided in **Table 7**.

In response to the health history questionnaire (HHQ), only N=1 participant indicated they were aware they had high blood pressure (defined as 140/90 mmHg) at the time of the study,

whereas N=4 participants indicated in response to a separate question, that they had high blood pressure (when "high blood-pressure" values weren't defined) at the time of the study, or at some other time in their life. N=1 participant indicated they had type 1 diabetes mellitus (T1DM) at the time of the study. This participant's fasting glucose concentration was removed from analysis as they were an outlier by >200 mg/dL. For the AL index (CFA method), the fasting glucose concentration from the participant with T1DM was removed from the analysis and treated as a missing variable, whereby, the biomarker weight for this participant was computed via a Full Information Maximum Likelihood (FIML) analysis conducted using the Lavaan package in R (256). For dichotomously scored AL indices (CC and SD methods), the fasting glucose value of the participant with T1DM was removed as to not influence the mean and SD of the population, and this participant was given a score =1 for the fasting glucose biomarker score (indicating high load for that biomarker). N=2 participants indicated that they had been diagnosed with high cholesterol at some point in their life, N=1 participant indicated that they had been diagnosed with epilepsy at some point in their life, and N=15 participants indicated that they had asthma at some point in their life. None of the participants indicated that they had ever had a heart attack, stroke, varicose veins, cancer, systemic lupus, arthritis, bronchitis, emphysema, pneumonia, and/or rheumatic or scarlet fever at any point in their lives.

N=2 participants answered "yes" to a question asking if they were currently taking antihypertensive, antianginal, or thyroid medication/s, metformin, insulin, antibiotics, prescription pain medications, and/or anticoagulation therapy. Of those two participants, one indicated T1DM, whereas the other did not indicate having any of the mentioned conditions.

None of the participants indicated smoking cigarettes at the time of the study, however, N=9 indicated current use of other types of tobacco. Among the N=9 who indicated current tobacco

use, N=5 indicated use of e-cigarettes and/or vapes at the time of the study. N=2 participants indicated that they smoked cigarettes in the past but have permanently quit at least one year prior to the study.

A total of N=16 female participants indicated use of hormonal contraception at the time of the study. Of those N=16 females, N=14 indicated use of oral contraceptive pills, N=1 indicated use of hormonal injections, and N=1 did not specify method of hormonal contraception. The remaining N=29 women indicated natural and regular menstruation at the time of the study. Normal menstruation was defined as "having a menstruation cycle every ~28-30 days with ~3-5 days of menstrual bleeding," in accordance with the International Federation of Gynecology and Obstetrics (FIGO) Recommendations on Terminologies and Definitions for Normal and Abnormal Uterine Bleeding (265).

Allostatic Load Biomarkers

Allostatic load was classified using the following nine variables: 1) diurnal slope of salivary cortisol; 2) serum C-reactive protein (CRP) concentrations; 3) resting heart rate (RHR); 4) resting systolic blood pressure (SBP); 5) resting diastolic blood pressure (DBP); 6) total body fat percentage (%TBF); 7) fasting serum glucose concentrations; 8) fasting serum creatinine concentrations; and 9) fasting total-cholesterol-to-high-density-lipoprotein-cholesterol (TC/HDL-C) concentrations. The measures of allostatic load (AL) were designed to summarize dysregulation across multiple physiological systems (cardiovascular, metabolic, anthropometric, immune, and neuroendocrine). Biomarkers comprising indices of allostatic load were selected based on two major criteria: 1) theory about their role in major regulatory systems; and 2) they could be collected within the logistical and financial constraints of the project. Selection of the biomarkers was confirmed by results of confirmatory factor analyses, as shown in **Figure 4** (266).

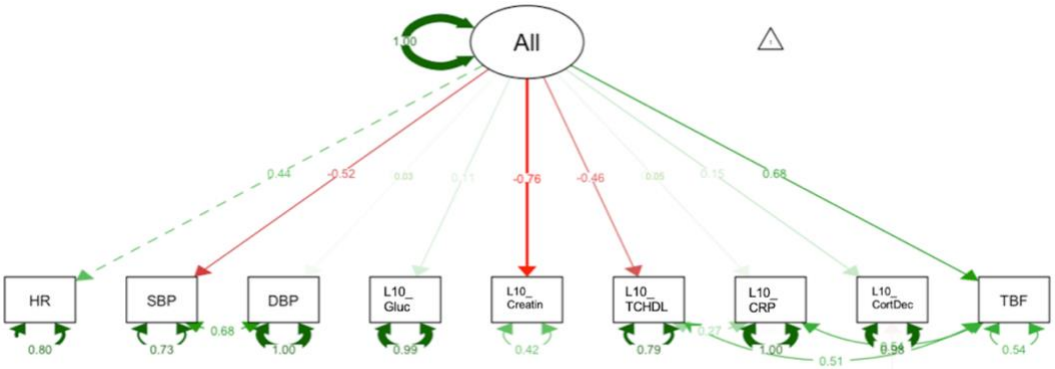


Figure 4: Confirmatory Factor Analysis Model for the Scoring of Allostatic Load.

Figure 4 Key: HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; L10_Gluc, log10(glucose); L10_Creatin, log10(creatinine); L10_TCHDL, log10(total-cholesterol-to-high-density-lipid cholesterol ratio); L10_CRP, log10(C-reactive protein); L10_CortDecr, log10(diurnal slope of salivary cortisol); TBF, total body fat.

The model in **Figure 4** had the best fit parameters (DF=24, χ^2; P=0.439, CFI; P=0.997, TLI; P=0.995, robust CFI; P=0.997, robust TFI; P=0.992, RMSEA; P=0.015, and SRMR; P=0.073). The residual variance was fixed to be equal to 1 for HR (indicated by the dotted line on **Figure 4**).

The average values for each biomarker are presented in **Table 7**. Missing data were relatively few: 100% of participants (N=78) had complete biomarker data for measurements of the cardiovascular (RHR, SBP, and DBP), metabolic (fasting glucose, creatinine, TC/HDL), and anthropometric (%TBF) components of AL; 97% of participants (N=76) had complete biomarker data for measurement of the immune (CRP) component of AL; and 77% of participants (N=60) had complete biomarker data for measurement of the neuroendocrine (diurnal slope of salivary cortisol) component of AL. Missing data were a result of late addition of the participants' data and failure of participants to return saliva samples. CRP data was missing from N=2 male participants. Salivary cortisol samples were missing from N=13 male and N=5 female participants.

Table 7: Aim 1 - Participant Characteristics and Raw Biomarkers.

	Total Sample	Female Participants	Male Participants	Sex Differences P-Value
N	78	45	33	-
Demographics				
Age, y	19.9±1.3	19.5 ±1.3	20.3±1.1	0.0053
Height, m	1.70 ± 0.09	1.65 ± 0.07	1.77 ± 0.08	<0.0001
Weight, kg	75.86 ± 19.41	70.24 ± 16.97	83.52 ± 18.60	0.0016
AL Biomarkers				
Diurnal Cortisol Slope, μg/dL	-0.26 ± 0.21	-0.23 ± 0.21	-0.30 ± 0.19	0.1949
RHR, bpm	67.79 ± 10.88	71.42 ± 11.03	62.85 ± 8.18	0.0003
SBP, mmHg	117.78 ± 11.83	112.11 ± 8.63	125.52 ± 11.29	<0.0001
DBP, mmHg	73.54 ± 8.33	73.13 ± 8.22	74.09 ± 8.58	0.6192
Serum CRP, ng/mL	0.17 ± 0.20	0.19 ± 0.21	0.15 ± 0.18	0.4077
Fasting glucose, mg/dL	87.18 ± 29.79	89.69 ± 38.96	83.76 ± 5.27	0.3885
Creatinine, mg/dL	0.83 ± 0.17	0.73 ± 0.13	0.97 ± 0.14	<0.0001
TC/HDL-C	2.77 ± 0.64	2.59 ± 0.55	3.01 ± 0.68	0.0034
TBF, %	30.03 ± 10.40	35.44 ± 7.37	22.51 ± 9.18	<0.0001

Table 7 Key. Values are means ± standard deviation. Y, years; AL, allostatic load; RHR, resting heart rate; bpm, beats per minute; SBP, systolic blood pressure; DBP diastolic blood pressure; CRP, C-reactive protein; TC/HDL-C, total-cholesterol-to-high-density-lipid-cholesterol-ratio; and TBF, total body fat. Provided P-values were derived from unpaired t-tests between females and males.

Allostatic Load Scoring

The average AL indices from each scoring method are provided in **Table 8**. For the CFA method, AL indices for each participant were scored on a continuous scale between -1 and 1. For the CC and SD methods, AL indices for each participant were scored out of a possible 9 points (one point per biomarker of AL). For all AL index scoring methods (CFA, CC, and SD), higher values indicate higher AL.

AL index (CFA method) data were normally distributed in female (N=45) and male participants (N=33). Results of an unpaired t-test between sexes indicate that AL index (CFA) was higher among female participants than male participants (**Table 8**). Data from dichotomous scoring of AL indices (CC and SD methods) were not normally distributed in female (CC: N=45,

and SD: N=45), or male participants (CC: N=33, and SD: N=33). Results of Mann-Whitney tests between sexes indicate that dichotomously scored AL indices (CC and SD methods) were similar between female and male participants (**Table 8**).

Table 8: Aim 1 - Average Allostatic Load Indices by Sex.

AL Index Scoring Method	Total Sample (N=78)	Female Participants (N=45)	Male Participants (N=33)	Sex Differences P-Value
CFA	0.001 ± 0.45	0.31±0.26	-0.42±0.28	<0.0001
CC	1.55 ± 1.23	1.36 ± 1.25	1.82 ± 1.19	0.0801
SD	1.54 ± 1.35	1.56 ± 1.32	1.52 ± 1.40	0.8399

Table 8 Key: CFA, confirmatory factor analysis; SD, standard deviation. Values are means ± SD. CFA was scored on a scale from -1 to 1. Clinical Cutoff and SD AL scores were scored on a scale of 0-9. Higher scores are associated with higher AL for all three methods.

Vascular Function and Structure

The average values for measures of vascular function and structure are presented in **Table 9.** 97% of participants (N=76) had complete data for measurements of %FMD (unscaled and scaled) and BD; 96% of participants (N=75) had complete data for measurements of RH-AUC; and 90% of participants (N=70) had complete data for measurements of cfPWV, AIx and AIx75. Missing data were a result of instrumentation failures and/or measurement difficulties. %FMD (unscaled and scaled) and BD data are missing from N=2 male participants. RH-AUC data are missing from N=3 male participants. AIx and AIx75 data are missing from N=8 female participants. cfPWV data are missing from N=1 male and N=7 female participants.

Self-Perceived Scales

Means and standard deviations from the SDoH-S, EDS, and PSS are displayed in **Table 9**. One male participant chose not to answer any questions on the EDS nor PSS. Therefore, they were

removed from analysis. An additional participant (female) failed to answer one of the questions on the SDoH-S, therefore, they were also removed from analysis. Among the total sample of participants (N=77), the SDoH-S scores, EDS scores, and PSS scores were not normally distributed. There were no outliers among the total scores for the SDoH-S, EDS, nor PSS. According to Mann-Whitney tests, the total scores for the SDoH-S, EDS, and PSS were similar between female and male participants (**Table 9**). According to Spearman's correlation analyses, among the total sample, there were no significant associations between total SDoH-S score and AL index (CFA: P=0.2150; CC: P=0.5773; SD: P=0.2883; **Table 10**). Further, when separated by sex, there were no significant correlations between total SDoH-S score and AL index (CFA: females P=0.3207, males P=0.1427; CC: females P=0.2937, males P=0.8673; SD: females P=0.6982, males P=0.2099; **Table 10**). According to Spearman's correlation analyses, among the total sample, there were no significant associations between total EDS score and AL index (CFA: P=0.8912; CC: P=0.3577; SD: P=0.4850; **Table 10**). Further, when separated by sex, there were no significant correlations between total EDS score and AL index (CFA: females P=0.1953, males P=0.8108; CC: females P=0.1564, males P=0.9959; SD: females P=0.3654, males P=0.9702; **Table 10**). Lastly, according to Spearman's correlation analyses, among the total sample, there were no significant associations between total PSS score and AL index (CFA: P=0.1704 CC: P=0.8221; SD: P=0.1219; **Table 10**). Further, when separated by sex, there were no significant correlations between total PSS score and AL index (CFA: females P=0.8529, males P=0.1299; CC: females P=0.4886, males P=0.7328; SD: females P=0.2531, males P=0.2039; **Table 10**).

Table 9: Descriptive Statistics of Measures of Vascular Function and Structure and Total Scores From Self-Perceived Scales.

	Total Sample	**Female Participants**	**Male Participants**	**Sex Differences P-Value**
Vascular Function				

Unscaled FMD, %	5.92 ± 3.64	6.42 ± 3.68	5.20 ± 3.50	0.1503
Scaled FMD, %	7.46 ± 3.19	6.24±3.02	9.25±2.55	<0.0001
BD, mm	3.52 ± 0.60	3.20 ± 0.45	3.98 ± 0.47	<0.0001
RH-AUC, AU	340.01±149.29	374.02±150.23	317.34±145.93	0.1076
Vascular Structure				
cfPWV, m/s	5.86 ± 0.73	5.72 ± 0.78	6.02 ± 0.63	0.0789
AIx, %	-2.84±13.23	-3.87±15.51	-1.97±10.38	0.5551
AIx75, %	-8.88 ± 14.17	-8.51 ± 16.33	-9.31 ± 11.42	0.8173
Self-Perceived Scales				
Total SDoH-S	21.06 ± 3.95	21.14 ± 3.52	20.97 ± 4.50	0.8558
Total EDS	13.23 ± 8.23	13.82 ± 8.93	12.41 ± 7.20	0.4606
Total PSS	21.82 ± 6.91	22.91 ± 6.30	20.28 ± 7.53	0.1002

Table 9 Key: FMD, flow-mediated dilation; BD, baseline diameter; RH-AUC, reactive-hyperemia area under the curve; AU, arbitrary units; cfPWV, carotid-to-femoral pulse wave velocity; AIx, augmentation index; AIx75, augmentation index standardized to a heart rate of 75 bpm; SDoH-S, Social Determinants of Health Scale; EDS, Everyday Discrimination Scale; PSS, Perceived Stress Scale.

Macrovascular Function

While there were no sex differences in unscaled %FMD values, according to an unpaired t-test, male participants had significantly larger BDs as compared to female participants (P<0.0001; **Table 9**). Among the total sample, a Pearson's product-moment correlation and simple linear regression were run to assess the relationship between BD and %FMD. Preliminary analyses showed the relationship to be linear with both variables normally distributed, and there were no outliers. There was a significant moderate negative Pearson's correlation between BD and %FMD (N=76; r=-0.3014; P=0.0082; **Figure 5**). The simple linear regression model further demonstrated that BD significantly predicted 9% of the variability in %FMD (R^2=0.0908; $F(1,74)$=7.39; Y=1.84*X+12.40; P=0.0082; **Figure 5**), suggesting for allometric scaling of %FMD.

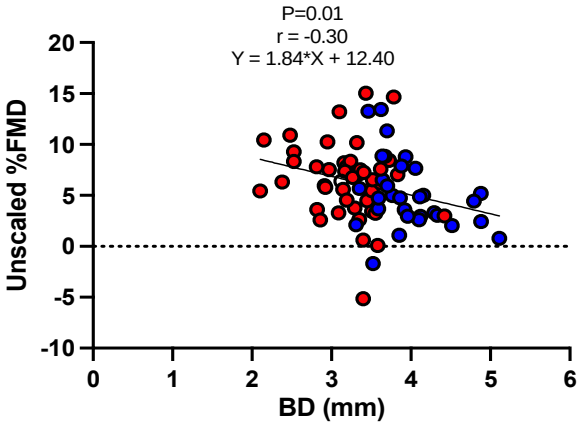


Figure 5: Correlation Between Unscaled %Flow-Mediated Dilation and Baseline Diameter.

Figure 5 Key: % FMD, Percent flow-mediated dilation; BD, baseline diameter; mm, millimeters. Data are from N=76 participants (N=45 females). Pearson's correlation: r=-0.3014, P=0.0082; Simple linear regression: R^2=0.0908, Y=-1.840*X+12.40, P=0.0082. ● Indicate female participants and ● indicate male participants. ** indicates significant (P<0.01) Pearson's correlation and linear regression.

Allometrically scaled %FMD values were estimated for each participant using the sex-specific β-coefficients (as in **Equation 2**). Estimated allometrically scaled %FMD values are normally distributed, and no statistical outliers were identified. The estimated allometrically scaled %FMD values of males were significantly greater than the estimated allometrically scaled %FMD values of females (P<0.0001; **Table 9**).

Allostatic Load and Macrovascular Function

Allostatic Load (CFA Method) and Macrovascular Function

A Pearson's product-moment correlation was run to assess the relationship between AL index (as calculated with a CFA) and unscaled %FMD. Preliminary analyses showed the relationship to be linear with both variables normally distributed, and there were no outliers. There was a statistically significant, small positive Pearson's correlation between AL index (CFA method) and unscaled %FMD (N=76; r=0.2610; P=0.0228; **Figure 6A**), whereby AL index (CFA method) statistically explained 7% of the variability in unscaled %FMD in young Black adults (R^2=0.0681; *F*(1,74)=5.41; Y=2.11*X+5.91; P=0.0228; **Figure 6A**).

When %FMD values were allometrically scaled, results differed. Preliminary analyses showed the relationship between AL index (CFA method) and allometrically scaled %FMD to be linear, with both variables normally distributed and no presence of outliers. There was a statistically significant small negative Pearson's correlation between AL index (CFA method) and allometrically scaled %FMD (N=76; r=-0.2547; P=0.0264; **Figure 6D**), whereby AL index (CFA method) statistically explained 7% of the variability in allometrically scaled %FMD in young Black adults (R^2=0.0649; *F*(1,74)=5.13; Y=-1.80*X+7.48; P=0.0264; **Figure 6D**).

There was also a significant moderate negative Pearson's correlation between AL index (CFA method) and BD (N=76; r=-0.6277; P<0.0001; **Figure 6G**). A simple linear regression further established that AL index (CFA method) significantly predicted 39% of the variability in BD in young Black adults (R^2=0.3941; *F*(1,74)=48.12; Y=-0.83*X+3.52; P <0.0001; **Figure 6G**).

Allostatic Load (CC and SD Methods) and Macrovascular Function

Spearman's rank-order correlations were run to assess the associations between AL index (as calculated with the CC and SD methods) and unscaled %FMD in young Black adults. Preliminary analyses showed the AL indices from both dichotomous scoring methods to be non-normally distributed. There were no outliers. There were no significant Spearman's correlations between AL indices (CC and SD methods) and unscaled %FMD in young Black adults (CC: N=76; r=0.1585; P=0.1714; **Figure 6B**, and SD: N=76; r=0.1563; P=0.1776; **Figure 6C**). When %FMD values were allometrically scaled, results differed. Results of a Spearman's rank-order correlation revealed a significant positive association between AL index (CC method) and allometrically scaled %FMD in young Black adults (N=76; r=0.2714; P=0.0177; **Figure 6E**). While data analysis did not detect any outlying values, upon graphical inspection, we chose to remove two points influential values to see if the correlation was still significant (**Figure 6E**). Upon removal of these

values, there was no significant Spearman's correlation between AL index (CC method) and scaled %FMD (N=74; r=0.2163; P=0.0642). There was no significant Spearman's correlation between AL index (SD method) and allometrically scaled %FMD in young Black adults (N=76; r=0.1348; P=0.2458; **Figure 6F).** There were also no significant Spearman's correlations between dichotomously scored AL indices (CC and SD methods) and BD in young Black adults (CC: N=76; r=0.2005; P=0.0825; **Figure 6H**, and SD: N=76; r=0.0307; P=0.7923; **Figure 6I**).

