The Effect of Curcumin on Cardiovascular Health in Obese Men

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THE EFFECT OF CURCUMIN ON CARDIOVASCULAR HEALTH IN OBESE MEN

DISSERTATION

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Exercise Science in the College of Education at the University of Kentucky

By
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Lexington, KY
2016

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THE EFFECT OF CURCUMIN ON CARDIOVASCULAR HEALTH IN OBESE MEN

Cardiovascular disease is the leading cause of death in the United States, and conventional treatment often emphasizes pharmaceutical approaches. Research has recently started exploring non-pharmaceutical approaches, including nutritional interventions. This research study was conducted to test the effectiveness of a novel nutritional approach, curcumin, on the improvement of cardiovascular health in young, obese males (BMI≥30 kg/m²). This study included 22 men, matched based on BMI and randomly assigned to the intervention (n=11) or placebo group (n=11). The intervention consisted of 12 weeks of curcumin supplementation (1.0 g/day) with fenugreek added to enhance the curcumin bioavailability; the placebo consisted of 12 weeks of equal parts fenugreek to that found in the intervention. To determine cardiovascular improvements, arterial stiffness via gold-standard carotid-femoral pulse wave velocity (cfPWV), endothelial dysfunction via reactive hyperemia index (RHI), and inflammation via plasma cytokine concentrations were measured. There were no overall differences in cfPWV (p=0.428) or RHI (p=0.951) between groups following the 12 weeks of intervention. However, some individuals did respond to the curcumin treatment with reductions in cfPWV, while others did not. Subjects who did respond to the curcumin treatment (n=6) entered the study with higher baseline values of cfPWV than those that did not respond (n=5) (6.81 m/s v. 5.84 m/s, p = 0.045). This suggests a potential role for curcumin to improve arterial stiffness in individuals with stiffer arteries at baseline. A possible mechanism to explain the difference in responsiveness is a trending increase in IL-13 (p=0.052), an anti-inflammatory cytokine that has been associated with amelioration of collagen content in the arteries. Also, 12 weeks of curcumin intervention resulted in reductions in brachial pulse pressure (p<0.05), a surrogate marker of arterial stiffness. This change in brachial pulse pressure in the curcumin group could be explained by an increased trend in anti-inflammatory cytokine IL-10 (p=0.071), but further studies are required to confirm this finding. Based on the findings of this study, curcumin might serve as a non-pharmaceutical intervention to improve vascular health in young obese men, especially when arteries are stiffer than age-matched counterparts.

KEYWORDS: Turmeric, Arterial stiffness, Carotid-femoral pulse wave velocity (cfPWV), Cardiovascular disease (CVD), Obesity
THE EFFECT OF CURCUMIN ON CARDIOVASCULAR HEALTH IN OBESE MEN

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April 28, 2016
Dedicated to my beloved brother

Johnathan Joseph Campbell
ACKNOWLEDGEMENTS

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# Table of Contents

**Acknowledgements** .......................................................................................................................... iii

**Table of Contents** ................................................................................................................................. iv

**List of Tables** ....................................................................................................................................... vii

**List of Figures** ....................................................................................................................................... viii

**Chapter I** .............................................................................................................................................. 1

  **Dissertation Introduction** ...................................................................................................................... 1

  **Introduction** ........................................................................................................................................... 1

  **Abbreviations** ....................................................................................................................................... 4

**Chapter II** ............................................................................................................................................... 5

  **Review of Literature** ............................................................................................................................. 5

  **Introduction** ........................................................................................................................................... 5

  **Defining Arterial Stiffness** .................................................................................................................... 6

  **Known Contributing Factors to Arterial Stiffness** .............................................................................. 9

    **Collagen and Elastin** ............................................................................................................................... 10

    **Inflammation** ....................................................................................................................................... 11

    **Advanced Glycation End-Products** .................................................................................................... 12

    **Oxidative Stress** ................................................................................................................................. 13

    **Nitric Oxide** ....................................................................................................................................... 14

  **Risk Factors for Increased Arterial Stiffness** ....................................................................................... 15

    **Non-modifiable Risk Factors** ............................................................................................................... 15

    **Modifiable Risk Factors** ..................................................................................................................... 17

    **Other Risk Factors** ............................................................................................................................. 22

  **Assessing Arterial Stiffness** ................................................................................................................... 22

    **Pulse Wave Velocity** ............................................................................................................................... 22

    **Pulse Wave Analysis** ............................................................................................................................... 24

    **Pulse Pressure** ....................................................................................................................................... 26

    **Ultrasound** ......................................................................................................................................... 27

    **Magnetic Resonance Imaging** ............................................................................................................... 27

  **Association Between Arterial Stiffness and Endothelial Dysfunction** .............................................. 28

    **Defining Endothelial Function** ............................................................................................................ 29
**LIST OF TABLES**