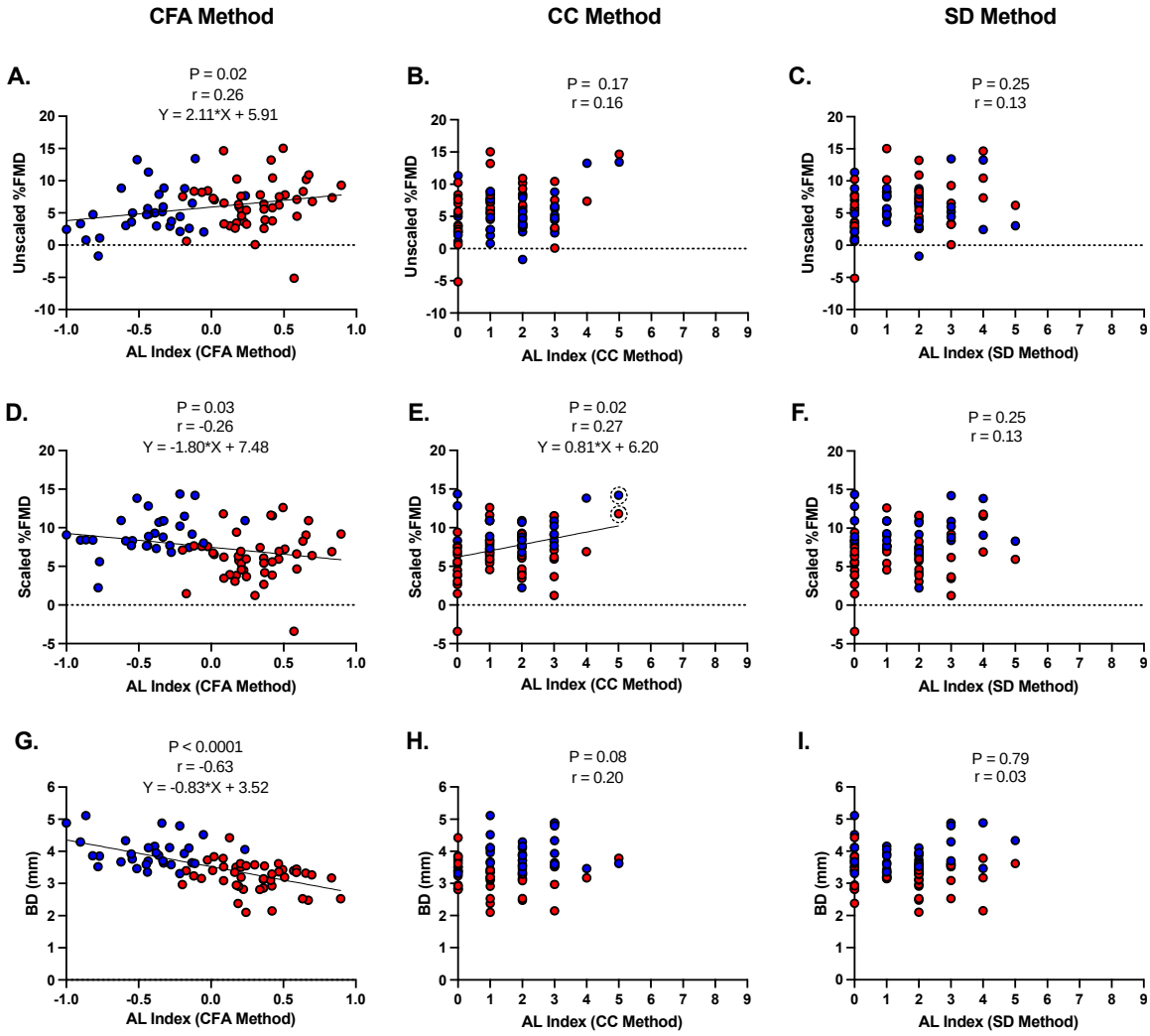


Figure 6: Correlations Between Allostatic Load and Measures of Macrovascular Function.

Figure 6 Key: AL, allostatic load; CFA, confirmatory factor analysis; CC, clinical cutoff; SD, standard deviation; %FMD, percent flow-mediated dilation; BD, baseline diameter; mm, millimeters. **A.** Significant Pearson's correlation and linear regression between AL index (CFA method) and unscaled %FMD; **B.** No

significant Spearman's correlation between AL index (CC method) and unscaled %FMD; **C.** No significant Spearman's correlation between AL index (SD method) and unscaled %FMD; **D.** Significant Pearson's correlation between AL index (CFA method) and allometrically scaled %FMD; **E.** Significant Spearman's correlation between AL index (CC method) and allometrically scaled %FMD. Values circled with dotted lines (◌) were removed from analysis to determine if they were influencing the correlation (P-value, r, and linear regression line equation on **7E** are with influential values included). When influential values are excluded, there is no significant Spearman's correlation between AL index (CC method) and allometrically scaled %FMD (N=74; P=0.06; r=0.22). **F.** No significant Spearman's correlation between AL index (SD method) and allometrically scaled %FMD. **G.** Significant Pearson's correlation and linear regression between AL index (CFA method) and BD; **H.** No significant Spearman's correlation between AL index (CC method) and BD; **I.** No significant Spearman's correlation between AL index (SD method) and BD. ● Indicate female participants and ● indicate male participants.

Moderating Role of Sex on Associations Between AL Index and Macrovascular Function

When separated by biological sex, the unscaled %FMD, scaled %FMD, and BD data were normally distributed in female participants. Among male participants, the unscaled %FMD and scaled %FMD data were normally distributed; however, BD data were not normally distributed. There were no statistical outliers in data for measures of macrovascular function in female or male participants.

Among female participants, there were no significant Pearson's correlations between AL index (CFA method) and unscaled %FMD (N=45; r=0.1242; P=0.4161; **Table 10**), scaled %FMD (N=45; r =0.1556; P=0.3073; **Table 10**), nor BD (N=45; r=-0.2674; P=0.0758; **Table 10**).

Among male participants, while there was no significant Pearson's correlation between AL index (CFA method) and unscaled %FMD (N=31; r=0.3529; P=0.0515; **Table 10**), nor a significant Spearman's correlation between AL index (CFA method) and BD (N=31; r=-0.1925; P=0.2995, **Table 10**), there was a significant positive Pearson's correlation between AL index (CFA method) and scaled %FMD (N=31; r=0.3724; P=0.0391; **Table 10**). Further, according to a simple linear regression, the male participants' AL index (CFA method) and scaled %FMD data were significantly linearly related (R^2=0.1387; $F(1,29)$=4.670; Y=3.42*X+10.70; P=0.0391; data not shown). Due to the significant association in males, a hierarchical multiple regression analysis

was run to assess the statistical significance of the interaction term between AL index (CFA method) and sex to a main effects model, whereby AL index (CFA method) was the independent variable, scaled %FMD was the dependent variable, and sex was the moderating variable. Sex did not moderate the association between AL index (CFA method) and scaled %FMD in young Black adults, as evidenced by the addition of the interaction term which explained an additional 0.55% of the total variance in scaled %FMD (P=0.2719).

Among female participants, there were no significant Spearman's correlations between dichotomously scored AL indices (CC and SD methods) and unscaled %FMD (CC: N=45; r=0.1543; P=0.3115, nor SD: N=45; r=0.1659; P=0.2760, **Table 10**), scaled %FMD (CC: N=45; r=0.2239; P=0.1393, nor SD: N=45; r=0.1655; P=0.2773, **Table 10**), nor BD (CC: N=45; r=0.0663; P=0.6652, nor SD: N=45; r=0.0319; P=0.8354, **Table 10**).

Among male participants, there were also no significant Spearman's correlations between dichotomously scored AL indices (CC and SD methods) and unscaled %FMD (CC: N=31; r=0.2544; P=0.1673, nor SD: N=31; r=0.1589; P=0.3931, **Table 10**), scaled %FMD (CC: N=31; r=0.1288; P=0.4900, nor SD: N=31; r=0.0793; P=0.6717, **Table 10**), nor BD (CC: N=31; r=0.0383; P=0.8378, nor SD: N=31; r=0.1099; P=0.5562, **Table 10**).

Mediating Role of AL Index on Associations Between Self-Perceived Stressors and Macrovascular Function

Spearman's rank-order correlations were run to assess the associations between responses to the SDoH-S, EDS, and PSS with measures of macrovascular function in young Black adults. Despite our initial hypotheses, there were no significant Spearman's correlations between the total SDoH-S scores and unscaled %FMD (N=75; r=0.0610; P=0.6031; **Figure 7A**), scaled %FMD (N=75; r=-0.1367; P=0.2422; **Figure 7D**), nor BD (N=75; r=-0.0114; P=0.9228; **Figure 7G**).

Therefore, we were unable to conduct a mediation analysis between AL, total SDoH-S scores, and macrovascular function, as originally intended. Similarly, in contrast with our initial hypotheses, there were no significant Spearman's correlations between the total EDS scores with unscaled %FMD (N=75; r=-0.0228; P=0.8460; **Figure 7B**), scaled %FMD (N=75; r=-0.0615; P=0.6001; **Figure 7E**), nor BD (N=75; r=-0.0743; P=0.5262; **Figure 7H**). Therefore, we were also unable to conduct a mediation analysis between AL, total EDS scores and macrovascular function, as originally intended. Lastly, while there were no significant Spearman's correlations between total PSS scores and unscaled %FMD (N=75; r=0.0379; P=0.7469; **Figure 7C**) nor scaled %FMD (N=75; r=-0.1196; P=0.3069; **Figure 7F**), there was a significant negative Spearman's correlation between total PSS score and BD (N=75; r=-0.2968; P=0.0097; **Figure 7I**). Further, according to a simple linear regression, the participants' total PSS scores and BD data were significantly linearly related (R^2=0.0577; $F(1,73)$=4.47; Y=-0.02*X+3.98; P=0.0379; **Figure 7I**).

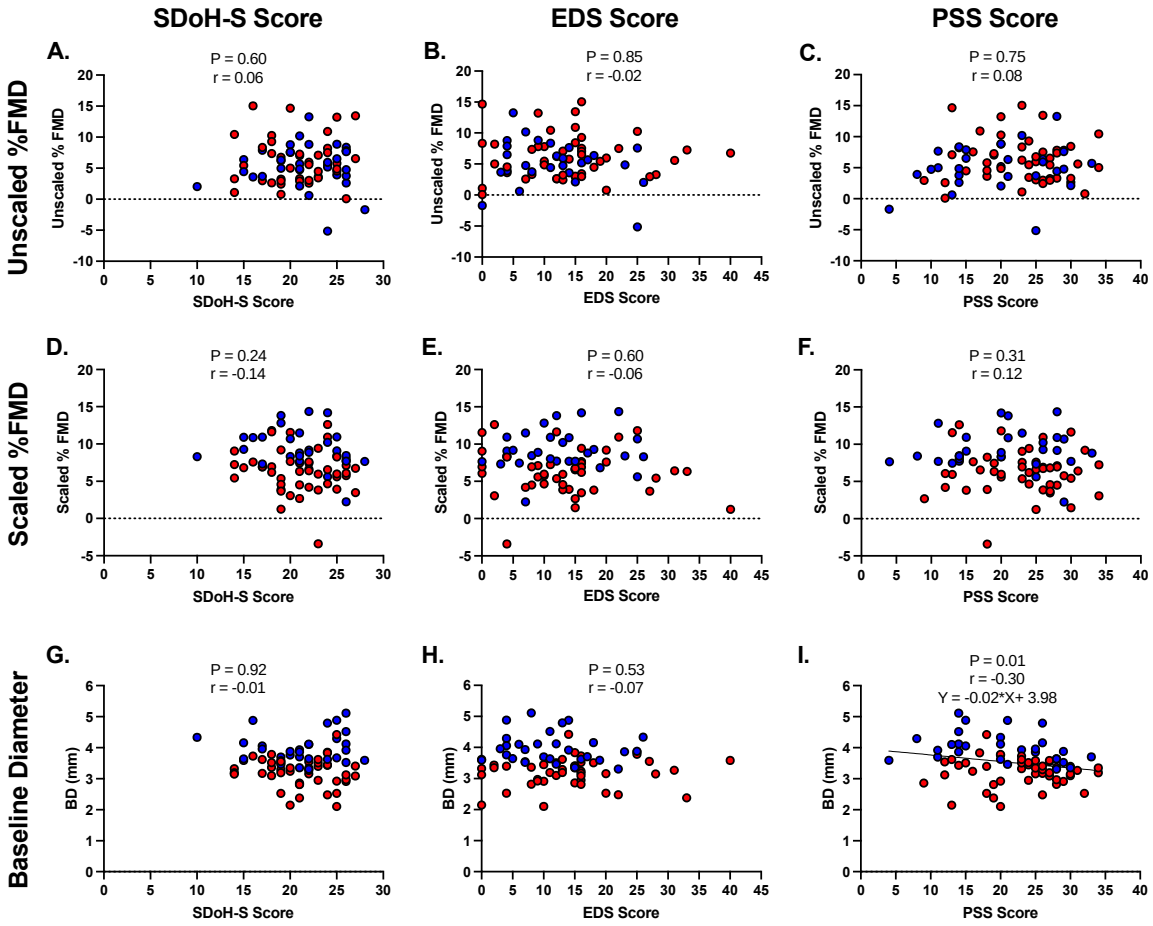


Figure 7: Correlations Between SDoH-S, EDS, and PSS Scores and Measures of Macrovascular Function.

Figure 7 Key: SDoH-S, social determinants of health scale; EDS, everyday discrimination scale; PSS, perceived stress scale; FMD, flow-mediated dilation; BD, baseline diameter; mm, millimeters. N=75 for all. **A.** No significant Spearman's correlation between the total SDoH-S score and unscaled %FMD. **B.** No significant Spearman's correlation between the total EDS score and unscaled %FMD. **C.** No significant Spearman's correlation between the total PSS score and unscaled %FMD. **D.** No significant Spearman's correlation between the total SDoH-S score and scaled %FMD. **E.** No significant Spearman's correlation between the total EDS score and scaled %FMD. **F.** No significant Spearman's correlation between the total PSS score and scaled %FMD. **G.** No significant Spearman's correlation between the total SDoH-S score and BD. **H.** No significant Spearman's correlation between the total EDS score and BD. **I.** Significant Spearman's correlation and linear regression between the total PSS score and BD. ● Indicate female participants and ● indicate male participants.

Microvascular Function

Microvascular function was assessed via RH-AUC. Preliminary analyses showed that RH-AUC was not normally distributed among the total sample. However, there were no significant outliers in measures of RH-AUC when the data was pooled into the full sample of young Black adults. There were no sex differences in measures of RH-AUC (**Table 9**).

Allostatic Load and Microvascular Function

Allostatic Load (CFA Method) and Microvascular Function

Spearman's rank-order correlations were run to assess the associations between AL index (as calculated with the CFA method) and RH-AUC. Preliminary analyses showed AL index (CFA method) to be normally distributed with no outliers. Results revealed that there was no significant Spearman's correlation between AL index (CFA method) and RH-AUC in young Black adults (N=75; r=0.1448; P=0.2152; **Figure 8A**).

Allostatic Load (CC and SD Methods) and Microvascular Function

Spearman's rank-order correlations were run to assess the associations between AL indices (as calculated with the CC and SD methods) and RH-AUC. There were no significant Spearman's correlations between dichotomously scored AL indices (CC and SD methods) and RH-AUC in young Black adults (CC: N=75; r=0.1799; P=0.1225; **Figure 8B**, and SD: N=75; r=0.0771; P=0.5110; **Figure 8C**).

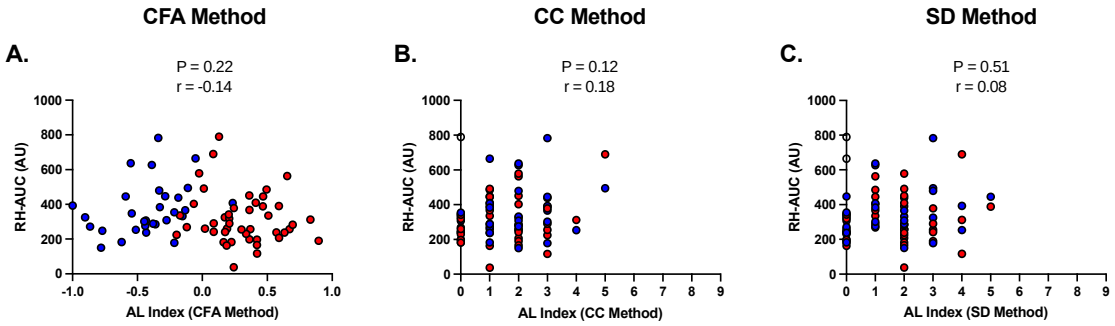


Figure 8: Correlations Between Allostatic Load Indices and Reactive Hyperemia Area Under the Curve.

Figure 8 Key: AL, allostatic load; CFA, confirmatory factor analysis; CC, clinical cutoff; SD, standard deviation; RH-AUC, reactive hyperemia area under the curve; AU, arbitrary units. **A.** No significant Spearman's correlation between AL index (CFA method) and RH-AUC; **B.** No significant Spearman's correlation between AL index (CC method) and RH-AUC; **C.** No significant Spearman's correlation between AL index (SD method) and RH-AUC. ● Indicate female participants and ● indicate male participants.

Moderating Role of Sex on Associations Between AL Index and Microvascular Function

When separated by biological sex, the RH-AUC data are not normally distributed in female or male participants. Within the female participants, one participant's RH-AUC data was considered a statistically significant outlier as compared to the other female participants' RH-AUC data. Upon removal of the outlier, RH-AUC was normally distributed in female participants. There were no significant outliers among male participants.

Among female participants, there was no significant correlation between AL index (CFA method) and RH-AUC data when the outlier was included (N=45; r=-0.1365; P=0.3713; **Table 10**) nor when removed from analysis (N=44; r=-0.0960; P=0.5355; **Table 10**). However, among male participants, there was a positive Spearman's correlation between AL index (CFA method) and RH-AUC (N=30; r=0.3976; P=0.0296; **Table 10**). Despite the significant correlation, according to a simple linear regression, the data were not significantly linearly related (R^2=0.0929; *F*(1,28)=2.87; Y=168.18*X+443.65; P=0.1015; **Table 10**). Regardless, a hierarchical multiple regression analysis was run to assess the statistical significance of the interaction term between

AL index (CFA method) and sex to a main effects model, whereby AL index (CFA method) was the independent variable, RH-AUC was the dependent variable, and sex was the moderating variable. Sex did not moderate the association between AL index (CFA method) and RH-AUC, as evidenced by the addition of the interaction term which explained an additional 0.95% of the total variance in RH-AUC (P=0.3913).

Among female participants, there were no significant Spearman's correlations between dichotomously scored AL indices (CC or SD methods) and RH-AUC when the outlier was included (CC: N=45; r=0.1292; P=0.3976, nor SD: N=45; r=0.0327; P=0.8312, **Table 10**), nor when it was removed from analysis (CC: N=44; r=0.1842; P=0.2313, nor SD: N=44; r=0.0827; P=0.5937, data not shown). Among male participants, there were also no significant Spearman's correlations between AL indices (CC or SD methods) and RH-AUC (CC: N=30; r=0.1817; P=0.3365, nor SD: N=30; r=0.1510; P=0.4257, **Table 10**).

Mediating Role of AL Index on Associations Between Self-Perceived Stressors and Microvascular Function

Spearman's rank-order correlations revealed no significant associations between the self-perceived scale's scores with measures of RH-AUC (SDoH-S: N=74; r=-0.1916; P=0.1020, EDS: N=74; r=0.0600; P=0.6115, PSS: N=74; r=-0.1179; P=0.3173, **Figure 9A-C**). Because there were also no significant associations between AL index and RH-AUC, the goal to assess the mediating role of the AL on the associations between responses to self-perceived scales and microvascular function became obsolete.