Table 4-1. Baseline characteristics of the curcumin and placebo groups ..........................70
Table 4-2. Baseline characteristics of responders and non-responders to the curcumin intervention ........................................................................................................................75
Table 4-3. Changes in cfPWV throughout the intervention in curcumin responders and non-responders ...................................................................................................................77
Table 4-4. Changes in IL-13 pre and post intervention in curcumin responders and non-responders ..........................................................................................................................79
Table 4-5. Effects of treatment on augmentation index ............................................................................................................................81
Table 4-6. Effects of treatment on endothelial function and inflammatory cytokines ....84
Table 4-7. Effects of treatment on BP and HR measures .................................................88
Table 4-8. Effects of treatment on body composition ........................................................................................................................................................................91
Table 4-9. Baseline differences in dietary intake for curcumin and placebo groups ......93
Table 4-10. Changes in dietary intake from baseline to the end of the study .................94
Table A-1. Predicting inflammatory cytokines with waist circumference ......................118
Table A-2. Univariate linear regression for correlations between baseline subject characteristics and cfPWV ...........................................................................................................119
Table A-3. Multiple linear regression for correlations between baseline subject characteristics and cfPWV ...........................................................................................................121
Table A-4. Univariate linear regression correlations between baseline subject characteristics and RHI ...............................................................................................................................................122
Table A-5. Univariate linear regression for correlations between baseline subject characteristics and Brachial SBP ...........................................................................................................124
Table A-6. Univariate linear regression for correlations between baseline subject characteristics and Brachial DBP ...........................................................................................................125
Table A-7. Univariate linear regression for correlations between baseline subject characteristics and Brachial PP ...........................................................................................................126
Table A-8. Univariate linear regression for correlations between baseline subject characteristics and Brachial MAP ...........................................................................................................127
Table A-9. Univariate linear regression for correlations between baseline subject characteristics and Central SBP ...........................................................................................................128
Table A-10. Univariate linear regression for correlations between baseline subject characteristics and Central DBP ...........................................................................................................129
Table A-11. Univariate linear regression for correlations between baseline subject characteristics and Central PP ...........................................................................................................130
Table A-12. Univariate linear regression for correlations between baseline subject characteristics and Central MAP ...........................................................................................................131
LIST OF FIGURES

Figure 3-1. Timeline of visits and associated measures .............................................58
Figure 4-1. Changes in cfPWV in curcumin and placebo groups throughout the intervention ........................................................................................................................72
Figure 4-2. Baseline cfPWV of responders and non-responders to curcumin treatment 76
Figure 4-3. Changes in cfPWV throughout the intervention in curcumin responders ......78
Figure 4-4. Changes in AIx in curcumin and placebo groups throughout the intervention ........................................................................................................................82
Figure 4-5. Changes in AIx75 in curcumin and placebo groups throughout the intervention ........................................................................................................................82
Figure 4-6. Changes in RHI in curcumin and placebo groups pre and post intervention ..85
Figure 4-7. Changes in anti-inflammatory cytokine IL-10 in curcumin and placebo groups pre and post intervention ...............................................................................................................86
Figure 4-8. Changes in Brachial PP in curcumin and placebo groups throughout the intervention ........................................................................................................................89
Figure 4-9. Changes in Central PP in curcumin and placebo groups throughout the intervention ........................................................................................................................89
CHAPTER I

DISSERTATION INTRODUCTION

Introduction

Cardiovascular diseases (CVDs) lead to more deaths in the United States than any other cause (226). Large artery stiffness is an independent risk factor for CVD and is an overall indicator of vascular health (324). More specifically, carotid-femoral pulse wave velocity (cfPWV), a measure of the stiffness of the thoracic and abdominal aorta, may serve as a better predictor of CVD than more traditional markers, such as resting blood pressure (BP) (216). Additionally, cfPWV is widely accepted as the non-invasive gold standard for measuring arterial stiffness in humans (185). Furthermore, vascular endothelial dysfunction, which includes an inability of the inner lining of the blood vessels to properly dilate and constrict, may contribute to stiffness of the arteries. The interplay between arterial stiffness and endothelial dysfunction is not well understood, but they are both indicators of vascular health (51, 60). In recent years, as more information has been reported on arterial properties and their indication of overall cardiovascular health, interest in strategies to reduce large artery stiffness in many populations has escalated. However, the contribution of obesity to large artery stiffness has not been fully elucidated.

Thirty-four percent of U.S. adults are classified as obese (114). Obesity has been clearly linked to CVD (259) and is associated with increased large artery stiffness (368). Some studies have been conducted to assess the ability of exercise and dietary interventions to reduce arterial stiffness in this population, but more work needs to be done to address CVD associated with obesity. A nutritional intervention of recent interest
is curcumin, which has been reported to have beneficial effects in many disease states, most notably cancer (18). The benefits of curcumin on cardiovascular health, and specifically arterial stiffness, are not well understood. Some studies have reported a potential benefit of curcumin in reducing stiffness of the arteries (9, 113, 230, 322), though none have evaluated an obese population. Furthermore, reducing arterial stiffness could lead to a reduction in risk for developing CVD. A previous study also examined the effects of curcumin on endothelial dysfunction, but this was limited to post-menopausal (PM) women (8). The present work studied the responsiveness of cardiovascular health to 12 weeks of supplementation with curcumin in obese men.

The purpose of this study was to determine if 12 weeks of curcumin supplementation could ameliorate arterial health in young, obese men. The primary outcome in this study was non-invasive gold standard arterial stiffness measure, cfPWV. We hypothesized that obese males in the intervention (curcumin + fenugreek) group would experience significant improvement in arterial stiffening at the 12-week visit when compared to obese males in the control group (fenugreek only).