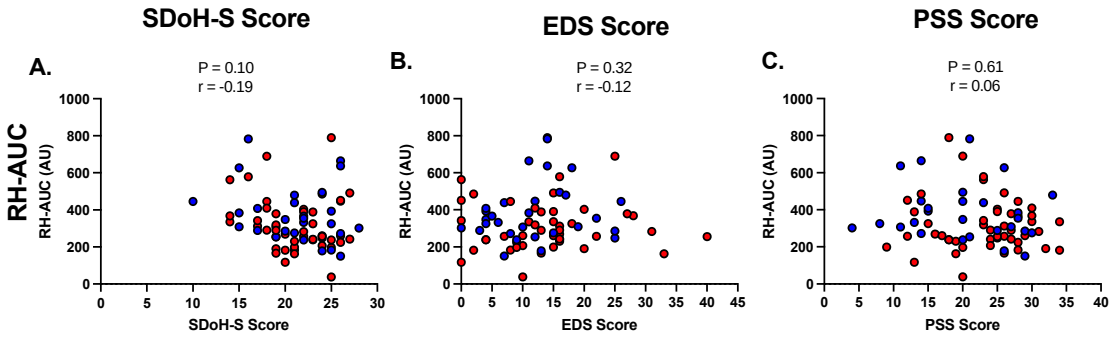


Figure 9: Correlations Between Self-Perceived Scales With Measures of Microvascular Function.

Figure 9 Key: SDoH-S, social determinants of health scale; EDS, everyday discrimination scale; PSS, perceived stress scale; RH-AUC, reactive hyperemia area under the curve; A.U., arbitrary units. N=74 for all. **A.** No significant Spearman's correlation between the total SDoH-S score and RH-AUC. **B.** No significant Spearman's correlation between the total EDS score and RH-AUC. **C.** No significant Spearman's correlation between the total PSS score and RH-AUC. ● Indicate female participants and ● indicate male participants.

Vascular Structure

Vascular structure was assessed via measures of arterial stiffness (cf-PWV) and wave reflection (AIx and AIx75). Preliminary analyses showed that measures of cfPWV, AIx, and AIx75 were normally distributed and there were no significant outliers among the total sample of young Black adults. There were no sex differences in measures of cf-PWV, AIx, or AIx75 (**Table 8**).

Allostatic Load and Vascular Structure

Allostatic Load (CFA Method) and Vascular Structure

Among the total sample, there were no significant Pearson's correlations between AL index (CFA method) and measures of vascular structure (cfPWV: N=70; r=-0.1709; P=0.1571; **Figure 10A**, AIx: N=70; r=-0.0058; P=0.9623; **Figure 10D**, and AIx75: N=70; r=0.1536; P=0.2043; **Figure 10G**).

Allostatic Load (CC and SD Methods) and Vascular Structure

There were no significant Spearman's correlations between dichotomously scored AL indices (CC and SD methods) and cfPWV in young Black adults (CC: N=70; r=0.1496; P=0.2166; **Figure 10B**, and SD: N=70; r=0.1367; P=0.2593; **Figure 10C**). However, there were significant positive Spearman's correlations between dichotomously scored AL indices (CC and SD methods) and AIx in young Black adults (CC: N=70; r=0.3092; P=0.0092; **Figure 10E**, and SD: N=70; r=0.3612; P=0.0021; **Figure 10F**). The dichotomously scored AL indices (CC and SD methods) were also positively correlated with AIx75 in young Black adults (CC: N=70; r=0.3106; P=0.0089; **Figure 10H**, and SD: N=70; r=0.3972; P=0.0007; **Figure 10I**).

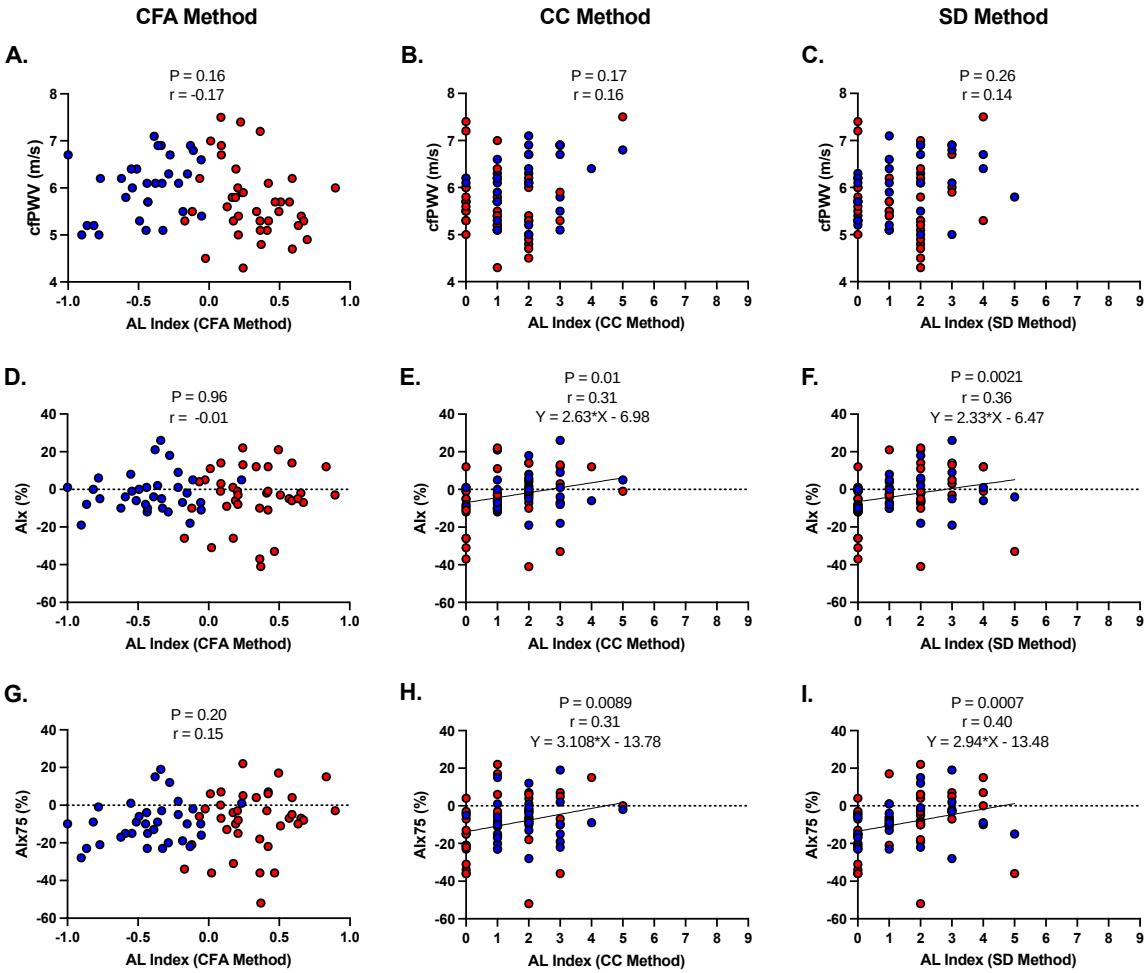


Figure 10: Correlations Between Allostatic Load and Vascular Structure.

Figure 10 Key: AL, allostatic load; CFA, confirmatory factor analysis; CC, clinical cutoff; SD, standard deviation; cfPWV, carotid-to-femoral pulse wave velocity; AIx, augmentation index; AIx75, augmentation index standardized to a heart rate of 75 bpm. **A.** Insignificant Pearson's correlation between AL index (CFA method) and cfPWV; **B.** Insignificant Spearman's correlation between AL index (CC method) and cfPWV; **C.** Insignificant Spearman's correlation between AL index (SD method) and cfPWV; **D.** Insignificant Pearson's correlation between AL index (CFA) and AIx; **E.** Significant Spearman's correlation between AL index (CC method) and AIx; **F.** Significant Spearman's correlation between AL index (SD method) and AIx; **G.** Insignificant Pearson's correlation between AL index (CFA method) and AIx75; **H.** Significant Spearman's correlation between AL index (CC method) and AIx75; **I.** Insignificant Spearman's correlation between AL index (SD method) and AIx75. ● Indicate female participants and ● indicate male participants.

Moderating Role of Sex on Associations Between AL Index and Vascular Structure

When separated by biological sex, the cfPWV and AIx75 data were normally distributed in female participants. However, AIx data were not normally distributed in female participants. Among male participants, data for all measures of vascular structure were normally distributed. There were no statistical outliers in data for measures of vascular structure in female or male participants.

Among the female participants, there were no significant Pearson's correlations between AL index (CFA method) and all measures of vascular structure (cfPWV: N=38; r=-0.2297; P=0.1653, AIx: N=37; r=0.0393; P=0.8175, and AIx75: N=37; r=0.2054; P=0.2226, **Table 10**).

Among the male participants there were also no significant correlations between AL index (CFA method) and all measures of vascular structure (cfPWV: N=32; r=0.3073; P=0.0871, AIx: N=33; r=0.1083; P=0.5485, and AIx75: N=33; r=0.2591; P=0.1453, **Table 10**).

Among the female participants, there were no significant Spearman's correlations between dichotomously scored AL indices (CC and SD methods) and cfPWV (CC: N=38; r=-0.0126; P=0.9400, and SD: N=38; r=0.0437; P=0.7943, **Table 10**). However, in female participants, according to Spearman's correlations, dichotomously scored AL indices (CC and SD methods) were positively correlated with AIx (CC: N=37; r=0.3911; P=0.0167, and SD: N=37, r=0.3635;

P=0.0270, **Table 10**) and AIx 75 (CC: N=37; r=0.4014; 0.0138, and SD: N=37; r=0.4308; P=0.0078, **Table 10**). Despite significant Spearman's correlations, when assessed with simple linear regressions, there were no significant associations between AL index (CC and SD methods) and AIx (CC: R^2=0.0761; *F*(1,35)=2.88; Y=3.36*X-8.40; P=0.0985; data not shown, SD: R^2=0.0611; *F*(1,35)=2.28; Y=2.77*X-8.29; P=0.1402; data not shown) nor AIx75 (CC: R^2=0.0965; *F*(1,35)=3.74; Y=0.02*X+1.56; P=0.0613; data not shown, SD: R^2=0.0873; *F*(1,35)=3.35; Y=0.03*X+1.81; P=0.0873; data not shown) in female participants.

Within the male participants, there were no significant Spearman's correlations between AL index (CC method) and measures of vascular structure (cfPWV: N=32; r=0.2907; P=0.1065, AIx: N=33; r=0.2092; P=0.2426, and AIx75: N=33; r=0.2959; P=0.0945, **Table 10**). There was also no significant Spearman's correlation between AL index (SD method) and cfPWV among male participants (N=32; r=0.2884; P=0.1094, **Table 10**). However, like the females, among male participants, AL index (SD method) was positively correlated with AIx (N=33; r=0.3476; P=0.0474, **Table 10**) and AIx75 (N=33; r=0.3705; P=0.0338, **Table 10**). Similar to females, despite the significant Spearman's correlations, when assessed with simple linear regressions, there were no significant associations between AL index (SD method) and AIx (R^2=0.06993; *F*(1,31)=2.33; Y=1.97*X-4.95; P=0.1370; data not shown) nor AIx75 (R^2=0.0832; *F*(1,31)=2.81; Y=0.04*X+1.85; P=0.1035; data not shown) in male participants.

Despite no significant linear regression, due to the significant correlations between dichotomously scored AL indices (CC and SD methods) and AIx and AIx75, hierarchical multiple regression analyses were run to assess the statistical significance of 1) the interaction term between AL index (CC method) and sex to a main effects model, and 2) the interaction term between AL index (SD method) and sex to a main effects model. In these analyses, AL index (CC or SD

method) was the independent variable, AIx or AIx75 was the dependent variable, and sex was the moderating variable. Sex did not moderate the associations between AL indices (CC or SD method) and AIx, as evidenced by the addition of the interaction terms, which explained an additional 0.72% (CC: P=0.4777), and 0.21% (SD: P=0.7015), respectively, of the total variance in AIx. Similarly, sex did not moderate the associations between AL indices (CC or SD method) and AIx75, as evidenced by the addition of the interaction terms, which explained an additional 0.48% (CC: P=0.5595), and 0.32 % (SD: P=0.6302), respectively, of the total variance in AIx75.

Table 10: Correlation Coefficients From Associations of Allostatic Load Indices With Measures of Vascular Function and Structure and Total Scores From Self-Perceived Scales.

	Total Sample			Female Participants			Male Participants		
	AL Index (CFA)	**AL Index (CC)**	**AL Index (SD)**	**AL Index (CFA)**	**AL Index (CC)**	**AL Index (SD)**	**AL Index (CFA)**	**AL Index (CC)**	**AL Index (SD)**
Vascular Function									
Unscaled FMD, %	0.2610 *	0.1585	0.1563	0.1242	0.2382	0.1759	0.3529	0.2544	0.1589
Scaled FMD, %	-0.2547 *	0.2714 *	0.1348	0.1556	0.2239	0.1655	0.3724 *	0.1288	0.0793
BD, mm	-0.6277 *	0.2005	0.0307	-0.2674	0.0663	0.0319	-0.1925	0.0383	0.1099
RH-AUC, AU	-0.1448	0.1799	0.0771	-0.1365	0.1292	0.0327	0.3976 *	0.1817	0.1510
Vascular Structure									
cfPWV, m/s	-0.1709	0.1496	0.1367	-0.2297	-0.0126	0.0437	0.3073	0.2907	0.2884
AIx, %	-0.0058	0.3092 *	0.3612 *	0.0393	0.3911 *	0.3635 *	0.1083	0.2092	0.3476 *
AIx75, %	0.1536	0.3106 *	0.3972 *	0.2054	0.4014 *	0.4308 *	0.2591	0.2959	0.3705 *
Self-Perceived Scales									
Total SDoH-S Score	-0.1429	-0.0645	-0.1225	-0.1532	-0.1619	-0.0601	-0.2608	0.0303	-0.2241
Total EDS Score	-0.0159	0.1063	0.0808	-0.1967	0.2148	0.3654	0.0441	0.0009	0.0069
Total PSS Score	0.1578	0.0261	0.1778	0.0284	0.1059	0.1740	0.2735	0.0628	0.2307

Table 10 Key: FMD, flow-mediated dilation; BD, baseline diameter; RH-AUC, reactive-hyperemia area under the curve; A.U., arbitrary units; cfPWV, carotid-to-femoral pulse wave velocity; AIx, augmentation index; AIx75, augmentation index standardized to a heart rate of 75 bpm; SDoH-S, Social Determinants of Health Scale; EDS, Everyday Discrimination Scale; PSS, Perceived Stress Scale. Values are Pearson's or Spearman's correlation coefficients (r). * Indicates significant correlation ($P<0.05$) between column-by-row variables.

Mediating Role of AL Index on Associations Between Self-Perceived Stressors and Vascular Structure

Spearman's rank-order correlations were run to assess the associations between responses to the SDoH-S, EDS, and PSS with measures of vascular structure in young Black adults. Despite our initial hypotheses, there were no significant Spearman's correlations between the total SDoH-S scores with cfPWV (N=69; r=-0.0908; P=0.4581; **Figure 11A**), AIx (N=69; r=-0.1776; P=0.1443; **Figure 11D**), nor AIx75 (N=69; r=-0.1647; P=0.1762; **Figure 11G**). Further, there were no significant associations between AL indices (CFA, CC, nor SD methods) and cfPWV (CFA: P=0.2150; CC: P=0.5773; SD: P=0.2883; **Table 10**). Therefore, assessment of the mediation effect of AL on the associations between total SDoH-S scores and measures of vascular structure (as originally intended) was deemed unwarranted.

Similarly, in contrast with our initial hypotheses, there were no significant Spearman's correlations between the total EDS scores with cfPWV (N=69; r=0.2211; P=0.0679; **Figure 11B**), AIx (N=69; r=0.1340; P=0.2724; **Figure 11E**), nor AIx75 (N=69; r=0.1150; P=0.3468; **Figure 11H**). Therefore, due to these null finding, coupled with the insignificant associations between AL indices (CFA, CC, and SD methods) and total EDS scores (CFA: P=0.8912; CC: P=0.3577; SD: P=0.4850; **Table 10**), assessment of the mediating effect of AL on associations between total EDS scores and measures of vascular structure (as originally intended) was deemed unwarranted.

Lastly, while there were no significant Spearman's correlations between total PSS scores with cfPWV (N=69; r=0.1561; P=0.2002; **Figure 11C**), nor AIx (N=69; r=0.2295; P=0.0578; **Figure 11F**), there was a significant negative Spearman's

correlation between total PSS score and AIx75 (N=69; r=0.2687; P=0256; **Figure 11I**). Further, according to a simple linear regression, the total PSS scores and AIx75 data were significantly linearly related (R^2=0.0754; *F*(1,67)=5.461; Y=-0.02*X+3.98; P=0.0224; **Figure 11I**). Despite the significant associations between dichotomously scored AL indices (CC and SD methods) and AIx75, and the significant association between total PSS scores and AIx75, since there were no significant associations between AL indices (CC or SD) and total PSS scores (CC: P=0.8221; SD: P=0.1219; **Table 10**), there were no grounds for assessing the mediation effect of AL on the associations between total PSS scores and measures of vascular structure as originally intended.

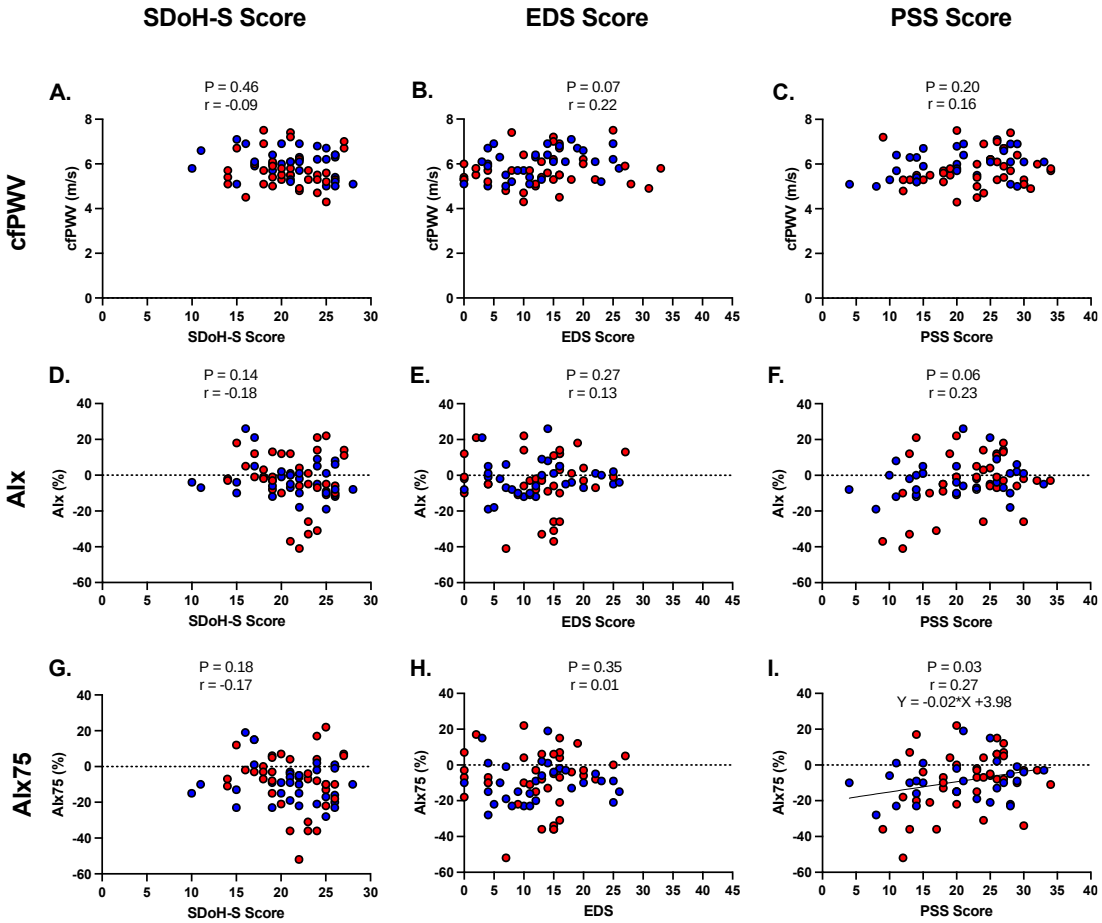


Figure 11: Correlations Between Responses to Self-Perceived Scales and Measures of Vascular Structure.