Additionally, pulse wave analysis (PWA) and resting blood pressure (BP) measures, including aortic BP measures, aortic augmentation index, and brachial BP measures, were assessed as secondary outcomes for arterial stiffness. The reactive hyperemia index, a measure of endothelial function, was monitored as a secondary outcome for vascular health. Furthermore, changes in inflammatory blood profiles were assessed because of the association between inflammation and both arterial stiffness and endothelial dysfunction (204, 357). We hypothesized that obese males in the intervention (curcumin + fenugreek) group would experience: (1) improvements in cardiovascular
hemodynamics, (2) improvements in endothelial function, and (3) improvements in inflammatory blood markers at the 12-week visit when compared to obese males in the control group (fenugreek only).
## Abbreviations

**ACh**: Acetylcholine  
**AGEs**: Advanced glycation end-products  
**AIx**: Augmentation index  
**AIx75**: Augmentation index adjusted for a heart rate of 75  
**BP**: Blood pressure  
**BMI**: Body mass index  
**CCTS**: Center for Clinical and Translational Sciences  
**cfPWV**: Carotid-femoral pulse wave velocity  
**CNP**: C-type natriuretic peptide  
**CVD**: Cardiovascular disease  
**DBP**: Diastolic blood pressure  
**DHA**: Docosahexaenoic acid  
**DHQ**: Dietary History Questionnaire  
**DXA**: Dual-energy X-ray absorptiometry  
**EKG**: Electrocardiogram  
**EPA**: Eicosapentaenoic acid  
**FMD**: Flow-mediated dilation  
**FFQ**: Food frequency questionnaire  
**GSH**: Glutathione  
**HDL**: High density lipoprotein  
**HEI**: Healthy Eating Index  
**HR**: Heart rate  
**HTN**: Hypertension  

**IFN**: Interferon  
**IL**: Interleukin  
**LDL**: Low density lipoprotein  
**MAP**: Mean arterial pressure  
**MRI**: Magnetic resonance imaging  
**MMPs**: Matrix metalloproteinases  
**NHANES**: National Health and Nutritional Examination Surveys  
**NIH**: National Institutes of Health  
**NO**: Nitric oxide  
**PAT**: Peripheral arterial tonometry  
**PWA**: Pulse wave analysis  
**PWV**: Pulse wave velocity  
**RHI**: Reactive hyperemia index  
**PM**: Postmenopausal  
**PP**: Pulse pressure  
**RFAB**: Risk Factor Assessment Branch  
**SAS**: Statistical Analysis Software  
**SBP**: Systolic blood pressure  
**SD**: Standard deviation  
**SOD**: Superoxide dismutase  
**THC**: Tetrahydrocurcumin  
**TNF**: Tumor Necrosis Factor
CHAPTER II
REVIEW OF LITERATURE

Introduction

Cardiovascular diseases (CVDs) persist as the leading cause of death in the United States and worldwide (226, 380). Obesity is also a rising epidemic, and with obesity, there is a 50-100% higher risk of death than normal weight counterparts, due primarily to CVDs (259). Arterial dysfunction, and more specifically aortic stiffness, has great predictive power in determining future cardiovascular events and all-cause mortality (356). Furthermore, endothelial dysfunction, as measured by the reactive hyperemia index (RHI) through peripheral arterial tonometry (PAT), has been associated with increased adverse cardiovascular events, including cardiac death, myocardial infarction, revascularization, or cardiac hospitalization (277). Therefore, identifying interventions for preventing or reducing arterial stiffness and endothelial dysfunction in humans may have immense clinical significance in the reduction of overall CVD (58).

Some studies have looked at using nutritional interventions to reduce arterial stiffness. Evidence from a few studies has suggested that a novel nutritional intervention, curcumin, serves to protect against various cardiovascular problems, though the mechanisms are not well understood (378). The impact curcumin plays on reducing the stiffness of the arteries may be partially responsible for this protective effect. Curcumin has been found to affect vascular health in mice specifically by: (1) reducing the stiffness of the arteries, as measured by PWV; (2) ameliorating endothelial dysfunction associated with diminished nitric oxide (NO) bioavailability; and (3) decreasing oxidative stress (113). Studies in PM women have shown that curcumin supplementation has resulted in
significant improvements in arterial compliance and endothelial dysfunction similar to aerobic exercise, a well-established method for reducing arterial stiffness (8, 9, 322).

To date, studies reporting the positive effects of curcumin on arterial stiffness and endothelial function have been limited to aged male mice and aged women but have not considered the effects of curcumin on obese individuals or men. Furthermore, a majority of studies looking at interventions for arterial stiffness have focused on aged populations. More recently, the link between obesity and arterial stiffness was reported, but few studies have focused on mechanisms that underlie arterial stiffness and nutritional interventions to treat arterial stiffness in obese populations. The present review is designed to look at how nutritional interventions affect large artery stiffness and endothelial function of overweight and obese individuals, with specific interest given to curcumin.

**Defining Arterial Stiffness**

Arterial stiffness, or arteriosclerosis, is an encompassing term that includes the compliance, distensibility, and elasticity of the arteries (317). Distensibility of the arteries is determined by the relative change in diameter of a vessel given a certain amount of pressure, whereas compliance is the absolute change in diameter given a certain amount of pressure. Moreover, “elastic modulus” refers to the pressure change required to increase the diameter of the vessel 100% (247). Though these terms may be used synonymously at times, arterial stiffness is the more encompassing, broader term that addresses these various aspects related to the rigidity of the arterial walls. Arterial health cannot be downplayed, as it has an integral role in the determination of overall
cardiovascular health. Throughout the cardiac cycle, the ability of the arteries to expand and recoil will be determined by the stiffening of the arteries.

Arterial stiffening is a known phenomenon that happens due to various causes, but it does not impact the arteries of the body systematically. The arterial system can be broken down categorically: large elastic arteries, medium-sized muscular (or peripheral) arteries, and small arterioles. The large elastic arteries, such as the thoracic aorta and carotid arteries, are characterized by their striking ability to respond to changes in pressure due to their high elastin content, which allows for greater adaptability than other arteries in the body. The muscular arteries, such as radial and splenic arteries, are characterized by decreased elastin content and increased vascular smooth muscle. The arterioles, the smallest arteries, connect to capillaries and are responsible for more resistance than the other two functional groups of arteries. Studies have shown that the large elastic arteries may be the primary target of arterial stiffness with age and that the muscular arteries may not be as readily affected as the large elastic arteries (39). This is not to suggest that no change occurs at the peripheral arteries with age, but in cases excluding peripheral arterial disease, the arteries of the periphery are not as easily or dramatically stiffened. Additionally, peripheral arteries are not as affected by traditional methods for lowering arterial stiffness as the large elastic arteries (135, 328). This could possibly be explained by the differing structure of the arteries along the arterial system, including collagen, elastin, and vascular smooth muscle content. Furthermore, the roles of the arteries along the arterial tree may also determine their sensitivity to stiffness. The large elastic arteries buffer pulsations, peripheral arteries modify the propagation velocity, and arterioles are reflection sites for the pulse wave (281). During youth, when
arteries are more compliant, large central arteries, such as the aorta, are more elastic in nature to buffer pulsations, whereas peripheral arteries are more rigid for their functionality in affecting the velocity of blood flow (39). The dilating capacity of the large elastic arteries is required to be 3-4.5 times as great as that of the muscular arteries, which suggests that the elastic arteries fatigue more with aging because of increased workload placed upon them throughout life (246). Consequently, the changes in arterial stiffness that primarily affect the large elastic arteries are likely a result of both the structure and function of these arteries. With the structural and functional changes that occur in these large elastic arteries, a consequent change in the pulse pressure wave will occur.

When the left ventricle of the heart contracts, blood is ejected from the heart into the ascending aorta. This causes the aorta to expand, and a pulse pressure wave is sent to the peripheral arteries and arterioles by way of the large elastic arteries. The stiffer an artery is, the faster the pulse pressure wave will travel. In contrast, healthy arteries are elastic and compliant in nature, allowing for necessary adjustments in blood flow to occur quickly and readily. Thus, healthy arteries show slower traveling pulse wave velocities than arteries that are stiffer. As the pulse wave reaches the periphery, where greater impedance occurs, a reflected wave is sent back to the aorta (74). Together, the initial (or incident) pulse wave and the reflected wave make up the pressure waveform. When arteries are stiffer, this waveform will be augmented by the increased transmission velocities; the reflected wave will arrive earlier in the aorta, causing pressure to augment during systole (217). This will, in turn, cause aortic systolic pressure to increase and aortic diastolic pressure to decrease (246).
Arterial stiffness, as measured by cfPWV, is an important risk factor, independent of conventional risk factors, in determining cardiovascular events (201). Additionally, it is predictive in determining cardiovascular and all-cause mortality (186). Remarkably, a rise in aortic PWV by 1.0 m/s increases cardiovascular risk by 15% (356). Therefore, vascular stiffness is very important in determining cardiovascular risk. It should also be noted that vascular dysfunction does not merely affect the heart but can also cause impaired blood flow to the brain, kidney, and other organs, which could lead to cognitive decline, renal impairment, or other problems throughout the body (213, 254, 326).

Arterial health is often overlooked in the role that it plays in the global health of an individual, so more attention needs to be given to screening for arterial stiffness.

**Known Contributing Factors to Arterial Stiffness**

Arterial stiffness leads to known structural changes of the arteries, but the mechanisms contributing to the stiffness are still under investigation. Arteries are composed of three layers: the tunica adventitia, the tunica media, and the tunica intima. Additionally, endothelial cells separate the arterial wall from the blood. As discussed earlier, the arteries have a different structure based on their function. The degree to which each of the known contributing factors to arterial stiffness can impact the artery is dependent upon the location and function of the artery. In general terms, stiffened arteries have been marked by: increased collagen content, decreased elastin content, inflammatory activity, increased matrix metalloproteinases, increased content of reactive oxygen species, and increased advanced glycation end-products (AGEs).
Proteins known as collagen and elastin, found within the extracellular matrix of the arteries, are two of the most well-known elements of the arterial wall that are affected with stiffness. Elastin serves to allow for pliability of the arterial wall when pressures are loaded during the cardiac cycle, whereas collagen provides structural support and strength when high pressures are placed on the arteries (128). Both collagen and elastin are stabilized by their ability to cross-link (273). This cross-linking allows for durability of these proteins present in the artery, but when the cross-linking is affected, structural damage at the artery can enhance the stiffness of the arteries. Arterial stiffness is amplified as the elastin fibers are fragmented or thinned (128). Elastin is found in higher content in the portions of the arterial tree that are closer to the aorta (153), allowing for resilience and extensibility, as well as recovery from stretch (90). In contrast, collagen functions to limit the distension that is able to occur when increased loads are placed on the arteries and the pressure exceeds physiologic loads (377). Therefore, both proteins are important for the integrity of the arterial wall – elastin working more at lower pressures and collagen working more at higher pressures. One study suggested that alterations in elastin are more likely to lead to changes in the geometry of a vessel but not changes in the mechanical properties, whereas alterations in collagen are more likely to have an effect on the mechanical properties while having little effect on the geometry, which will cause collagen alterations to have a greater effect on the stiffness (91). When the balance of these proteins is offset in cases of arterial stiffness, the arteries become more rigid from higher collagen content and lower elastin content.
**Inflammation**