Figure 11 Key: SDoH-S, social determinants of health scale; EDS, everyday discrimination scale; PSS, perceived stress scale; cfPWV, carotid-to-femoral pulse wave velocity; AIx, augmentation index; AIx75, augmentation index standardized to a heart rate of 75 beats per minute. N=69 for all. **A.** No significant Spearman's correlation between the total SDoH-S score and cfPWV. **B.** No significant Spearman's correlation between the total EDS score and cfPWV. **C.** No significant Spearman's correlation between the total PSS score and cfPWV. **D.** No significant Spearman's correlation between the total SDoH-S score and AIx. **E.** No significant Spearman's correlation between the total EDS score and AIx. **F.** No significant Spearman's correlation between the total PSS score and AIx. **G.** No significant Spearman's correlation between the total SDoH-S score and AIx75. **H.** No significant Spearman's correlation between the total EDS score and AIx75. **I.** Significant Spearman's correlation between the total PSS score and BD. ● Indicate female participants and ● indicate male participants.

Discussion

Novel findings from this study are that among young Black adults, i) higher AL index is associated with smaller resting BD and higher unscaled %FMD, but with lower measures of allometrically scaled %FMD; ii) higher AL index is associated with larger wave reflection; and iii) AL index is not associated with measures of microvascular function or central artery stiffness. Additionally, this study's exploratory analyses revealed that among young Black adults, i) sex does not moderate the observed correlations between AL indices and measures of vascular health; and ii) there are significant correlations between higher scores on the PSS with smaller BD, and with greater wave reflection.

Allostatic Load and Macrovascular Function

The first of our main findings, that higher AL is correlated with greater unscaled %FMD, is contradictory with the initial hypothesis that higher AL would beget less-favorable macrovascular function. This hypothesis was formed based on robust evidence of chronic stress-induced increases in neuroendocrine dysfunction (via

chronic increases in cortisol, and thus flatter diurnal slopes of cortisol), and evidence of subsequential immune (61, 241), cardiovascular (234), and metabolic (267, 268) deviations from the healthy allostatic response. However, participants' BD in the present study may partially explain the discrepancy between our hypothesis and results. Given that lower AL index was correlated with larger BD, it is possible that the individuals with larger arteries were at maximum vasodilation during rest and were therefore unable to further vasodilate in response to the reactive hyperemia. Additionally, due to the young men having larger BDs than the young women in our study, a common discrepancy observed between sexes in young adults (239, 269–274), we assessed the variance between sexes to determine if sex was moderating the significant associations of AL index with the unscaled measures %FMD. Therefore, by assessing the interaction of sex in the associations we observed between AL and unscaled %FMD, we were able to correct for sex-specific discrepancies between the BD and peakD through the estimation of allometrically scaled %FMD. Allometrically scaling the %FMD values in the total population reversed the correlation between AL index and macrovascular function. These findings align with the hypothesis that higher AL would beget less favorable macrovascular function. When combined, the findings suggest that the BD is largely influential in the determination of macrovascular function in young Black adults, and that sex is a not a moderating variable in this association. These findings are consistent with Akins et al. (2022), who report an interplay between BD and %FMD in young Black men and women.

It is plausible that higher AL was correlated with impaired endothelial function (via scaled %FMD) and amplified wave-reflection (via AIx) due to early aging of the

vasculature. Early aging of the vasculature refers to premature structural and functional alterations in blood vessels occurring before the expected chronological age (275). Physiologically, this phenomenon is characterized by impaired vasodilation and increased vasoconstriction due to oxidative stress, inflammation, and diminished nitric oxide bioavailability (275). Indeed, impaired endothelial function and unfavorable structural changes in the vasculature occur with advancing biological age, which put an individual at an increased risk for CVD (275).

Allostatic Load and Microvascular Function

Contrary to our initial hypothesis, there are no correlations between AL index (assessed with the CFA, CC, or SD method) and microvascular function (assessed via RH-AUC) in young Black adults. The preservation of microvascular function in the presence of high AL may be due to the compensatory role of endothelium-derived hyperpolarizing factors (EDHFs; such as, epoxyeicosatrienoic acids, hydrogen peroxide, and potassium ions) in the vasculature. While NO primarily acts as the endothelium-dependent vasodilator in conduit arteries, EDHFs play a more significant role in the smaller arteries and arterioles of the microcirculation, especially in individuals with impaired NO activity (276–278).

In conditions like arterial hypertension, where NO availability is often reduced (279), EDHF-mediated vasodilation becomes particularly relevant in regulating vascular tone and systemic BP. Specifically, among hypertensive patients, the responsiveness to bradykinin (a vasodilatory agent) is unaffected by NOS-inhibition but is blunted in the presence of the sodium-potassium-ATPase pump blockade,

ouabain, indicating the involvement of EDHF as an alternative vasodilator pathway in smaller resistance vessels when NO is less readily available (280).

Moreover, racial differences in EDHF activity have been observed, with Black adults demonstrating a greater reliance on EDHF-mediated vasodilation compared to White adults, even in the absence of known cardiovascular risk factors (81). This could be due to decreased NO availability often observed in Black adults when compared to their White adult counterparts (81, 82). Specifically, it has been found that while NO contributes more to resting tone and stimuli-mediated vasodilation in White adults, EDHFs will partially compensate for the reduced NO release in Black adults (81). These findings suggest that Black individuals may have adapted to maintain microvascular function in the face of high AL by relying more on EDHF-mediated vasodilation, which helps preserve microvascular health, despite potential limitations in NO bioavailability.

Allostatic Load and Vascular Structure

In the present study, the dichotomous scoring methods for calculating AL index (CC and SD methods) were positively associated with measures of wave reflection (AIx and AIx75). Increased wave reflections have been associated with increased risk for CVD in healthy individuals (281). While previous studies have established associations between acute stressors and elevated wave reflections in young, apparently healthy adults (173, 282), the associations between chronic psychological and physiological stressors with indices of wave reflection in young, apparently healthy adults had not yet been explored. It is likely that augmented AIx is occurring in young Black adults due to early vascular aging. Specifically, arterial stiffening arising from

changes in arterial wall composition, such as augmented collagen deposition and reduced elastin content, can alter the smooth muscle tone and contribute to enhanced vasoconstriction. These alteration in vascular structure may arise from increased sympathetic tone, and manifest as smaller BD, even at rest. Changes in sympathetic function and arterial structure, such as increased AIx, may precede alterations in vascular function during early vascular aging due to several physiological processes. Firstly, increased SNA can lead to vasoconstriction and heightened arterial stiffness through the release of neurotransmitters like norepinephrine, which directly influence smooth muscle tone and arterial wall properties. Consequently, changes in sympathetic function may induce early modifications in arterial structure, as reflected by increased AIx, before noticeable changes in vascular function become apparent. Secondly, alterations in arterial structure, such as increased collagen deposition and reduced elastin content, contribute to arterial stiffness by diminishing the vessel's ability to expand and contract in response to changes in blood flow. These structural changes may occur relatively early in the aging process and can influence AIx measurements. However, changes in vascular function, such as decreased %FMD, may take longer to manifest, as changes in endothelial function of larger vessels involve more complex molecular and cellular processes that are subject to multiple regulatory mechanisms. Therefore, while changes in sympathetic function and arterial structure may occur early in vascular aging, alterations in vascular function may lag slightly, making stiffness assessments like AIx a potentially sensitive marker for early detection of vascular aging.

Allostatic Load, Vascular Health, and Perceived Stressors

Results of the present study differ from our hypotheses that lower perceptions of SDoH and/or higher perceptions of everyday discrimination stress would be associated with greater vascular dysfunction, higher arterial stiffness, and elevated wave reflections. Indeed, our results revealed no associations between responses to the SDoH-S or EDS with measures of vascular health. Similarly, there were no significant correlations between responses to the PSS and vascular function. However, results confirmed our hypothesis of a positive correlation between results of the PSS and wave reflection. Interestingly, there were no correlations between responses to the SDoH-S, EDS, or PSS and AL indices. Therefore, our goal to detect the mediating effect of AL index on the associations between results of the SDoH-S, EDS, and PSS with measures of vascular health became obsolete.

Among a cohort of young healthy men, those reporting higher self-perceived depressive symptomology (without meeting the threshold for major depressive disorder), had significantly elevated wave-reflections following CPT as compared to young men with lower self-perceived depressive symptomology (282). The hyperactive cardiovascular response (via increased AIx) to the CPT in the more depressed group of male participants is postulated to be driven by chronic increases in adrenergic stimulation, owing to altered plasma catecholamines concentrations, which may ultimately increase smooth muscle vascular tone (282). While perceived depression was not assessed in the present study, results of this scale have been correlated with responses to the PSS (121), indicating that there may be some overlapping constructs

between the two scales that could, down the line, play a role in elevating wave reflections to the heart. Indeed, given the positive correlations between i) AL index (CC and SD methods), and ii) PSS score with AIx75 in the present study, a plausible interpretation of our results may be that chronic perceived stress is a catalyst for multi-system physiological dysregulation in young Black adults, which can be detected by elevations in AIx75 and smaller BD. While higher scores on the PSS have shown no association with %FMD (283), there have been established associations between higher PSS and adverse physiological changes, including increased SNA (284) and inflammation (241, 284). Therefore, it's plausible that individuals experiencing higher levels of perceived stress may exhibit greater SNA and altered vascular tone, leading to increased stiffness in the peripheral arteries and thus higher AIx75 values.

Limitations and Future Directions

There are some limitations of our study that should be acknowledged. Firstly, our study focused exclusively on young adults, which may limit the generalizability of our findings to older age groups or clinical populations. Future research should aim to replicate these findings across diverse age ranges and clinical cohorts to ascertain the broader applicability of our results. Secondly, we did not collect information from participants regarding lifestyle and behavioral factors, such as dietary patterns and exercise habits. These variables could potentially serve as confounding factors influencing the calculation and interpretation of the total AL indices, as well as introducing variability within individual biomarkers. Future studies should incorporate comprehensive assessments of these factors to better elucidate their impact on AL. Additionally, while we collected a wide range of variables, the AL index utilized in our

study was based on a selection of nine biomarkers for the sake of simplicity. This selection was made based on our understanding of their relevance to vascular health and function, of which is mostly informed by studies predominantly White populations. However, it is possible that other biomarkers not included in our analysis could be important contributors to AL, particularly within marginalized demographic groups such as Black individuals. We recommend future investigations to explore additional biomarkers to refine the AL index further and ensure the applicability of certain biomarkers over others when assessing specific populations. Lastly, it's worth noting that our study had a higher proportion of female participants compared to males. This sex imbalance could potentially introduce bias into our findings, particularly considering that males exhibited higher attrition rates for saliva drop-off, which was a primary mediating biomarker for AL. Ideally, there will be more balanced representation in subsequent studies to mitigate the impact of potential sex biases on study outcomes.

Chapter 3: Allostatic Load and Functional Sympatholysis in Young Black Adults

Chapter Overview

Hypertension remains a prevalent public health concern globally, with significant implications for cardiovascular morbidity and mortality. In the U.S., Black adults have a higher prevalence and incidence of hypertension than their peers of other racial identities (2, 6). Heightened sympathetic nervous activity (SNA) from various chronic stressors increases the likelihood for development of hypertension (96, 99, 101). Specifically, chronic elevations in SNA perpetuate a state of heightened physiological arousal. Within states of heightened arousal, pre- and post-ganglionic nerves from the sympathetic nervous system (SNS) release NE, a catecholamine that acts on α- and β-adrenergic receptors on the heart and arteries to increase heart rate (HR), cardiac contractility, and peripheral vascular resistance (36). As a result of the systemic actions of catecholamines, augmented SNS activation inherently increases arterial pressure, thus heightening the risk for hypertension. Further, prolonged activation of the SNS disrupts the body's ability to maintain allostasis among various physiological systems such as the immune and metabolic systems resulting in increased systemic inflammation, and higher resting concentrations of glucose and cholesterol. With prolonged exposure, the cumulative effects of chronic SNA thus contribute to

allostatic load (AL), a measure of the wear and tear on the body caused by chronic stress exposure, which in turn increases the risk of various adverse health outcomes.

In the context of chronic elevations in SNA, it's important to note that Black adults often experience higher levels of chronic stress due to various psychosocial, economic, and environmental factors, including systemic discrimination and socioeconomic disparities. Specifically, chronic stressors such as racism and discrimination are thought to contribute to higher blood pressure (96, 105–108, 151), and increased chronic inflammation (as measured by circulating concentrations of CRP) (111–113, 152). Indeed, when conventionalized, AL has been shown to be higher in Black adults as compared to identities of other racial backgrounds in the U.S. (24–34). Along these lines, *The Weathering Hypothesis* proposes that chronic exposure to social and/or economic disadvantages promotes acceleration of the normal aging process, and thus, earlier onset of adverse health conditions (24, 154). Various researchers have described *The Weathering Hypothesis*, with AL as a metric of weathering to understand the impact of chronic stress on allostasis (24, 147, 154). The Weathering Hypothesis has been hypothesized as an explanation for the higher rates of hypertension (147, 154) and greater AL scores (24, 285) among older Black adults. However, the assessment of weathering via measures of AL to understand the impact of chronic stress on sympathetic regulation of the vasculature has yet to be studied in young Black adults.

Increased SNA to a laboratory stressor reflects increased systemic vascular resistance (150, 151), and is an indicator of increased risk for hypertension (151). Therefore, observations of greater SNA to a given stressor, even among young

normotensive Black individuals (37, 74, 157, 286, 287), may partially explain this group's greater risk for developing hypertension.

Functional sympatholysis is the physiological process by which the SNS's vasoconstrictive effects are attenuated in active skeletal muscles (locally) during exercise, allowing for adequate blood flow to meet the increased metabolic demands of exercising tissues (194). Impairments in functional sympatholysis can manifest as inadequate vasodilation in response to exercise, leading to compromised blood flow regulation and impaired oxygen delivery to active muscles. The compromised blood flow regulation may be indicative of underlying hyperactive SNS or impaired vascular endothelial function both of which are common contributors to hypertension.

Dysfunctional SNS responses and impaired vascular function via elevated AL may consequentially hamper the body's ability to modulate vascular tone and adequately vasodilate in response to exercise-induced metabolic demands, thus impairing functional sympatholysis. However, the relationship between AL and functional sympatholysis has yet to be studied. Therefore, it is unclear if individuals experiencing greater relative physiological wear-and-tear (e.g. increased AL) are more or less likely to demonstrate impaired functional sympatholysis as young adults, let alone among Black adults, who have higher prevalence of hypertension (2, 6) and higher AL than their White peers (24–34).

The purpose of this study was to test whether there are associations between indices of AL and the functional sympatholytic response in young Black adults. We hypothesized that higher indices of AL would be significantly correlated with a reduced magnitude of the functional sympatholytic response (MoS) in young Black adults.

The vasoconstrictive effects that are typically associated with increased SNA to a CPT are blunted among young women as compared to young men (164). Indeed, there is a potent vasodilatory response mediated by NE binding to β-adrenergic receptors. Therefore, sex differences in the hemodynamic response to a CPT are likely due to 1) higher concentrations of estrogen among young women as compared to young men, and 2) and the propensity of estrogen to increase β-adrenergic receptor sensitivity within the peripheral vasculature (170). Despite this, one study has observed no sex-differences in the magnitude of functional sympatholysis between young women and men (273). However, the impact of chronic stress (particularly among Black adults) was not explored in this former study. Therefore, an exploratory aim of the current study was to determine if sex was moderating the association between AL and the magnitude of functional sympatholysis in young Black adults. It was hypothesized the association between AL and the magnitude of functional sympatholysis would be moderated by sex.

Our hypothesis was, in part, formed based on the "Superwoman Schema" – a term used to describe a set of cultural expectations and pressures placed on Black women, particularly in the U.S., to fulfill multiple roles and responsibilities while maintaining strength, resilience, and selflessness in the face of adversity (288). This concept stems from the intersectionality of race and gender, highlighting unique experiences and challenges faced by Black women in society. The constant juggling of multiple roles and responsibilities, coupled with the pressure to appear strong and resilient, can contribute to chronic stress, fatigue, and burnout (288). Research suggests that Black women may experience disparities in physical and mental health outcomes,

including higher rates of hypertension (4), which may be influenced by the Superwoman Schema and its associated stressors (289). Therefore, we expected chronic stress (as evidenced by higher AL) to be present even among young Black women, heightening their SNA, and therefore impairing their ability to attenuate the vasoconstrictive response to CPT during rhythmic handgrip exercise.

Methods

Ethical Approval

Methods are the same as previously explained in Chapter 2.

Participants

A sub-sample of participants from the study in Chapter 2 were requested to complete the functional sympatholysis protocol. This protocol occurred during the same visit as the previously explained methods. It was completed last as not to interfere with other vascular testing. Methods for recruitment, inclusion/exclusion criteria, and visit preparation are the same as previously explained in Chapter 2, except participants were excluded from participating in the functional sympatholysis protocol if they confirmed as having or being diagnosed with Raynaud's syndrome on the HHQ.

Experimental Protocol

All testing was completed in the Human Integrative Physiology Laboratory, housed within the School of Public Health, at the University of Maryland, College Park. To optimize testing capacity, the laboratory was split by noise cancelling curtains into two testing setups. Participants were tested on one set up vs. the other at random.

The first setup was equipped with a Hitachi-Aloka Arietta 70 ultrasound device (Tokyo, Japan), a single-lead electrocardiograph (ECG), and finger plethysmography device (ADInstruments, Colorado Springs, CO, U.S.A.). Beat-to-beat measures of heart rate (HR) and mean arterial pressure (MAP) were obtained via ECG and finger plethysmography, respectively. Additionally, beat-to-beat cardiac output (Q), stroke volume (SV), and total peripheral resistance (TPR) were estimated from the blood pressure waveform acquired through the finger plethysmograph (Non-Invasive Cardiac Output [NICO] software extension, ADInstruments).

The second setup was equipped with a Philips EPIQ 5G ultrasound device (Amsterdam, Netherlands) and a single-lead ECG (ADInstruments, Colorado Springs, CO, U.S.A.). Beat-to-beat measures of HR were obtained via ECG. To capture MAP throughout the functional sympatholysis protocol, an automated oscillometric device (Omron Corporation, Kyoto, Japan) collected SBP and DBP measurements with 30 seconds left in every minute of the protocol. MAP was later calculated as = (SBP + 2*DBP) /3.

For both setups, the duplex-mode ultrasound devices were utilized to image participants' left brachial arteries via a probe operating at 5-18 MHz for the Doppler to permit concurrent collection of the diameter and blood velocity profiles. The brachial artery was imaged longitudinally and continuously throughout each trial while the probe was stabilized. In between Trials A and B, the skin was marked for probe placement to ensure measurements were being taken in the same location. During measurement, the insonation angle was fixed at 60°, and the sample volume was maximized to blood vessel size for each participant. blood flow analyses were

performed offline using a semiautomated program (Cardiovascular Suite; Quipu, Pisa, Italy), which determined vessel diameter from the continuous measurements of the median distance from the lumen-intima interface of the near wall to the far wall, and blood velocity by tracing the Doppler flow velocity waveforms. The second-by-second mean antegrade and retrograde blood velocities were summed to calculate mean blood velocity (MBV). Forearm blood flow (FBF) was calculated as in **Equation 6**. Forearm vascular conductance was calculated as = FBF/MAP. Forearm volume (FAV) was measured using water displacement, whereby, participants placed their left arm (up to the antecubital fossa) into a column of water with a known volume, the resulting volume of water overflow from the column being measured, and the volume of the left hand being subtracted to represent pure forearm volume.

Diurnal Slope of Salivary Cortisol

Methods are the same as previously explained in Chapter 2.

Resting Heart Rate and Blood Pressure

Methods are the same as previously explained in Chapter 2.

Total Body Fat Percentage

Methods are the same as previously explained in Chapter 2.

Blood Collection and Analysis

Methods are the same as previously explained in Chapter 2.

Allostatic Load Scoring

Methods are the same as previously explained in Chapter 2.

Functional Sympatholysis

A series of tests encompassing placing each participant's foot in a bucket of cold water and/or having them engage in a dynamic handgrip exercise were carried out to measure changes in blood flow distribution. The responses to these trials represent the functional sympatholytic response.

Forearm volume (FAV) was measured on the left forearm via water displacement. Total body lean mass and left arm lean mass were assessed via dual energy X-ray absorptiometry (DEXA) assessments. Handgrip strength was determined via maximal voluntary contraction (MVC) of the left hand with a manual handgrip dynamometer. MVCs were repeated 3-6 times with at least one minute of resting recovery between each trial. The three closest MVC trials were averaged to represent the participant's overall MVC. Before starting Trial A, the participant rested supine for 10 minutes. The left arm's brachial artery diameter, and positive and negative blood flow velocities were imaged with a high-resolution ultrasound probe equipped with a 5-18 MHz linear transducer (Hitachi-Aloka Arietta 70, Tokyo, Japan or Philips EPIQ 5G, Amsterdam, Netherlands). The brachial artery was imaged longitudinally and continuously throughout each trial while the probe was stabilized. In between Trial A and B, the skin was marked for probe placement to ensure measurements were being taken in the same location. Second-by-second mean blood velocity (MBV) was calculated as the sum of the positive and negative blood velocities. Image capture was attained with Quipu Cardiovascular Suite FMD Studio (Pisa, Italy). Forearm blood

flow (FBF) was calculated as in **Equation 6**. Forearm vascular conductance (FVC) was calculated as FBF/MAP.

Equation 6.

$$FBF\ \left(\frac{mL}{min}\right) = (MBV) \times \left({}^{arterial\ diameter\ (cm)}/_{2}\right)^{2} \times \pi \times 60\ (s)$$

Trial A. Resting Cold-Pressor Trial: Blood flow through the participant's left arm was measured for two-minutes of rest. Following rest, the participant's right foot was emersed up to the right-side ankle in a basin filled with cold water (0-4°C) for two-minutes.

Trial B. Handgrip Exercise and Cold-Pressor Trial: Following 10 minutes of rest, participants were asked to use their left hand to rhythmically exercise with the handgrip device set at 20% of their average MVC. Participants performed the intermittent handgrip to the rhythm of a metronome (20 handgrips*min^{-1}: 50% duty cycle) at 20% of their average MVC for a total of 5 minutes. This level of handgrip exercise was chosen because it does not alone increase muscle SNA among healthy individuals (290). A resistance of 20% of their MVC was chosen because even a single dynamic forearm contraction at resistances of 5-70% elicits transient increases in blood flow, with blood flow peaks occurring within about five cardiac cycles (291). This transient increase in blood flow occurs because of the role of local vasodilatory responses in promoting feed-forward mechanisms for exercise hyperemia (292). At three minutes into the handgrip exercise, the participant's right foot was emersed up to the right-side ankle in a basin filled with ice water (0-4 degrees Celsius) for two minutes while they continued the dynamic handgrip exercise. After two minutes of ice water

emersion, the participant's foot was removed from the ice water, and they were instructed to stop exercising.

Collectively, the two functional sympatholysis trials allow for the assessment of changes in blood flow distribution in response to increased sympathetic activity (evoked by emersion of the foot in cold water).

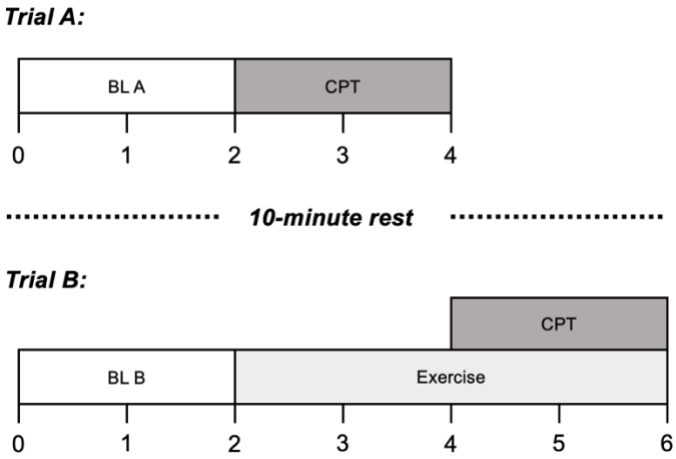


Figure 12: Protocol for Functional Sympatholysis Assessment.

Figure 12 Key: BL A, baseline A; CPT, cold-pressor task; BL B, baseline B. This protocol aimed at assessing: (Trial A) The vasoconstrictive effect of a systemic sympathetic stimulus (via CPT), and (Trial B) the capacity of handgrip exercise to blunt sympathetic vasoconstriction during CPT.

Assessing the Functional Sympatholytic Response

Percent changes in forearm blood flow (%ΔFBF) were determined in response to the cold pressor task (CPT) at rest, as per **Equation 7**, and during rhythmic handgrip exercise at 20% of MVC (Ex+CPT), as per **Equation 8**.

Equation 7.

$$\%\Delta\text{FBF (CPT)} = \left(\frac{(FBF\ during\ CPT - FBF\ during\ BL\ A)}{FBF\ during\ BL\ A}\right) \times 100$$

Equation 8.

$$\%\Delta\text{FBF (Ex + CPT)} = \left(\frac{(FBF\ during\ Ex + CPT - FBF\ during\ Ex)}{FBF\ during\ Ex}\right) \times 100$$

Percent changes in forearm vascular conductance (%ΔFVC) were determined in response to the CPT at rest, as per **Equation 9**, and during rhythmic handgrip exercise at 20% of MVC (Ex+CPT), as per **Equation 10**.

Equation 9.

$$\%\Delta\text{FVC (CPT)} = \left(\frac{(FVC\ during\ CPT - FVC\ during\ BL\ A)}{FVC\ during\ BL\ A}\right) \times 100$$

Equation 10.

$$\%\Delta\text{FVC (Ex + CPT)} = \left(\frac{(FVC\ during\ Ex + CPT - FVC\ during\ Ex)}{FVC\ during\ Ex}\right) \times 100$$

The relative MoS (%) was calculated for FBF as per **Equation 11**, and for FVC as per **Equation 12**. The FBF and FVC MoS values were divided by FAV to determine if the size of the participant's forearms was a confounding variable in the determination of their %FBF and/or %FVC MoS.

Equation 11.

$$\%\text{FBF MoS} = [\%\Delta\text{FBF (Ex + CPT)}] - [\%\Delta\text{FBF (CPT)}]$$

Equation 12.

$$\%\text{FVC MoS} = [\%\Delta\text{FVC (Ex + CPT)}] - [\%\Delta\text{FVC (CPT)}]$$

Data Analyses

Analyses were performed using SPSS Version 29.0.0 (SPSS Inc., Chicago, IL, U.S.A.), GraphPad Prism Version 10.1.1 (San Diego, CA, U.S.A.), and R (Vienna, Austria). Data from all variables are reported as means (±SD). Univariate normality was assessed using Shapiro-Wilk tests and outliers were identified using the ROUT method (Q=1%). The significance level for all analyses was set at a P-value of <0.05.

Among the total sample, dichotomous scoring methods for AL index (CC vs. SD methods) were compared using a Wilcoxon matched pairs signed rank test. Baseline descriptive characteristics, raw-biomarker values, and AL indices were compared between sexes using unpaired t-tests when data from both sexes were normally distributed, or using Mann-Whitney tests when data from one or both sexes were non-normally distributed.

All continuous data were sampled at 1,000 Hz and stored for offline analysis. In all cases, HR, MAP, diameter, and MBV data were time aligned with FBF to calculate FVC. When participants were tested on setup 1, we were able to acquire measures of cardiac output (Q), stroke volume (SV), and total peripheral resistance (TPR); these data were also time-aligned with FBF data (**Supplemental Table 1**). Data were averaged over 45-60-second periods during each condition.

As an arbitrary cutoff for data inclusion, we ensured there was no more than a 30% difference in the average of each of the cardiovascular parameters (HR, MAP, diameter, MBV, FBF, and FVC) between the BL conditions from Trials A and B among each participant. Further, paired t-tests or Wilcoxon matched-pairs signed rank tests were conducted to assess whether there were any significant differences in the cardiovascular parameters between BL A and BL B.

To test sex-by-condition interactions, raw cardiovascular data (HR, MAP, diameter, MBV, FBF, and FVC) were analyzed during Trial A and Trial B, respectively, with two-way repeated-measures ANOVAs for complete data sets or with mixed-effects analyses for data sets with missing values. If there were significant effects of sex in variables during Trial A, post-hoc Šidák's multiple comparisons tests

were performed to examine if sex-differences were occurring during the BL A and/or the CPT condition. Similarly, if there was a significant effect of sex in variables during Trial B, post-hoc Šidák's multiple comparisons tests were performed to determine whether sex-differences were occurring during the BL B condition, Ex condition, and/or Ex+CPT condition.

The functional sympatholysis protocol provides two separate values of the vasoconstrictor responses to CPT: one applied at rest, and another during exercise. Therefore, like previous studies (273, 293–296), the MoS (i.e., how much vasoconstriction was prevented) was calculated as the difference in the CPT-induced percent changes in FBF and FVC between the handgrip exercise and rest (i.e., Δ% CPT during exercise – Δ% CPT at rest).

Pearson's product-moment or Spearman's rank-order correlations were performed to assess the associations between the various methods for scoring AL index (CFA, CC, and SD methods) and the MoS (%FBF and %FVC). When correlations were significant, simple linear regression analyses were performed to further establish the magnitude of the associations between AL indices and the MoS (%FBF and %FVC). Additionally, Pearson's product-moment or Spearman's rank-order correlations were performed to assess the associations between the various methods for scoring AL index (CFA, CC, and SD methods) and the MoS (%FBF and %FVC) when controlled for FAV. Similarly, when correlations were significant, simple linear regression analyses were performed to further establish the magnitude of the associations between AL indices and the MoS (%FBF and %FVC) controlled for FAV.

Results

Data from all variables are reported as means ± standard deviation (SD). Data are presented with and without the identified outliers.

Participant Characteristics

Fifty-two (N=52) young Black adults met screening eligibility and came to the laboratory for testing. Of the N=52 participants, N=5 participants were removed from analysis because researchers were unable to perform a blood draw, N=1 was removed because they requested not to complete Trial B of the functional sympatholysis protocol, and N=13 participants were removed due to unreliable ultrasound data recordings during one or both trials of the functional sympatholysis protocol. Therefore, a total of N=35 young Black adults who participated in and completed this study are included in the analysis (Females, N=24; Males, N=11). Demographic characteristics of the participants and descriptive statistics of their AL variables are provided in **Table 11**. FAV was obtained on all participants except one (N=34). The missing FAV measure belonged to a male participant who needed to leave before we were able to acquire the measurement. While independent sample t-tests indicated no sex-differences in age, they did reveal that male participants were significantly taller, weighed more, had stronger MVC, larger FAV, and greater left arm lean mass as compared to female participants (**Table 11**).

In response to the health history questionnaire (HHQ), none of the participants indicated they were aware they had high blood pressure (defined as 140/90 mmHg) at the time of the study, whereas N=1 participant indicated in response to a separate question, that they had high blood pressure (when “high blood-pressure” values weren’t

defined) at the time of the study, or at some other time in their life. N=6 participants indicated that they had asthma at some point in their life. N=1 participant indicated they had type 1 diabetes mellitus (T1DM) at the time of the study. This participant's fasting glucose concentration was removed from analysis as they were an outlier by >200 mg/dL. For the AL index (CFA method), the fasting glucose concentration from the participant with T1DM was removed from the analysis and treated as a missing variable, whereby, the biomarker weight for this participant was computed via a Full Information Maximum Likelihood (FIML) analysis conducted using the Lavaan package in R (256). For dichotomously scored AL indices (CC and SD methods), the fasting glucose value of the participant with T1DM was removed as to not influence the mean and SD of the population, and this participant was given a score =1 for the fasting glucose biomarker score (indicating high load for that biomarker). None of the participants indicated that they had been diagnosed with high cholesterol at any point in their life. None of the participants indicated ever having a heart attack, stroke, epilepsy, varicose veins, cancer, systemic lupus, arthritis, bronchitis, emphysema, pneumonia, and/or rheumatic or scarlet fever at any point in their lives. Further, none of the participants, aside from the participant with T1DM, were taking antihypertensive, antianginal, or thyroid medication/s, metformin, insulin, antibiotics, prescription pain medications, and/or anticoagulation therapy at the time of the study.

None of the participants indicated smoking cigarettes at the time of the study, however, N=3 participants indicated current use of other types of tobacco products. Among the N=3 who indicated current tobacco use, N=2 indicated use of e-cigarettes

and/or vapes at the time of the study. N=1 participant indicated that they smoked cigarettes in the past but have permanently quit at least one year prior to the study.

A total of N=10 female participants indicated use of hormonal contraception at the time of the study. Of those N=10 females, N=8 indicated use of oral contraceptive pills, N=1 indicated use of hormonal injections, and N=1 did not specify method of hormonal contraception. The remaining N=14 women indicated natural and regular menstruation at the time of the study. Normal menstruation was defined as "having a menstruation cycle every ~28-30 days with ~3-5 days of menstrual bleeding," in accordance with the International Federation of Gynecology and Obstetrics (FIGO) Recommendations on Terminologies and Definitions for Normal and Abnormal Uterine Bleeding (265).

Allostatic Load Biomarkers

As described in Chapter 2, AL was classified using the following nine biomarkers: 1) diurnal slope of salivary cortisol; 2) serum C-reactive protein (CRP) concentrations; 3) resting heart rate (RHR); 4) resting systolic blood pressure (SBP); 5) resting diastolic blood pressure (DBP); 6) total body fat percentage (%TBF); 7) fasting serum glucose concentrations; 8) fasting serum creatinine concentrations; and 9) fasting total-cholesterol-to-high-density-lipoprotein-cholesterol (TC/HDL-C) concentrations.

The average values for each biomarker are presented in **Table 11**. Missing data was relatively low: 100% of participants (N=35) had complete biomarker data for measurements of the cardiovascular (RHR, SBP, and DBP), metabolic (fasting glucose, creatinine, TC/HDL), anthropometric (%TBF), and immune (CRP) components of AL;

and 83% of participants (N=29) had complete biomarker data for measurement of the neuroendocrine (diurnal slope of salivary cortisol) component of AL. Missing data were a result of failure of participants to return saliva samples. Salivary cortisol samples were missing from N=3 male and N=3 female participants.

According to independent sample t-tests, there were no sex-differences in average measures of diurnal cortisol slope, DBP, serum CRP, fasting glucose, nor TC/HDL-C (**Table 11**). Female participants had significantly higher RHR and %TBF, and significantly lower SBP and creatinine values, as compared to male participants (**Table 11**).

Table 11: Aim 2 - Participant Characteristics and Raw Biomarkers.

	Total Sample	**Female Participants**	**Male Participants**	**Sex Differences P-Value**
N	35	24	11	-
Demographics				
Age, y	20 ± 1	20 ± 1	20 ± 1	0.0690
Height, m	1.68 ± 0.09	1.65 ± 0.06	1.76 ± 0.09	0.0005
Weight, kg	73.85 ± 14.83	68.59 ±11.39	83.14 ± 17.63	0.0099
MVC, kg	31.15 ± 8.27	27.31 ± 5.11	39.55 ± 7.74	<0.0001
FAV, mL	878.56 ± 237.18	781.13 ± 136.71	1112.40 ± 269.14	<0.0001
Left Arm Lean Mass, kg	2.66 ± 0.91	2.21 ± 0.33	3.75 ± 0.94	<0.0001
AL Biomarkers				
Diurnal Cortisol Slope	-0.28 ± 0.26	-0.24 ± 0.27	-0.37 ± 0.24	0.2680
RHR, bpm	67.86 ± 11.11	71.29 ± 10.01	60.36 ± 9.98	0.0051
SBP, mmHg	116.50 ± 9.92	113.60 ± 9.07	122.64 ± 9.19	0.0104
DBP, mmHg	73.34 ± 8.85	74.00 ± 8.23	71.91 ± 10.34	0.5243
Serum CRP, ng/mL	0.17 ± 0.21	0.19 ±0.24	0.13 ± 0.12	0.4987
Fasting glucose, mg/dL	83.71 ± 4.69	83.70 ± 5.16	83.73 ± 3.74	0.9857
Creatinine, mg/dL	0.79 ± 0.16	0.71 ± 0.10	0.96 ± 0.11	<0.0001
TC/HDL-C	2.55 ± 0.50	2.51 ± 0.54	2.65 ± 0.39	0.4281
TBF, %	31.03 ± 9.96	34.57 ± 7.59	23.33 ±10.45	0.0010

Table 11 Key: Values are means ± standard deviation. Y, years; MVC, maximal voluntary contraction; FAV, forearm volume; AL, allostatic load; RHR, resting heart rate; bpm, beats per minute; SBP, systolic blood pressure; DBP diastolic blood pressure; CRP, C-reactive protein; TC/HDL-C, total-cholesterol-to-high-density-lipid-cholesterol-ratio; and TBF, total body fat. Provided P-values were derived from unpaired t-tests between females and males.

Allostatic Load Scoring

In the total sample of participants (N=35), AL indices (CFA method) were normally distributed, but dichotomous scoring methods for AL index (CC and SD methods) were not normally distributed. There were no significant outliers among AL indices (CFA, CC, or SD methods). Per a Wilcoxon matched-pairs signed rank test, among the total sample of participants, there were no significant differences between the two dichotomous scoring methods for AL index (CC vs. SD method; P=0.8734; **Table 12**).

When separated by sex, AL indices (CFA method) were normally distributed in both female and male participants, and there were no significant outliers among either sex. An unpaired t-test confirmed that AL index (CFA method) was significantly higher among female participants than male participants (P<0.0001; **Table 12**).

When separated by sex, AL indices (CC method) were not normally distributed among female participants but were normally distributed among male participants. There were no significant outliers in AL indices (CC or SD methods) in either sex. According to a Mann-Whitney test, there was no significant difference in AL index (CC method) between female and male participants (P=0.8117; **Table 12**).

When separated by sex, AL indices (SD method) were not normally distributed in female or male participants. According to a Mann-Whitney test, there was no

significant difference in AL index (SD method) between female and male participants (P=0.4535; **Table 12**). As reported by Wilcoxon matched-pairs signed rank tests, there were no significant differences between dichotomous scoring methods for AL indices (CC vs. SD method) among female (P=0.2734) or male participants (P=0.2734).

Table 12: Aim 2 - Average Allostatic Load Indices by Sex.

AL Index Scoring Method	**Total Sample** (N=35)	**Female Participants** (N=24)	**Male Participants** (N=11)	**Sex Differences** ***P-Value***
CFA	0.12 ± 0.43	0.33 ± 0.27	-0.35 ± 0.33	<0.0001
CC	1.54 ± 1.27	1.46 ± 1.10	1.73 ± 1.62	0.8117
SD	1.57 ± 1.42	1.67 ± 1.34	1.36 ± 1.63	0.4535

Table 12 Key: AL, allostatic load; CFA, confirmatory factor analysis; CC, clinical cutoff; SD, standard deviation. Values are means ± SD. CFA was scored on a scale from -1 to 1. CC and SD AL indices were scored on a scale of 0-9. Higher scores are associated with higher AL for all three methods.