Large artery stiffness has been directly associated with acute and chronic inflammation (204, 357). Inflammation is associated with the structural changes that occur in the artery, as inflammation leads to increases in collagen production and decreases in elastin production, corrupting the normal balance that exists between collagen and elastin in the arteries (159). Inflammation reportedly affects the structural components of the arteries through matrix metalloproteinases (MMPs), which are secreted by cells such as fibroblasts, osteoblasts, endothelial cells, macrophages, neutrophils, and lymphocytes (352). The arterial wall is specifically affected by MMPs that are secreted by vascular smooth muscle cells and macrophage foam cells (386). MMPs are catabolic in nature, and they function as collagenases, elastases, and other factors that indirectly degrade the collagen and elastin content in the arteries (89). Therefore, inflammation leads to structural deterioration of the arteries, which can further promote stiffening. Activation of the NFκB pathway induces transcription of pro-inflammatory cytokines, such as IL-1β, IL-6, IFN-γ, and TNF-α, which directly affects the inflammation of the arteries (188). Inflammatory factors are often present in states of arterial stiffness, though it is not yet known whether a reduction in chronic inflammation at a vascular level will improve large elastic artery stiffness (109). However, the acute effects of a known anti-inflammatory drug, aspirin, reported that reductions in inflammation nullified acute increases in aortic arterial stiffness (357). In this randomized, placebo-controlled study, a *Salmonella typhi* or sham vaccination was administered; the *Salmonella typhi* vaccination increased cfPWV by 0.43 m/s in association with pro-inflammatory markers, C-reactive protein (CRP), Interleukin (IL)-6,
and MMP-9. In contrast, when participants were pre-treated with 1200 mg aspirin treatment, neither cfPWV nor the pro-inflammatory markers were increased. The authors report a cause-and-effect relationship between acute systemic inflammation and aortic stiffness. They speculate that inflammation could affect aortic stiffness through an unfavorable effect of inflammation on NO bioavailability, MMPs acting upon either the structural or functional changes of the aortic wall, or through further inflammatory cytokines that are not measured within the study. The role of chronic inflammation on aortic stiffening is still under investigation.

**Advanced Glycation End-Products**

Advanced glycation end-products (AGEs) also have an effect on collagen and elastin in the artery. These AGEs form irreversible cross-links with both collagen and elastin, leading to more rigid arteries (163). Cross-linking with AGEs results in a reduced capacity of collagen and elastin to turnover, leading to an accumulation of collagen and elastin molecules that are unable to function properly within the artery (353). When elderly individuals (163) and rodents (316) were treated with an agent that breaks the cross-linking due to AGEs, it was found that arterial stiffening was attenuated. Moreover, in hypertensive subjects, AGEs are associated with aortic stiffness independent of age and BP (207). In type 2 diabetics, AGEs are increasingly prevalent in the extracellular matrix of the arteries, and the formation of AGEs on lipids can become a relevant concern (125). Specifically, AGEs form on LDL and can reduce NO production, which suppresses LDL uptake and clearance and promotes dyslipidemia in diabetics (54, 125, 264). Furthermore, type 2 diabetics have increased aortic stiffness, where obesity is a risk factor, and weight loss of ~8% has been reported to improve arterial stiffness in this
population. Therefore, the association between AGEs and arterial stiffening is clear; however, the mechanisms of AGEs activation impacting arterial stiffening has not been completely elucidated (109).

**Oxidative Stress**

Oxidative stress has also been identified as a key mechanism of arterial stiffness, though its exact involvement has not yet been elucidated (180, 243, 289). Oxidative stress is characterized by an imbalance between reactive oxygen species and the antioxidant defense capacity, and it leads to cellular damage and dysfunction (111). Specifically, in mice, superoxide production is increased in the aorta (302), which is believed to contribute to arterial aging (111). Increased superoxide production within the aorta is associated with increased secretion of inflammatory cytokines, suggesting that oxidative stress may contribute to vascular aging by promoting inflammation (110). Even when other contributing factors to arterial stiffness are taken into account, oxidative stress is positively associated with arterial stiffness (256). A study looking at the responsiveness of the arteries to statin treatment found that reductions in arterial stiffness were correlated with reductions in oxidative stress (r = -0.340, p = 0.003) (360). Another study looking at arterial stiffness in aging mice indicated that that older mice with increased aortic PWV had higher levels of oxidative stress, collagen expression, pro-inflammatory cytokines, and AGEs (111). When aged mice with increased arterial stiffness were treated with TEMPOL, an antioxidant which replicates the action of superoxide dismutase (SOD), arterial stiffness was ameliorated, endothelial function was restored, collagen levels were reduced, inflammation was reduced, and oxidative stress was decreased. The ability of an antioxidant to normalize vascular function in old mice suggests that oxidative stress may
be playing a crucial role in the development of arterial stiffness. While the relationship between oxidative stress and arterial stiffness has not been clearly deduced, oxidative stress seems to have an effect on the arterial stiffness progression.