Because of the significant differences between sexes in measures of 1) FAV (**Table 11**); 2) left arm lean mass (**Table 11**); and 3) AL index (CFA method; **Table 12**), we assessed the correlation of AL index (CFA method) with FAV and left arm lean mass. Indeed, there were significant negative Pearson's correlations between AL index (CFA method) and FAV (N=34; P=0.0003; r=-0.5809; **Figure 13A**) as well as between AL index (CFA method) and left arm lean mass (N=34; P<0.0001; r=-0.8076; **Figure 13B**). Simple linear regression models further demonstrated that AL index (CFA method) significantly predicted 34% of the variability in FAV ($F(1,32)=16.30$; $R^2=0.3374$; Y=-329.99*X+923.69; P=0.0003; **Figure 13A**) and 65% of the variability in left arm lean mass ($F(1,32)=60.02$; $R^2=0.6522$; Y=-1.71*X+2.88; P<0.0001; **Figure 13B**).

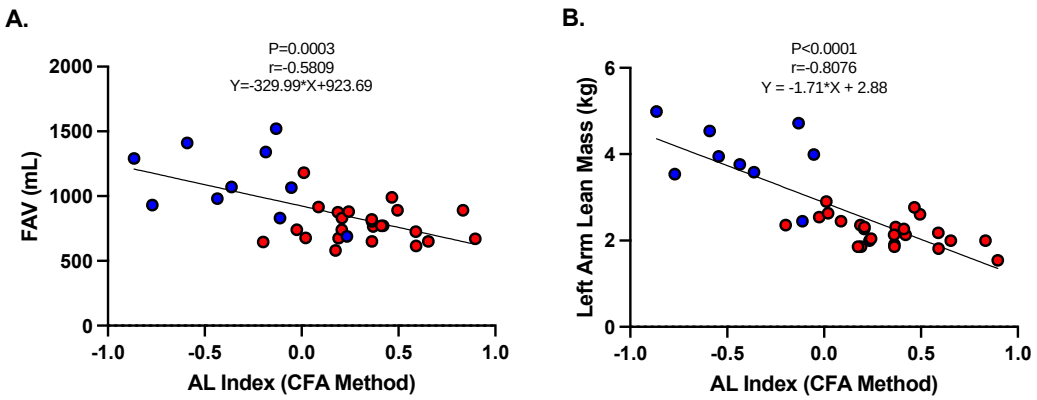


Figure 13: Correlations Between Allostatic Load, Forearm Volume, and Left Arm Lean Mass.

Figure 13 Key: AL, allostatic load; CFA, confirmatory factor analysis; FAV, forearm volume. ● Indicate female participants and ● indicate male participants.

Cardiovascular Responses to Functional Sympatholysis

The resting baseline data collection for Trial A (BL A) lasted 2 minutes (i.e., time 0:00-2:00 of Trial A; **Figure 12**). To calculate the resting responses of HR, MAP, BD, MBV, FBF and FVC, measures were averaged for the last 60 seconds prior to CPT application (i.e., time 1:00-2:00 of Trial A) among 77% of participants (N=27), and the last 45 seconds prior to CPT application (i.e., time 1:15-2:00 of Trial A) among 3% of participants (N=1). Among the remaining 20% of participants (N=7), the last 30-45 seconds before the CPT were unusable due to participant movement or anticipatory responses to the CPT stimulus (e.g., sudden increase in HR). Therefore, the 45-60 seconds of data preceding the unusable 30-45 seconds prior to CPT application (i.e., time 0:30-1:30 for ~14% of participants (N=5), and time 0:30-1:15 for ~6% of participants (N=2) during Trial A) were extracted and averaged to represent the resting BL A response.

The CPT stimulus was applied immediately after the 2 minutes of BL A and lasted an additional 2-minutes (i.e., time 2:00-4:00 of Trial A; **Figure 12**). To calculate

the response of HR, MAP, BD, MBV, FBF and FVC to CPT, measures were averaged for the last 60-seconds of CPT application (i.e., time 3:00-4:00 of Trial A) among ~66% of participants (N=23) and for the last 45-seconds (i.e., time 3:15-4:00 of Trial A) among ~9% of participants (N=3). Among the remaining ~26% of participants (N=9), the last 30-seconds of the 2-minute CPT were unusable due to participant movement and/or loss in ultrasound signal. Therefore, the time corresponding to 2:30-3:30 of Trial A was averaged and used to represent the response to the CPT in these ~26% of participants (N=9).

The resting baseline data collection for Trial B (BL B) lasted 2 minutes (i.e., time 0:00-2:00 of Trial B; **Figure 12**). To calculate the resting responses of HR, MAP, BD, MBV, FBF and FVC for Trial B, measures were averaged for the last 60-seconds prior to initialization of the handgrip exercise (i.e., time 1:00-2:00 of Trial B) among 80% of participants (N=27). Among the remaining 20% of participants (N=7), the last 30-45 seconds of data collection during BL B (before handgrip exercise) were unusable due to participant movement or anticipatory responses to the onset of handgrip exercise (e.g., sudden increase in FBF). Therefore, the time corresponding to 0:15-1:15 of BL B was averaged and utilized for ~9% of participants (N=3), and the time corresponding to 0:30-1:30 of BL B was averaged and utilized for ~11% of participants (N=4).

The handgrip exercise was performed immediately after the 2 minutes of BL B and lasted for a total of 5 minutes (i.e., time 2:00-7:00; **Figure 12**). The last 2 minutes of the 5-minute-long handgrip exercise were accompanied by CPT (Ex+CPT condition, i.e., time 5:00-7:00; **Figure 12**). Therefore, the handgrip exercise only (Ex) condition occurred during time 2:00-5:00 of Trial B (**Figure 12**). To calculate the responses of

HR, MAP, BD, MBV, FBF and FVC to the Ex condition, measurements were averaged for the last 60-seconds of handgrip exercise before CPT application (i.e., time 4:00-5:00) among ~83% of participants (N=29), and for the last 45 seconds of handgrip exercise before CPT application (i.e., time 4:15-5:00) for ~3% of participants (N=1). Among the remaining ~14% of participants (N=5), the last 30 seconds of the Ex condition were unusable due to anticipatory responses to the CPT stimulus (e.g., sudden increase in HR). Therefore, the data corresponding to time 3:30-4:30 of Trial B was averaged and utilized for these ~14% of participants (N=5).

To calculate the responses of HR, MAP, BD, MBV, FBF and FVC to the Ex+CPT condition, measurements were averaged for the last 60 seconds of Trial B (i.e., time 6:00-7:00) among 40% of participants (N=14). Among the remaining 60% of participants (N=21), the last 20-45 seconds of the Ex+CPT condition were unusable due to participant movement and/or loss of ultrasound signal. Therefore, the data corresponding to time 5:15-6:15 of Trial B was averaged and used for ~9% of participants (N=3), time 5:30-6:30 of Trial B was averaged and used for ~43% of participants (N=15), and time 5:40-6:40 of Trial B was used for ~9% of participants (N=3).

Heart Rate

Heart rate (HR) data were collected on N=35 participants; however, due to technological difficulties, HR data are missing from all conditions of the functional sympatholysis protocol (Trials A and B) from N=1 female participant, and from Trial A conditions (BL A and CPT) of another N=1 female participant. Means ± standard

deviations of HR throughout each condition of the functional sympatholysis protocol from N=33 participants are presented in **Table 13**.

Among the total sample, HR data were normally distributed and there were no significant outliers during all conditions of the protocol. Of note, among the total sample, a Wilcoxon matched pairs signed rank test revealed there were no significant differences in HR between BL A and BL B (P=0.3902; **Table 13**). The difference in HR values between BL A and BL B ranged from 0-15.8% and averaged 3.4% among the N=33 participants. When separated by sex, the HR data were normally distributed in female (N=23) and male (N=11) participants and there were no significant outliers throughout all conditions of the functional sympatholysis protocol.

According to a two-way repeated measures ANOVA of HR data, during Trial A there was a significant effect of condition (P<0.0001) and sex (P=0.0185), but no significant effect of the condition-by-sex interaction (P=0.8023). To test the effect of sex, post-hoc multiple comparisons tests were run to examine if there were sex-differences in HR during BL A and/or CPT. According to Šidák's multiple comparisons tests, HR was similar between sexes during BL A (P=0.0610; **Table 13**) but was significantly higher among females as compared to males during CPT (P=0.0386; **Table 13**).

According to a two-way repeated measures ANOVA of HR data, during Trial B there was a significant effect of condition (P<0.0001), but not sex (P=0.0922), and no significant effect of the condition-by-sex interaction (P=0.8503). Post-hoc multiple comparisons tests were run to examine if there were differences in HR between conditions of Trial B among each sex. According to Šidák's multiple comparisons tests,

HR was significantly higher during Ex as compared to BL B (P<0.0001; **Table 13**), and higher during Ex+CPT as compared to BL B (P<0.0001; **Table 13**), but similar during the Ex and Ex+CPT conditions (P=0.33901; **Table 13**).

Mean Arterial Pressure

MAP data were collected on N=35 participants; however, due to technological difficulties, MAP data are missing from all conditions of the functional sympatholysis protocol on N=1 female participant, and from Trial A conditions (BL A and CPT) of another N=1 female participant. Means ± standard deviations of MAP throughout each condition of the functional sympatholysis protocol are presented in **Table 13**. Of note, among the total sample, a Wilcoxon matched pairs signed rank test revealed there were no significant differences in MAP between BL A and BL B (P=0.3902; **Table 13**). The difference in MAP values between BL A and BL B ranged from 0-19.6% and averaged 3.4% among the N=33 participants.

Among the total sample, MAP data were not normally distributed during all conditions of the functional sympatholysis protocol, except during the Ex condition. Four statistical outliers were identified among the MAP data: two outliers (N=2 were identified among the BL A condition; N=1 was identified among the CPT condition; and N=1 was identified among the Ex+CPT condition). Among the outliers, N=2 belonged to the same individual and were not considered to be within plausible normal physiological ranges. Therefore, these N=2 male outliers were removed from analysis. When separated by sex, the MAP data were not normally distributed in female participants during the BL A, CPT, BL B, or Ex+CPT conditions, but were normally

distributed during the Ex condition. Among male participants, MAP data were normally distributed during all conditions of the functional sympatholysis protocol.

According to a mixed-effects analysis of MAP data, during Trial A there was a significant effect of condition ($P<0.0001$) and sex ($P=0.0458$), but no significant condition-by-sex interaction effect ($P=0.1379$). Post-hoc multiple comparisons tests were run to examine if there were differences between the MAP during BL A and the MAP during CPT among each sex. According to Šidák's multiple comparisons tests, MAP was significantly higher during CPT than during BL A in both females and males ($P<0.0001$, and $P<0.0001$, respectively; **Table 13**). According to Šidák's multiple comparisons tests, MAP was similar between sexes during BL A, but higher among males than females during CPT ($P=0.4472$ and $P=0.0109$, respectively; **Table 13**).

According to a mixed-effects analysis of MAP data, during Trial B there was a significant effect of condition ($P<0.0001$), but not sex ($P=0.1246$), and no significant effect of the condition-by-sex interaction ($P=0.0886$). Post-hoc multiple comparisons tests were run to examine if there were differences between the MAP during BL B, Ex, and Ex+CPT in the total sample. According to Šidák's multiple comparisons tests of the total sample, MAP was significantly higher during Ex than during BL B ($P<0.0001$; **Table 13**), higher during Ex+CPT than BL B ($P<0.0001$; **Table 13**), and higher during Ex+CPT than Ex ($P<0.0001$; **Table 13**).

Brachial Artery Diameter

Diameter data were collected on N=35 participants. Means ± standard deviations of the diameter data throughout each condition of the functional sympatholysis protocol are presented in **Table 13**. There were no missing data. Among

the total sample, diameter data were normally distributed throughout all conditions of the functional sympatholysis protocol and there were no outliers. Of note, among the total sample, a paired t-test revealed there were no significant differences in diameters between BL A and BL B (P=0.2793; **Table 13**). The difference in diameter values between BL A and BL B ranged from 0-9% and averaged 2.4% among the N=35 participants.

When separated by sex, diameter data were normally distributed throughout all conditions of the functional sympatholysis protocol for female participants. Among male participants, data were normally distributed throughout all conditions of the functional sympatholysis protocol except for the BL B condition.

According to a two-way repeated measures ANOVA of diameter data, during Trial A there were significant effects of condition (P<0.0001; **Table 13**) and sex (P<0.0001; **Table 13**), but no significant effects of the condition-by-sex interaction (P=0.8860). Per Šidák's multiple comparisons tests, male participants had significantly larger diameters than female participants during BL A (P<0.0001; **Table 13**) and CPT (P<0.0001; **Table 13**).

According to a two-way repeated measures ANOVA of diameter data, during Trial B there were significant effects of condition (P<0.0001) and sex (P=0.0001), but no significant effect of the condition-by-sex interaction (P=0.6605). Post-hoc multiple comparisons tests were run to examine if there were differences between the diameter throughout the different conditions of Trial B in the total sample. According to Šidák's multiple comparisons tests of the total sample, diameter was significantly larger during Ex than during BL B (P<0.0001; **Table 13**), larger during Ex+CPT than BL B

(P<0.0001; **Table 13**), and larger during Ex than Ex+CPT (P=0.0238; **Table 13**). Post-hoc multiple comparisons tests were run to examine if there were sex differences in diameters data throughout Trial B. Per Šidák's multiple comparisons tests, male participants had larger diameters than female participants during BL B, Ex, and Ex+CPT (P<0.0001 for all; **Table 13**).

Additionally, due to males having significantly larger FAVs compared to females (**Table 11**), and significantly larger brachial artery diameters as compared to females during BL A and BL B (**Table 13**), we assessed the associations between FAV and brachial artery diameter during BL A and BL B. Indeed, there were significant Pearson's correlations between FAV and brachial artery diameter during BL A (BL A: N=34; r=0.5251; P=0.0014, BL B: N=34; r=0.5932; P=0.0002, data not shown)**.**

Mean Blood Velocity

Mean blood velocity (MBV) data were collected on N=35 participants. Means ± standard deviations of the MBV data throughout each condition of the functional sympatholysis protocol are presented in **Table 13**. Of note, among the total sample, a Wilcoxon matched pairs signed rank test revealed there were no significant differences in MBV between BL A and BL B when the outlier was included (P=0.3678; **Table 13**) or excluded from analysis (P=0.2778). The difference in MBV values between BL A and BL B ranged from 0-21.1% and averaged 7.1% among the N=35 participants.

There were no missing data. Among the total sample, MBV data were normally distributed during the Ex condition, but not during BL A, CPT, BL B, nor Ex+CPT conditions of the functional sympatholysis protocol. There was one outlier during the

BL A condition (N=1 female). When the outlier was removed from analysis, MBV data collected during BL A were normally distributed among the total sample.

When separated by sex, MBV data were normally distributed throughout all conditions of the functional sympatholysis protocol for female participants except during the Ex+CPT. Among male participants, data were normally distributed throughout all conditions of the functional sympatholysis protocol.

According to a two-way repeated measures ANOVA of MBV data, during Trial A there were significant effects of condition ($P<0.0001$; **Table 13**), but no significant effects of sex ($P=0.3546$; **Table 13)**, nor of the condition-by-sex interaction ($P=0.5979$). When the outlier was removed, assessments of MBV data during Trial A were similar (effect of condition, $P<0.0001$; effect of sex, $P=0.3742$; effect of interaction, $P=0.9566$).

According to a two-way repeated measures ANOVA of MBV data, during Trial B there was a significant effect of condition ($P<0.0001$), but not sex ($P=0.1010$), nor of the condition-by-sex interaction ($P=0.3527$). Post-hoc multiple comparisons tests were run to examine if there were differences between the MBV data throughout the different conditions of Trial B in the total sample. According to Šidák's multiple comparisons tests of the total sample, the MBV was significantly higher during Ex ($P<0.0001$; **Table 13**) and Ex+CPT ($P<0.0001$; **Table 13**) as compared to BL B, but similar between Ex and Ex+CPT ($P=0.9997$; **Table 13**).

Forearm Blood Flow

Forearm blood flow (FBF) data were collected on N=35 participants. Means ± standard deviations of FBF throughout each condition of the functional sympatholysis

protocol are presented in **Table 13**. There were no missing data. Among the total sample, raw FBF data were not normally distributed during the BL A, CPT, or BL B conditions of the functional sympatholysis protocol, but were normally distributed during the Ex and Ex+CPT conditions. Two statistical outliers were identified among the raw FBF data, occurring during BL A and CPT (both from N=1 male). Even when outliers were included, per results of a Wilcoxon signed rank test, all N=35 participants experienced a significant decrease in FBF between BL A and CPT (P<0.0001), with the average FBF during CPT being 47.55±25.13 mL/min (**Table 13**), indicating an average relative decrease in FBF by 24.2% from BL A.

When separated by sex, the raw FBF data were not normally distributed in female participants during the BL A, CPT, BL B, or Ex+CPT conditions, but were normally distributed during the Ex condition. When the outliers were included, the raw FBF data from BL A and CPT of male participants were not normally distributed. Upon removal of the outliers, raw FBF data were normally distributed among male participants during all conditions of the functional sympatholysis protocol.

According to a two-way repeated measures ANOVA of raw FBF data, during Trial A there was a significant effect of condition (P<0.0001; **Table 13**), but no significant effects of sex (P=0.0732), nor of the condition-by-sex interaction (P=0.3429). These results were unaffected when the outliers were removed from analysis (effect of condition, P<0.0001; effect of sex, P=0.2933; effect of interaction, P=0.5768).

According to a two-way repeated measures ANOVA of the raw FBF data, during Trial B there were significant effects of condition (P<0.0001), sex (P=0.0019),

as well as condition-by-sex interaction (P<0.0001). When assessing data to include or exclude, we ensured that participants were experiencing an exercise hyperemic response that was between 150-700% greater than their resting (BL B) FBF to be consistent with previous literature. Indeed, this was the case for all N=35 participants as their average raw FBF during exercise was 326.29±89.97 mL/min, indicating an average 461.4% increase in FBF due to the exercise stimulus (**Table 13**).

Post-hoc multiple comparisons tests were run to examine if there were differences between the raw FBF data during BL B, Ex, and Ex+CPT in the total sample. According to Šidák's multiple comparisons tests of the total sample, the raw FBF was significantly greater during Ex than during BL B (P<0.0001; **Table 13**), and Ex+CPT (P=0.0226; **Table 13**). The raw FBF was also significantly greater during Ex+CPT compared to BL B (P<0.0001; **Table 13**). The average change in raw FBF between the Ex and Ex+CPT conditions was by -3.6% (**Table 13**). As anticipated, most participants (N=30) experienced a decrease in FBF between Ex and Ex+CPT conditions. On the contrary, N=5 participants displayed an increase in FBF between Ex and Ex+CPT. However, these increases were marginal, with the raw FBF increases among the N=5 participants ranging between 1-3% and averaging 2%.

Among the total sample, a Wilcoxon matched pairs signed rank test revealed there were no significant differences in the raw FBF data between BL A and BL B (P=0.2793; **Table 13**). The difference in raw FBF values between BL A and BL B ranged from 0-26% and averaged 7.6% among the N=35 participants.

Post-hoc multiple comparisons tests were run to examine if there were differences between the raw FBF data during BL B, Ex, and Ex+CPT between sexes.

Per Šidák's multiple comparisons tests, there were no significant differences in raw FBF data between female and male participants during BL B (P=0.9086; **Table 13**); however, male participants had significantly higher raw FBF values than female participants during Ex (P=0.0002; **Table 13**) and Ex+CPT (P=0.0002; **Table 13**).

Forearm Vascular Conductance

Because MAP data were missing from all conditions of the functional sympatholysis protocol on N=1 female participant, and from Trial A conditions (BL A and CPT) of another N=1 female participant, we were unable to calculate forearm vascular conductance (FVC) for N=2 participants. Therefore, raw data for FVC throughout the functional sympatholysis protocol were analyzed from N=33 participants for the BL A and CPT conditions of Trial A and from N=34 participants for the BL B, Ex, and Ex+CPT conditions of Trial B. Means ± standard deviations of raw FVC data throughout each condition of the functional sympatholysis protocol are presented in **Table 13**.

Among the total sample, the raw FVC data were not normally distributed during the BL A, CPT, or BL B conditions of the functional sympatholysis protocol but were normally distributed during the Ex and Ex+CPT conditions of the functional sympatholysis protocol. Two statistical outliers were identified among the raw FVC data – occurring during the BL A and CPT conditions. Among the outliers, both belonged to the same male individual and were considered plausibly within normal physiological ranges. Therefore, results are presented with and without the identified outliers. Among the total sample, when outliers were removed from analysis, the raw

FVC data were normally distributed during all conditions of the functional sympatholysis protocol except during BL B.

When separated by sex, among female participants the raw FVC data were normally distributed during the BL A, CPT, and Ex+CPT conditions of the functional sympatholysis protocol but were not normally distributed during the BL B or Ex conditions. Among male participants, when the outliers were included, the raw FVC data were normally distributed during the BL B, Ex, and Ex+CPT conditions, but were not normally distributed during the BL A or CPT conditions. Among male participants, upon removal of the outliers, the raw FVC data were normally distributed throughout all conditions of the functional sympatholysis protocol.

According to a two-way repeated measures ANOVA of the raw FVC data, during Trial A there was a significant effect of condition ($P<0.0001$; **Table 13**), but no significant effects of sex ($P=0.2515$), nor of the condition-by-sex interaction ($P=0.2543$). These results were unaffected when the outliers were removed from analysis (effect of condition, $P<0.0001$; effect of sex, $P=0.7931$; effect of interaction, $P=0.6116$).

According to a two-way repeated measures ANOVA of the raw FVC data during Trial B, there were significant effects of condition ($P<0.0001$), and condition-by-sex interaction ($P=0.0344$), but not sex ($P=0.0747$). Post-hoc multiple comparisons tests were run to examine if there were differences between the raw FVC data during the BL B, Ex, and Ex+CPT conditions of the functional sympatholysis protocol in the total sample. According to Šidák's multiple comparisons tests of the total sample, raw FVC data was significantly higher during Ex than during BL B ($P<0.0001$; **Table 13**),

higher during Ex+CPT than BL B (P<0.0001; **Table 13**), and higher during Ex than Ex+CPT (P=0.0009; **Table 13**). Among the total sample, a Wilcoxon matched pairs signed rank test revealed there were no significant differences in the raw FVC data between BL A and BL B (P=0.6850; **Table 13**).

Post-hoc multiple comparisons tests were run to examine if there were sex differences in the raw FVC data during BL B, Ex, and Ex+CPT. Per Šidák's multiple comparisons tests, there were no significant differences in raw FVC data between female and male participants during BL B (P=0.9710; **Table 13**), nor during Ex+CPT (P=0.1527; **Table 13**); however, male participants had significantly higher raw FVC data than female participants during Ex (P=0.0402; **Table 13**).

Table 13: Cardiovascular Responses to the Functional Sympatholysis Protocol.

	Baseline A	CPT	Baseline B	Exercise	Exercise + CPT
HR, bpm (N=34)	66.40 ± 10.57	74.51 ± 11.66 #	65.41 ± 9.68	78.49 ± 13.87 #	81.07 ± 12.20 #
Females (N=23)	69.24 ± 8.73	77.59 ± 7.57 *	67.86 ± 7.39	80.39 ± 10.97	83.34 ± 9.30
Males (N=11)	60.72 ± 12.01	68.34 ± 15.89	60.28 ± 12.08	74.52 ± 18.57	76.31 ± 16.25
MAP, mmHg (N=34)	86.22 ± 10.69	102.09 ± 16.14 #	87.21 ± 13.01	95.02 ± 16.62 #	105.09 ± 20.23 # $
Females (N=23)	84.40 ± 9.16	97.50 ± 11.14 *	85.68 ± 11.50	91.98 ± 11.87	100.59 ± 15.69
Males (N=11)	89.87 ± 12.94	111.26 ± 20.83	90.39 ± 15.84	101.39 ± 23.14	114.50 ± 25.79
Diameter, mm (N=35)	3.47 ± 0.55	3.34 ± 0.55	3.44 ± 0.53	3.76 ± 0.54 #	3.68 ± 0.52 # $
Females (N=24)	3.21 ± 0.39 *	3.09 ± 0.40 *	3.19 ± 0.36 *	3.51 ± 0.39 *	3.43 ± 0.36 *
Males (N=11)	3.93 ± 0.44	3.79 ± 0.46	3.87 ± 0.50	4.16 ± 0.51	4.10 ± 0.48
MBV, cm/s (N=35)	10.85 ± 4.15	8.91 ± 3.51 #	11.22 ± 4.06	48.53 ± 10.24 #	48.52 ± 9.17 #
Females (N=24)	11.30 ± 4.13	9.27 ± 3.40	11.92 ± 4.23	50.14 ± 10.90	50.29 ± 9.51
Males (N=11)	9.93 ± 4.02	8.32 ± 3.72	10.18 ± 3.29	45.57 ± 7.73	45.42 ± 7.40
FBF, mL/min (N=35)	62.35 ± 30.97	47.54 ± 25.13 #	62.50 ± 26.31	326.29 ± 89.97 #	313.99 ± 85.07 #
Females (N=24)	56.18 ± 25.40	42.31 ± 18.72	58.13 ± 26.59	295.66 ± 85.55 *	283.43 ± 79.19 *
Males (N=11)	72.64 ± 36.78	56.99 ± 32.06	70.50 ± 23.24	373.67 ± 72.10	362.11 ± 67.75
FVC, mL/min*100mmHg (N=34)	74.67 ± 37.73	47.66 ± 23.71 #	74.17 ± 32.70	357.22 ± 114.86 #	310.85 ± 96.07 # $
Females (N=23)	69.16 ± 32.75	44.48 ± 19.03	70.15 ± 32.20	331.75 ± 100.87 *	291.07 ± 84.79
Males (N=11)	85.71 ± 45.85	54.03 ± 31.17	82.57 ± 33.67	410.46 ± 128.62	352.21 ± 108.90

Table 13 Key: CPT; cold-pressor task; HR, heart rate; bpm, beats per minute; MAP, mean arterial pressure; MBV, mean blood velocity; FBF, forearm blood flow; FVC, forearm vascular conductance. * indicates significantly different (P<0.05) from males during the same condition. # indicates significantly different (P<0.05) from the baseline condition of that trial. $ indicates significant difference (P<0.05) between the Ex and Ex+CPT conditions.

Forearm Blood Flow Magnitude of Sympatholysis

We compared the vasoconstrictive response to CPT (as compared to BL A) and to Ex+CPT (as compared to Ex) by assessing the percent changes in forearm blood flow (%ΔFBF). According to a two-way repeated measures ANOVA, there was a significant effect of condition (P<0.0001; **Figure 14A**), but no effects of sex (P=0.38; **Figure 14A**), nor condition-by-sex interaction effects (P=0.72; **Figure 14A**). The %FBF magnitude of sympatholysis (MoS) data are displayed in **Figure 14B**. The %FBF MoS data ranged from 5.40-38.15% and averaged 20.56±8.88%. Further, %FBF MoS were similar between female and male participants (P=0.72; **Figure 14B**) when uncontrolled for FAV.

We divided the %ΔFBF values by participants' forearm volume (FAV) and multiplied values by 1000 to account for differences in forearm size. According to a mixed-effects analysis of the %ΔFBF / FAV data, there were significant effects of condition (P<0.0001; **Figure 15A**), sex (P=0.0032; **Figure 15A**), and condition-by-sex interaction (P=0.0478; **Figure 15A**). We also divided the %FBF MoS values by participants' FAV and multiplied values by 1000 to account for differences in forearm size (%FBF MoS / FAV). The %FBF MoS / FAV data are displayed in **Figure 15B**. The %FBF MoS / FAV data ranged from 7.03-48.15% and averaged 24.30±10.72%. Further, the %FBF MoS / FAV were larger among female participants than male participants (P=0.0478; **Figure 15B**).

Results indicate that regardless of whether FAV was or was not controlled for, the vasoconstrictive influence of the CPT was abolished in the presence of handgrip exercise in young Black adults. However, only when FAV was controlled for was the

%FBF MoS greater among young Black female participants as compared to young Black male participants.

Forearm Vascular Conductance Magnitude of Sympatholysis

We compared the vasoconstrictive response to CPT (as compared to BL A) and to Ex+CPT (as compared to Ex) by assessing the average percent change in forearm vascular conductance (%ΔFVC). According to a mixed-effects analysis, there was a significant effect of condition (P<0.0001; **Figure 14C**), but no effects of sex (P=0.3257; **Figure 14C**), nor condition-by-sex interaction effects (P=0.9741; **Figure 14C**). The %FVC magnitude of sympatholysis (MoS) data are displayed in **Figure 14D**. The %FVC MoS data ranged from 3.13-42.52% and averaged 23.39±10.02%. Further, %FVC MoS data were similar between female and male participants when uncontrolled for FAV (P=0.9535; **Figure 14D**).

We also divided the %ΔFVC values by participants' forearm volume (FAV) and multiplied values by 1000 to account for differences in forearm size. According to a mixed-effects analysis, there was a significant effect of condition (P<0.0001; **Figure 15C**), but not sex (P=0.0825; **Figure 15C**), nor effects of the condition-by-sex interaction (P=0.1823; **Figure 15C**). We also divided the %FVC MoS values by participants' FAV and multiplied values by 1000 to account for differences in forearm size. The %FVC MoS / FAV are displayed in **Figure 15D**. The %FVC MoS / FAV data ranged from 3.19-49.97% and averaged 27.69±12.63%. Further, the %FVC MoS / FAV were similar between female and male participants (P=0.1517; **Figure 15D**).

Results indicate that regardless of whether FAV was or was not controlled for, the vasoconstrictive influence of the CPT was abolished in the presence of handgrip

exercise and that the %FVC magnitude of sympatholysis was similar between young Black female and male participants.

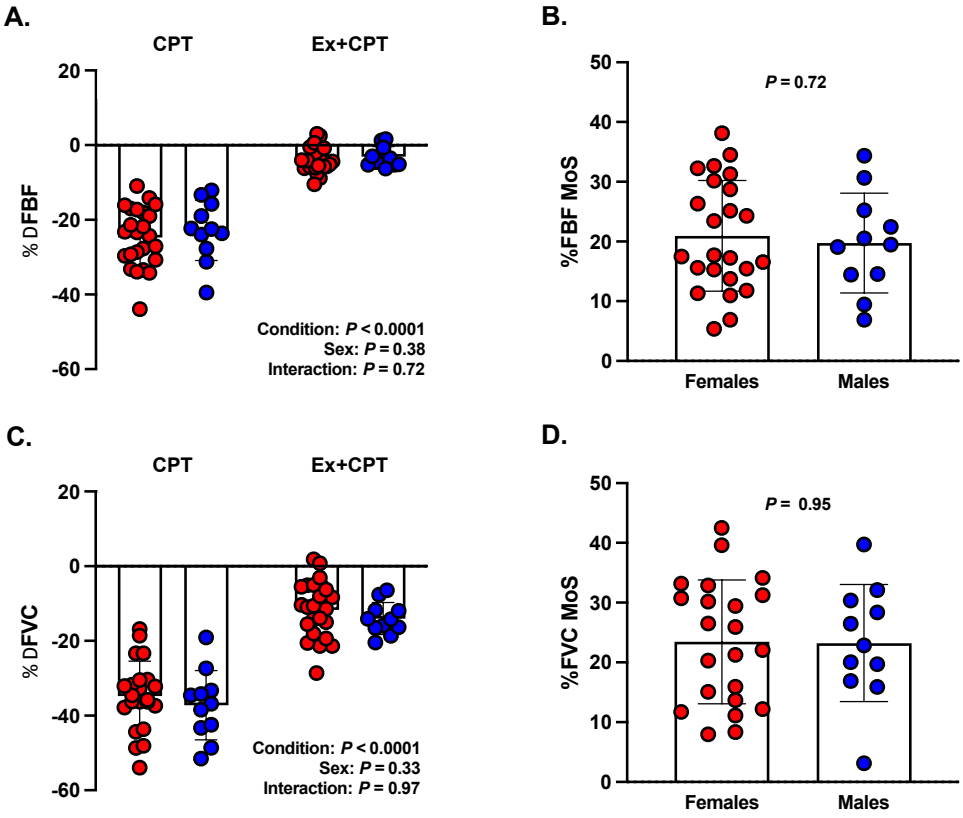


Figure 14: Forearm Blood Flow and Vascular Conductance Responses to the Functional Sympatholysis Protocol.

Figure 14 Key: Left panels represent individual data and group average changes in forearm blood flow (**A.** %ΔFBF: Females, N=24; Males, N=11), and **C.** forearm vascular conductance (**C.** %ΔFVC: Females, N=22; Males, N=11) in response to a cold pressor task at rest (CPT) and during rhythmic handgrip exercise at 20% maximal voluntary contraction (Ex+CPT). The right panels represent the magnitude of sympatholysis (MoS, e.g., the difference between CPT-induced percent changes during handgrip and at rest) for %FBF (**B.** %FBF MoS: Females, N=24; Males, N=11) and %FVC (**D.** %FVC MoS: Females, N=22; Males, N=11). Data were analyzed using two-way ANOVAs with repeated-measures for %ΔFBF, mixed-effects analyses for %ΔFVC, and independent sample t-tests for sex-differences in the %FBF MoS and the %FVC MoS. (●) Indicate female participants and (●) indicate male participants.

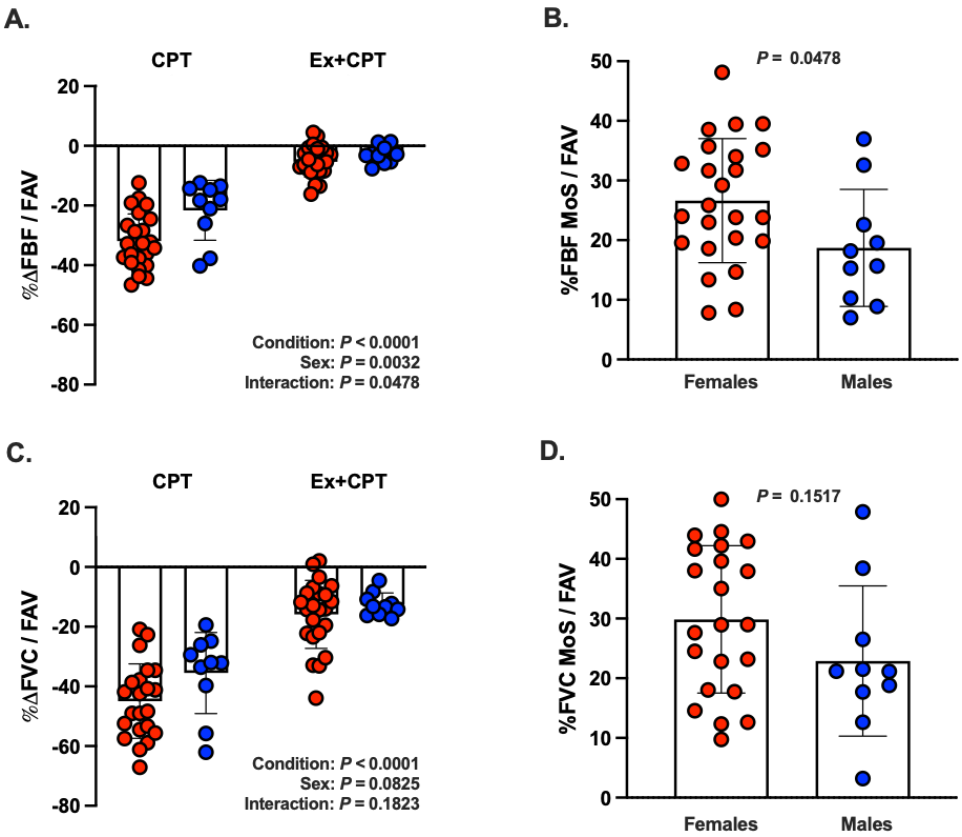


Figure 15: Forearm Blood Flow and Vascular Conductance Responses to the Functional Sympatholysis Protocol (Controlled for Forearm Volume).

Figure 15 Key: Left panels represent individual data and group average changes (%Δ) in forearm blood flow when controlled for forearm volume (**A.** %ΔFBF / FAV; Females, N=24; Males, N=10), and group average changes (%Δ) in forearm vascular conductance when controlled for FAV (**C.** %ΔFVC / FAV; Females, N=22; Males, N=10) in response to a cold pressor task at rest (CPT) and during rhythmic handgrip exercise at 20% maximal voluntary contraction (Ex+CPT). The right panels represent the magnitude of sympatholysis (MoS, e.g., the difference between CPT-induced percent changes during handgrip and at rest) for %FBF when controlled for FAV (**B.** %FBF MoS / FAV: Females, N=24; Males, N=10) and %FVC when controlled for FAV (**D.** %FVC MoS / FAV: Females, N=22; Males, N=10). Data were analyzed using two-way ANOVAs with repeated-measures for %ΔFBF / FAV, mixed-effects analyses for %ΔFVC / FAV, and independent sample t-tests for sex-differences in the %FBF MoS / FAV and the %FVC MoS / FAV. (●) Indicate female participants and (●) indicate male participants.

Allostatic Load and Magnitude of Sympatholysis

As displayed in **Figure 16A-C**, there were no significant Pearson's or Spearman's correlations between AL indices the %FBF MoS (CFA: N=35; r=0.1570; P=0.3678; **Figure 16A**, CC: N=35; r=0.2243; P=0.1953; **Figure 16B**, nor SD: N=35;

r=0.2703; P=0.1163; **Figure 16C**). Similarly, there were no significant Pearson or Spearman's correlations between AL indices and the %FVC MoS (CFA: N=33; r=0.1037; P=0.5657; **Figure 16D**; CC: N=33; r=0.3001, P=0.0897; **Figure 16E,** nor SD: N=33; r=0.2775; P=0.1179; **Figure 16F**).

However, when FAV was controlled for, there was a significant Pearson's correlation between AL index and the %FBF MoS / FAV (CFA: N=34; r=0.4623; P=0.0059; **Figure 17A**), despite there being no significant Spearman's correlations between dichotomously scored AL indices and %FBF MoS / FAV (CC: N=34; r=0.0579; P=0.7452; **Figure 17B**, nor SD: N=34; r=0.1797; P=0.3092; **Figure 17C**). Similarly, when FAV was controlled for, there was a significant Pearson's correlation between AL index and the %FVC MoS / FAV (CFA: N=32; r=0.3814; P=0.0313; **Figure 17D**), despite there being no significant Spearman's correlations between dichotomously scored AL indices and %FVC MoS / FAV (CC: N=32; r=0.1050; P=0.5674; **Figure 17E**, nor SD: N=32; r=0.1673; P=0.3601; **Figure 17F**).