*Nitric Oxide*

The role of NO in arterial stiffness is still being explored. Functional changes that occur with vasoactive properties of the arteries may be a result of NO bioavailability, and they may occur apart from structural changes within the artery (373). A study looking at blood flow variations in humans suggested that arterial stiffness is regulated by the release of NO (37). Local vasoactive mediators released from the endothelium influence smooth muscle tone of arteries and can functionally affect arterial stiffness (370). In rats, inhibiting NO synthase with L-NG-Nitroarginine Methyl Ester (L-NAME) significantly increased PWV acutely, and additional increases were seen after three weeks on L-NAME treatment while controlling for associated changes in BP (108). In this rat study, it was suggested that the endogenous NO system must be functioning properly to preserve vascular compliance. Additionally, NO has been shown to contribute to arterial elasticity (172). When basal NO production by the endothelium was inhibited in humans, arterial stiffness, as measured by augmentation index (AIx) (371) and PWV (172), were increased. Moreover, exogenous administration of foods rich in nitrates and nitrites can affect the nitrate-nitrite-NO pathway, and produce bioactive NO (302). Administering nitrite supplementation in middle-aged and older adults has resulted in improved arterial compliance and endothelial function (80). Therefore, the ability to modulate NO has been associated with the ability to affect arterial health.
Risk Factors for Increased Arterial Stiffness

Non-modifiable Risk Factors

Age

Age is known as the leading risk factor for arterial stiffness and CVD risk (229). Most of the research to date that has been conducted on arterial stiffness has focused on aged populations, as aging with increased stiffness in the arteries is common to all people, and the elderly are one of the most readily available populations for studying this common phenomenon. A study showed that, throughout the lifetime, cfPWV increases by roughly 0.1 m/sec each year, which is equivalent to about 1% per year (26). However, after age 55, some studies have shown that a more rapid increase starts to occur (179, 228). One study showed that the percentage of individuals with abnormal cfPWV rises from a few percent in individuals under the age of 50 to 70% after the age of 70 (215).

Race

Racial differences exist in vascular health. Black Americans have been shown to exhibit higher arterial stiffness, as measured by PWV, as well as greater endothelial dysfunction, as measured by the RHI via EndoPAT, even after adjustments are made for BP (224). Interestingly, one study looked at whether the differences that exist between African-American men and White men could be attenuated with exercise (138). They found that cfPWV was not significantly different at baseline in either group but that peripheral PWV responded to exercise in White men, yet it did not in African American men. Independent of BP, they conclude that racial differences exist in the arterial response in the peripheral vascular system, presumably due to blunted vasodilation in African American males. Also, a study conducted in Brazil looked at arterial stiffness in
four different ethnic groups: American Indians, individuals of Caucasian descent, individuals of African descent, or Mulatto (racially mixed) individuals (75). They found that individuals of African descent had higher PWV and BP than the other three groups. Additionally, when data were analyzed with the removal of hypertensive individuals, they found that American Indians had lower adjusted PWV values than the other groups, and individuals of African descent still had higher PWV values independent of BP. Accordingly, there is solid evidence to suggest that individuals of African descent are more apt to arterial stiffness than other individuals. More research needs to be done to elucidate whether other ethnicities are at risk for greater arterial stiffness.

Sex

Several studies have suggested that women have significantly stiffer and less compliant arteries than men (71, 240, 278). While some studies would suggest that this is true during all points of life, other studies only observed increased arterial stiffness in older, or PM, women when compared to men (123, 359). On the other hand, a study looking at sex differences in men and women pre- and post-puberty found that prepubescent females had stiffer arteries than their prepubescent male counterparts but that there was no difference in males and females after puberty (6). They concluded that, as females age, their large arteries become more distensible, whereas, as males age, their arteries become stiffer. They suggest that these differences are due to sex-specific steroids. They recognize that elderly women have stiffer arteries and suggest this is due to intrinsic differences between the sexes. Current evidence suggests that women may have stiffer arteries than their age-matched male counterparts, but this may not be true of all decades of life.
**Modifiable Risk Factors**