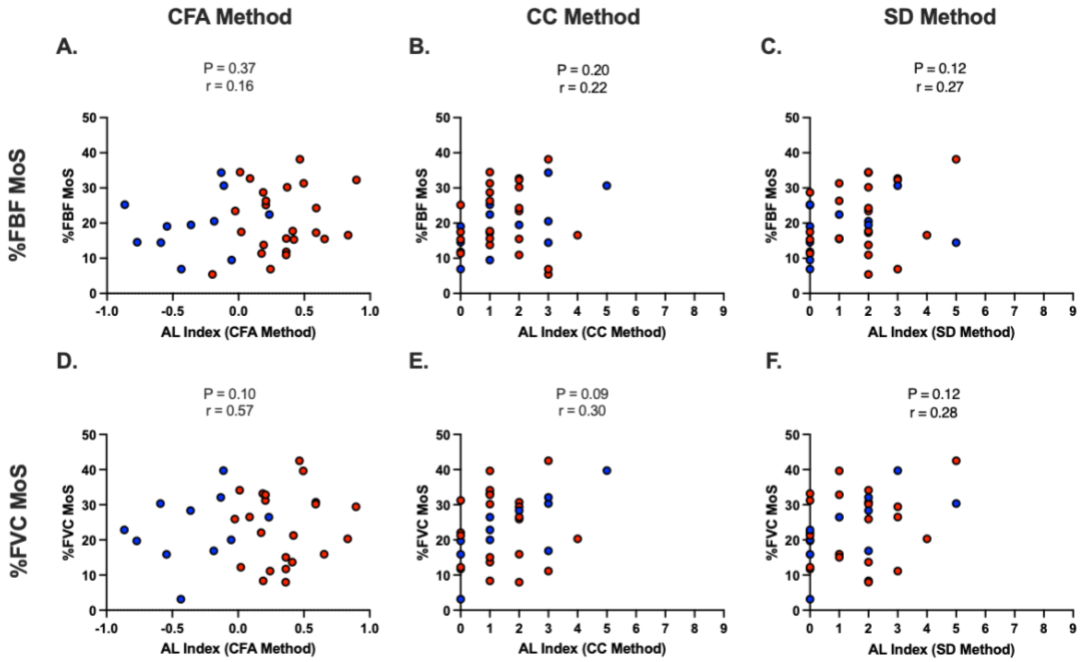


Figure 16: Correlations Between Allostatic Load and %Forearm Blood Flow and % Forearm Vascular Conductance Magnitude of Sympatholysis.

Figure 16 Key: AL, allostatic load; CFA, confirmatory factor analysis; CC, clinical cutoff; SD, standard deviation; FBF, forearm blood flow; FVC, forearm vascular conductance; MoS, magnitude of sympatholysis. **A.** No significant Pearson's correlation between AL index (CFA method) and % FBF MoS (N=35); **B.** No significant Spearman's correlation between AL index (CC method) and % FBF MoS (N=35); **C.** No significant Spearman's correlation between AL index (SD method) and % FBF MoS (N=35); **D.** No significant Pearson's correlation between AL index (CFA method) and % FVC MoS (N=33); **E.** No significant Spearman's correlation between AL index (CC method) and % FVC MoS (N=33); **F.** No significant Spearman's correlation between AL index (SD method) and % FVC MoS (N=33). ● Indicate female participants and ● indicate male participants.

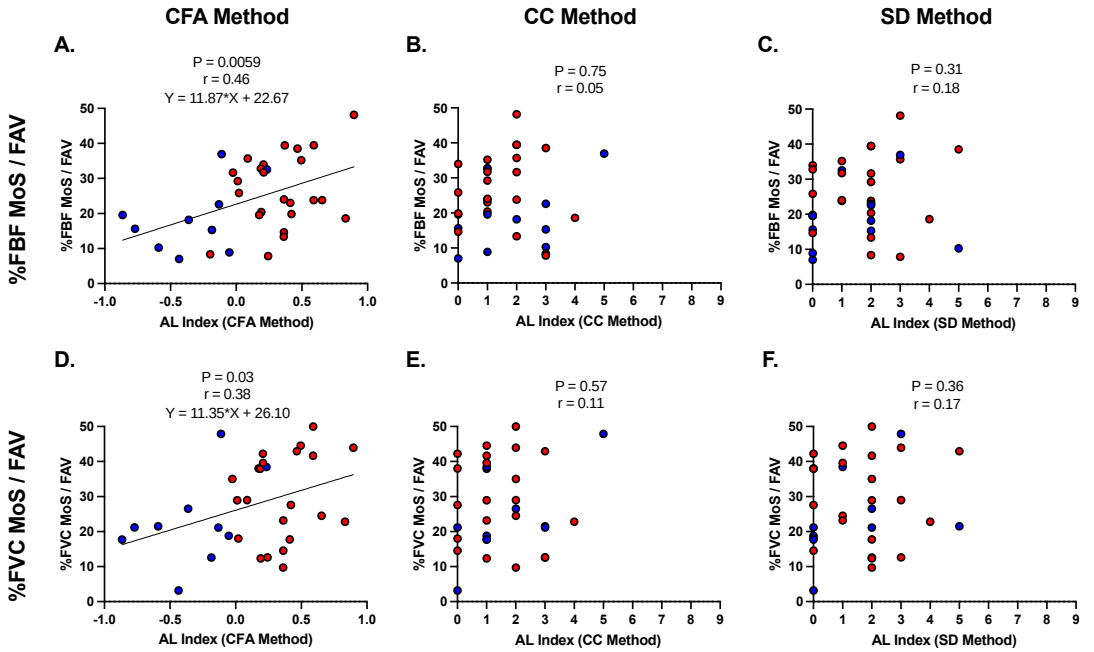


Figure 17: Correlations Between Allostatic Load and %Forearm Blood Flow and % Forearm Vascular Conductance Magnitude of Sympatholysis (Controlled for Forearm Volume).

Figure 17 Key: AL, allostatic load; CFA, confirmatory factor analysis; CC, clinical cutoff; SD, standard deviation; FBF, forearm blood flow; FVC, forearm vascular conductance; MoS, magnitude of sympatholysis; FAV, forearm volume. **A.** Significant Pearson's correlation between AL index (CFA method) and % FBF MoS / FAV (N=34); **B.** No significant Spearman's correlation between AL index (CC method) and % FBF MoS / FAV (N=34); **C.** No significant Spearman's correlation between AL index (SD method) and % FBF MoS / FAV (N=34); **D.** Significant Pearson's correlation between AL index (CFA method) and % FVC MoS / FAV (N=32); **E.** No significant Spearman's correlation between AL index (CC method) and % FVC MoS / FAV (N=32); **F.** No significant Spearman's correlation between AL index (SD method) and % FVC MoS / FAV (N=33). ● Indicate female participants and ● indicate male participants.

Discussion

The novel findings from this study are that among young Black adults, 1) there are significant positive associations between AL index and the %FBF and %FVC magnitude of sympatholysis (MoS), and 2) while the %FBF MoS is significantly greater among young Black females as compared to young Black males, there are no

sex differences in the %FVC MoS, thus suggesting a role of the blood pressure response.

Allostatic Load and Functional Sympatholysis

While contrary to our initial hypothesis, results from our study partially align with those of Caldwell et al. (2019), who reported no changes in the %FBF MoS of young adults before and after an acute high-salt diet (297). While we did not assess the impact of dietary, nor acute stressors on the MoS in the present study, we assessed chronic physiological stress via measures of AL, and instead, found a positive correlation between AL and the %FBF MoS in young Black adults. Taken together, results from these studies may suggest that among young adults, there are compensatory physiological mechanisms acting to maintain (or amplify) the functional sympatholytic response in the face of acute or chronic stressors. Along these lines, while NO and ATP are suggested to be the primary vasodilatory substances responsible for facilitating functional sympatholysis (200–202, 206, 221, 298–302), in conditions of diminished NO and/or ATP bioavailability there are compensatory roles of prostacyclin (PGI_2) (198, 199, 300) and/or adenosine (213, 303, 304) in facilitating exercise hyperemia and maintaining functional sympatholysis by attenuating α-adrenergic-mediated vasoconstriction. Despite evidence of impaired contribution of NO to the vasodilatory response among Black individuals (74, 81, 188, 305, 306), young healthy Black and White men have similarly preserved functional sympatholysis (293, 307), suggesting mediation of functional sympatholysis via substances independent of NO. In a similar manner, despite a substantial reduction in NO bioavailability among hypertensive individuals, microvascular vasodilation in response

to shear stress remains intact, primarily due to the compensatory function of H_2O_2 (308). Therefore, it is possible that the highly adaptive SNS and compensatory mechanisms maintaining functional sympatholysis in young Black adults effectively buffer against the effects of chronic stressors, thus explaining the positive association between AL and the %FBF and %FVC MoS among this population.

Although associations between AL and the functional sympatholytic response have not been explored previously, our hypothesis that there would be a negative association between AL and the MoS was formed based on evidence of impaired functional sympatholysis among individuals with significantly higher blood pressure (stage 1 hypertension) and resting heart rates as compared to healthy controls (220). One explanation for the discrepancy in findings among our participants as compared to participants in the aforementioned study by Vongpatanasin et al. (2011), could be due to their sample being made up of middle-aged adults. Additionally, despite significant differences in SBP, DBP, and RHR, the stage 1 hypertensive participants and healthy controls had similar concentrations of creatinine, total cholesterol, and fasting glucose (220). Indeed, while they do not report measurement of diurnal cortisol, CRP, %TBF, or HDL, normotensive and hypertensive middle-aged participants of the Vongpatanasin et al. (2011) study had numerically higher mean concentrations of creatinine (1.0 and 1.0 mg/dL vs. 0.79 mg/dL), total cholesterol (181 and 183 mg/dL vs. 152 mg/dL), and fasting glucose (94 and 99 mg/dL vs. 84 mg/dL) as compared to the young adult participants in our study, whereby they may be trending toward higher AL as compared to the participants of our study. Therefore, when elevations in the preceding biomarkers of AL are combined with aging and a potential clinical condition

like hypertension, may be when impaired functional sympatholytic responses begin to emerge. Another discrepancy between our participants and those of the Vongpatanasin et al. (2011) study, is that their study population was ~70% males, as compared to our study, which assessed a sample of young adults, of which were ~70% female, which calls into question the moderating role of sex on the functional sympatholytic response.

Sex Differences in Functional Sympatholysis

The current study was, to our knowledge, the first to assess sex differences in the %FBF and %FVC MoS among young Black adults. According to our results, the %FBF MoS was significantly larger among young Black women as compared to young Black men. In contrast to our findings, Teixera et al., (2022) found no moderating effect of biological sex on the %FBF functional sympatholytic response among young adults (273); however, this study did not indicate the racial identities of participants, nor whether male and female participants had similar or disparate FAV.

Physiologically, our findings of enhanced %FBF MoS among females as compared to males align with results from rodent studies, which have attributed these sex-differences to the role of estrogen in increasing NO bioavailability, and the consequential blunting of α_2-adrenogenic vasoconstriction during exercise (309). These suggestive effects of estrogen on the enhancement of the %FBF MoS are further supported by evidence of marked detriments in the %FBF MoS as women progress through the menopausal transition (219). Specifically, research on healthy post-menopausal women has revealed that their otherwise impaired functional sympatholytic response as compared to the intact response in healthy premenopausal women, can be reversed following one-month of oestradiol replacement therapy (219).

Therefore, estrogen has been implicated as a protective agent in the maintenance of functional sympatholysis (219, 309, 310). This could explain why female participants in our study, who, despite having significantly higher AL, also had a higher %FBF MoS as compared to their male counterparts, as they were all premenopausal.

While raw FBF data during the Ex and Ex+CPT conditions were significantly greater among young Black males as compared to young Black females in our study, there were no sex differences in the %FBF MoS when FAV was not controlled for. This finding aligns with a recent study that reported similar %FBF and %FVC MoS (uncontrolled for FAV) between sexes in young adults, despite reports of young male participants having significantly lower resting heart rates and larger brachial artery diameters than young female participants (273). However, after controlling for FAV, we observed a higher MoS among young Black females as compared to young Black males. This sex discrepancy therefore may have been a function of the female participants having significantly smaller resting brachial artery diameters and FAV, and significantly less left arm lean mass as compared to their male counterparts. Among the total sample of participants, larger FAV was correlated with higher left arm lean mass and larger resting brachial artery diameter. Larger FAV lends to increased blood vessel density, thus providing greater capacity for blood flow to exercising muscles, and consequentially impacting the degree to which sympatholysis occurs during exercise. By virtue of these factors, there was a more pronounced sympatholytic response among young Black females as compared to young Black males. This is likely because with smaller resting diameters came a larger magnitude of change come the onset of exercise hyperemia among the young Black females. Whereas, with male

participants, who already had larger resting brachial artery diameters, we posit that there existed an inability for the male participants to further vasodilate to the same magnitude as female participants. With this in mind, when assessing group differences, the controlling of the functional sympatholytic response for FAV may be an experimental consideration for future studies.

Limitations and Future Directions

Given that our sample of young Black adults were all University attending students, this could muddle the application of our findings to a larger sample of Black adults. It's been postulated that increased AL among Black individuals as compared to those of other racial and ethnic backgrounds is due to a combination of the unique social, economic, environmental, and psychological disparities that more commonly affect Black adults in the U.S. While the systemic and downstream effects of chronic stress, such as racism and discrimination, are not abolished on a college campus, these environments provide key resources such as housing, food, and health care, which may be more difficult to attain outside the university setting. Therefore, future studies should consider seeking inclusion of young Black adults who are and who are not undergraduate students to form a more comprehensive understanding of which aspects of increased AL and/or impaired functional sympatholysis are physiologically linked to increased hypertension prevalence in this population.

Chapter 4: Summary of Findings

Aim 1

The first main objective of this dissertation project was to investigate how the allostatic load (AL) profiles of young Black adults may correlate with measures of vascular dysfunction and stiffness. To this end, we measured AL utilizing nine unique biomarkers and three statistical models to paint a comprehensive portrait of the latent construct of AL. To evaluate vascular function, we utilized measures of flow-mediated dilation (%FMD) to assess the macrovasculature, and the reactive hyperemic response following an ischemic stimulus (RH-AUC) to assess the microvasculature. To evaluate vascular structure, we deployed measures of carotid-to-femoral pulse wave velocity (cfPWV) and wave-reflection (AIx and AIx75). While much of the literature has implied that there are vascular consequences by way of sustained psychological and/or physiological distress (57, 234, 235), actual investigation of the associations between vascular dysfunction and/or stiffness with AL as the method for capturing chronic stress are novel to this dissertation. Our choice to study a cohort of young Black adults was motivated by the disproportionate burden of hypertension in this demographic (2, 6), alongside its earlier onset (7, 8), and the continued need to understand the underlying mechanisms contributing to cardiovascular health disparities faced by Black individuals in the U.S. Our findings revealed a negative correlation between AL and allometrically scaled %FMD, indicating that higher AL was associated with impaired macrovascular function. Conversely, AL was positively correlated with

augmentation index, suggesting a potential link between elevated AL, and increased wave-reflection from the peripheral vasculature. Notably, there were no significant correlations between AL and microvascular function or cfPWV, indicating differential effects on macrovascular versus microvascular health.

Because women and men exhibit different responses to stress exposure (311), we chose to investigate whether sex moderated the association between AL and metrics of vascular health as an exploratory aim of this dissertation project. In contrast to our hypothesis, sex did not moderate any of the associations we observed between AL and measures of vascular health. This finding remained even despite significantly higher AL among female participants than male participants when AL was assessed using the CFA method. Interestingly, when scored with dichotomous scoring methods (CC and SD methods), AL was less variable within the population. Indeed, the highest AL index achieved by any participant in this study was equivalent to five out of a total nine points (55%). Therefore, our population of participants may have skewed healthier than the general population.

It has been proposed that the higher AL observed among Black individuals in the U.S. (24–34) may be attributed to a complex interplay of social, economic, environmental, and psychological distress, including socioeconomic disparities and discrimination, as salient exposures to chronic stress. Therefore, we also investigated the associations between participants' self-reported perceptions of social determinants of health, everyday discrimination, and stress with measures of vascular function and structure as an exploratory aim of this dissertation project.

Aim 2

This elevated AL may predispose Black adults to an increased risk for hypertension through dysregulation of physiological stress response systems, including the sympathetic nervous system (SNS). Therefore, the second main objective of this dissertation project was to investigate how the AL profiles of young Black adults may correlate with their ability to attenuate the vasoconstrictive response to acute sympathetic stimulation during rhythmic handgrip exercise (i.e. functional sympatholysis). Because of the synonymous role of heightened sympathetic activity in elevating AL and impairing the functional sympatholytic response, we hypothesized that these measures would be negatively correlated among young Black adults.

We also investigated the moderating role of sex on any associations between AL index and the magnitude of the functional sympatholytic response. When we controlled for forearm volume (FAV), results of this study identified positive correlations between AL and the %forearm blood flow (FBF) and %forearm vascular conductance (FVC) magnitude of sympatholysis (MoS) within the total sample of participants, and a higher %FBF MoS among young Black females as compared to their male counterparts, despite similar %FVC MoS between sexes. While the magnitude of the functional sympatholytic response may be able to predict CVD onset and/or progression, the maintained MoS among young Black adults in the face of increased AL among participants in this study does not parallel the development of CVD observed in this population (2). It is likely that this discrepancy stems from the age of the participants in the present study (e.g., ~20 years) when compared to the onset age of overt CVD (e.g., midlife), when the effects of prolonged physiological wear-and-

tear (i.e., AL) also become more pronounced. Additionally, due to the tendency for the MoS to be correlated with AL only when FAV was controlled for, we presume that larger FAV (of which was positively correlated with increased arterial diameter) may be indicative of arterial remodeling or adaptation in response to chronic stress or other factors associated with elevated AL, as increased arterial diameter is a structural adaptation that occurs with aging and/or among chronic disease states (179).

Collective Findings

Taken together, the results from these studies suggest that while elevated AL may predict macrovascular dysfunction, this relationship is dependent on the increased arterial diameters among this population. Indeed, the increased brachial artery diameter size may explain why we did not observe any correlations between AL and the %FBF and %FVC MoS in young Black adults until we controlled them for FAV. An increased brachial artery diameter and FAV may explain the positive correlations between AL and the functional sympatholytic response in young Black adults due to potential compensatory mechanisms in vascular function. A larger brachial artery diameter and FAV could indicate arterial remodeling or adaptation in response to chronic stress or other factors associated with elevated AL, as this is a structural adaptation seen in aging and diseased states (179). Indeed, these adaptations may allow for greater blood flow and oxygen delivery to the muscles during exercise, thus explaining the preserved functional sympatholysis, even in the presence of increased AL. These consistent observations of redundant physiological mechanisms present in young Black adults to maintain macrovascular function and responses to a sympathetic stimulus strengthen

our findings and further highlight the complex interplay between chronic stress, vascular function, and cardiovascular health outcomes in young Black adults.

Future Directions

Moving forward, several avenues for future research can be explored based on these findings. Firstly, longitudinal studies are needed to examine the causal relationship between AL and vascular function or stiffness over time among Black adults. Our cross-sectional study design precludes causal inferences and limits our ability to determine the temporal relationships between chronic stress exposure, AL development, and changes in vascular function over time. Therefore, longitudinal studies are needed to better understand the dynamic interplay between psychosocial factors, AL, and vascular health outcomes in young Black adults. Further investigation into the mechanisms underlying the relationship between perceived stress and augmentation index (AIx, AIx75) may specifically provide insights into the physiological pathways linking psychosocial stress to downstream onset of hypertension at increased rates in Black adults. Secondly, while our study provides valuable insights into the relationship between AL and vascular health among young Black adults, it is important to acknowledge that we only assessed university-attending individuals in our sample. University-attending individuals may not be reflective of the entire population as they likely perceive themselves as having greater access to education and may have greater access to more favorable socioeconomic resources compared to those who do not attend university. As a result, our findings may not fully generalize to all young Black adults, particularly those from lower socioeconomic backgrounds who may experience higher levels of chronic stress and greater

cardiovascular health disparities. Therefore, future research should strive to include diverse samples representative of the broader population, including individuals from varying socioeconomic backgrounds and educational attainment levels, to ensure the generalizability of findings. Lastly, interventions aimed at reducing stress and improving coping mechanisms may help mitigate the impact of AL on vascular health and promote vascular resilience in this population. Overall, addressing the impact of chronic stress on vascular function and stiffness represents a critical area for future research and public health interventions aimed at promoting health equity and reducing disparities in cardiovascular outcomes.

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