**Obesity**

Obesity is an independent risk factor for CVD (146) and arterial stiffness (384). Obesity is often defined by body mass index (BMI). BMI is simply determined by dividing weight in kilograms by height in meters squared (kg/m\(^2\)). While BMI can be limiting in that it does not measure the body composition of an individual, it is the measure most commonly used to define overweight and obesity (275). By these standards, a normal weight individual would have a BMI of 18.5-24.9 kg/m\(^2\), an overweight individual would have a BMI of 25.0-29.9 kg/m\(^2\), and an obese individual would have a BMI greater than or equal to 30.0 kg/m\(^2\) (59). Studies have shown that obesity, as defined by BMI, is linked to an increase in arterial stiffness independent of blood pressure, ethnicity, and age (280). Previous work has suggested that the age of obesity development may play a role in the degree of vascular damage found, although the mechanisms are not yet understood (1, 73). Nonetheless, individuals as young as 20-30 years have increased cfPWV compared to their normal weight counterparts, as determined by BMI (347). Furthermore, when healthy 20-40 year old individuals experienced two year weight gains, associated increases in cfPWV progression independent of age and BP were also seen (367). Furthermore, abdominal adiposity, as determined by waist circumference, shows a stronger relationship with increases in arterial stiffness than overall obesity (as determined by BMI), presumably due to inflammation or insulin resistance, leading to increased AGEs (280). The association between abdominal adiposity and arterial stiffness may be a possible mechanism by which central adiposity leads to increased risk for CVD (287). While some studies have
pointed to the importance of the age of obesity onset, other studies have suggested that long lasting abdominal adiposity is not required to observe stiffening of the arteries (287). Increased body weight, BMI, waist and hip circumference, and waist-to-hip ratio are strongly correlated with higher PWV (368). Due to the limiting nature of using BMI as an indicator of body composition, direct measures of body fat may be a better alternative in truly classifying obesity. Large artery stiffness correlates with body fat percentage as measured by skinfold calipers (2). In children, body fat percentage, as assessed by Dual-energy X-ray absorptiometry (DXA), was independently and positively associated with PWV (282). In older adults, DXA measures of total fat mass were independently and positively associated with PWV (325). Together, these studies show a strong link between various parameters of obesity and arterial stiffness.

**Hypertension**

Hypertension (HTN) has been associated with decreased compliance of the arteries (192). While HTN and arterial stiffness are both common to aging, HTN has been shown to accelerate the arterial stiffening process, especially when it is uncontrolled. A study of both hypertensive and normotensive subjects showed that hypertensive subjects had a greater progression in PWV with aging than their normotensive counterparts over a six-year follow up period with treatment for HTN (38). However, when BP was successfully controlled with medication, PWV progression was more than three times slower in the individuals with well-controlled BP than individuals who were on medication yet did not show well-controlled BP. Therefore, a clear association between arterial stiffness and BP seems to exist. Traditionally, it was believed that HTN led to aortic remodeling and stiffening as well as vascular smooth muscle cell hypertrophy.
(251). However, reduced arterial elasticity has been shown to progress to HTN after looking at a six year follow-up of normotensive men and women (189). Recently, in a study observing mice with diet-induced obesity, the mice presented with arterial stiffness before they presented with HTN (363). Therefore, there has been much question as to which comes first, HTN or arterial stiffness. It is imperative to develop a better understanding of what is happening mechanistically in these two conditions to understand this relationship better (119).

**Physical Activity**

Physical activity has been shown to improve cardiovascular health as a whole. In one study of aging individuals, it was discovered that older individuals who engaged in regular endurance exercise habitually presented with greater arterial compliance than their age-matched counterparts; they concluded that habitual vigorous aerobic exercise can be protective against the regular progression of arterial stiffening that occurs with age (329). In this study, it is important to note that decreased arterial compliance with aging was common to all individuals, whether they were regular exercisers or not, but the arterial compliance was significantly improved in men that participated in habitual and vigorous endurance exercise (vigorous aerobic-endurance ≥ 5 times per week) yet not in men who were recreationally active (light to moderate exercise ≥ 3 times per week). Also, a cross-sectional study looking at habitual physical activity in hypertensive subjects indicated that physical inactivity was positively correlated with arterial stiffness, as measured by both PWV and AIx (245). Physical activity was found to be a significant predictor of both AIx and PWV. Many studies have further explored the possible benefits of various modes of exercise interventions in reducing arterial stiffness in obese
populations, and several have reported significant reductions in arterial stiffness following exercise interventions, but these studies are beyond the scope of this review (33, 142, 164, 202, 206, 220, 354). Taken together, the above studies suggest that physical activity plays an important role in overall arterial health.

**Smoking**

Smoking can dramatically impact arterial health (170, 198). AIx is significantly higher in chronic smokers than nonsmokers; in effect, simply smoking one cigarette is potent enough to raise arterial stiffness on an acute basis (198). Furthermore, a study found that second-hand smoke is powerful enough to change elastic properties of the aorta of the passive smokers, though PWV was not measured (314). Additionally, another study indicated improvements in arterial stiffness, as measured by AIx (but not PWV), after four weeks of successful tobacco cessation (272). Interestingly, in smokers that quit but were unsuccessful, AIx was increased from initial smoking levels after resuming smoking. Moreover, when looking at cross-sectional measures of smokers, ex-smokers, and non-smokers, current and ex-smokers had higher PWV and AIx measures than nonsmokers; however, duration of smoking cessation had a significant negative correlation with PWV, and individuals who had been ex-smokers for more than 10 years showed PWV measures that were not higher than nonsmokers (154). Therefore, while smoking has been shown to have negative effects on arterial stiffness, cessation can help ameliorate these effects.

**Dietary Habits**

Several dietary habits have been associated with increased arterial stiffness. Populations with lower salt intakes have shown smaller increases in PWV with age than
those that have high salt intakes (29). In a study examining hypertensive subjects cross-sectionally, individuals who were identified as being salt sensitive had decreased arterial compliance compared to those who were salt resistant (93). Also, higher garlic consumption has been shown to lead to smaller increases in PWV with age (53). On the other hand, chronic and acute coffee consumption have been shown to contribute to arterial stiffness, though the PWV changes are transient after acute consumption (253, 358). Higher fish consumption in Japanese fishing and farming villages was associated with lower cfPWV (132). Alcohol consumption has shown some differing effects on arterial stiffness. One study showed that alcohol consumption had a J-shaped association with cfPWV in men (300). Thus, they concluded that moderate alcohol consumption (4-21 glasses/week) could lead to a decreased risk for cardiovascular disease, while more-than-moderate consumption (22-58 glasses/week) was associated with an increased risk. Another study found that acute consumption of red wine decreased arterial stiffness when compared to acute consumption of non-alcoholic red wine (PWV decreased by 0.7 m/s, p < 0.05; AIx decreased by 4%, p < 0.05), which was still significant after adjusting for BP (197). However, this study also concluded that, in chronic drinkers, excessive weekly intake (> 210 g in men and > 140 g in women) for at least one year increased central BP and AIx in men only. Therefore, these studies seem to agree that excess alcohol intake is associated with arterial stiffness, though moderate intake may be associated with reduced stiffness. Lifestyle decisions regarding nutrition can considerably impact arterial health. Furthermore, nutritional intervention studies have shown improvements in arterial stiffness (255). Therefore, making nutritional changes at any point in life should be
advocated for to improve arterial health. Specific nutritional interventions will be discussed below.

Other Risk Factors

Some other conditions have been associated with arterial stiffness, including various disease states and compromised functionality of different systems of the body. Certain disease conditions, such as type 1 and type 2 diabetes, dyslipidemia, heart failure, end-stage renal disease, and atherosclerosis have been associated with increased arterial stiffness (40). Also, elevated fasting glucose, higher HR, and lipid disorders are specifically associated with a stiffer aorta (215). Considerations for these various disease states and conditions is beyond the scope of this review.

Assessing Arterial Stiffness

Pulse Wave Velocity

Pulse wave velocity (PWV), a direct measure of arterial stiffness, is considered the gold standard for measuring arterial stiffness, and it is both valid and reliable (186). PWV serves as a predictive measure for both cardiovascular events and overall mortality (356). Knowing the speed of the pulse wave through different parts of the body gives indication of the health of the arteries. PWV is a simple calculation that looks at the transit time of the pulse wave through the body over the length the pulse wave is traveling. There are various ways to measure PWV, but the measure of interest for this review will be that which is taken by way of applanation tonometry.

To measure PWV, Doppler probes (or tonometers), are generally placed on two different arterial locations of the body. For example, the gold standard for measuring
central PWV utilizes the carotid and femoral arteries and gives an indication of the stiffness of the aorta. Doppler probes allow for applanation tonometry, whereby applanation means, “to flatten”, and tonometry means, “measuring of pressure” (232). By placing pressure on the artery with the tonometer, the artery is flattened against the underlying bone (194). The arterial pressure is transmitted from the vessel to the tonometer, which contains a strain gauge that allows for a pressure waveform to be recorded, and digital images are recorded for analysis (232). Pulse wave transit time is accounted for by the difference in arrival time of the pulse wave from the proximal to the distal site and is determined by looking at the foot of each wave (foot-to-foot method). If the carotid-femoral pulse wave velocity (cfPWV) is used, probes placed on each of these sites will reflect differing waves at each site. Computer systems, such as that which accompanies Sphygmocor, have been developed to calculate the change in time from the foot of one wave to the foot of another. The distance between the two arterial sites is measured with a measuring tape by the investigator, using standardized anatomical markers on the body, and is recorded in the program. Sphygmocor uses this simple calculation to determine PWV: distance between two sites / Δ time between two waves. For an average middle-aged adult, a PWV of 4.0 m/s in the ascending aorta, 5.0 m/s in the abdominal aorta, and 8.0 m/s in the iliac arteries is to be expected. cfPWV is used as a global estimate of PWV through the entire aorta (56). Normal cfPWV measures have been reported as 6.0 m/s in individuals younger than 30 years of age and up to 10.0 m/s in individuals older than 70 years of age (271). This is a crucial measure for understanding the central arterial stiffness, as it takes into account the large elastic arteries, and most notably, the aorta. Other sites have been recognized as good sites for determining
peripheral arterial stiffness. While this method does serve as the gold standard for measuring arterial stiffness, it does have some limitations. The most notable limitation is that the length of the arteries, which are measured on skin surfaces and based on reference markers, may not be the most accurate measurements for the length of deep arteries. When abdominal obesity or large bust size is present, this can be even more of an issue (346). If the vessel length estimation is off, this could alter the PWV measure (274). Also, acute raises in BP or heart rate (HR) can affect the PWV readings (27, 184). However, taking the two readings in immediate succession can reduce changes in HR, but if changes in HR > 5 beats per minute occur, additional readings should be taken (186). While changes in BP have also been shown to affect PWV in normotensive subjects more than hypertensive subjects (244), no parameters are set for acceptable changes in BP between readings because a second BP reading is not taken at each site. It is generally recommended that repeated measures of PWV are taken at the same time each day to limit changes in PWV due to BP fluctuations associated with eating; measurements should be taken 3-4 hours after a meal (unless they are taken first thing in the morning), and subjects should not consume caffeine within three hours of testing to limit changes in BP and HR (346). Therefore, some of the limitations present with cfPWV can largely be controlled with careful consideration taken by the tester. Despite the limitations that exist, cfPWV is still considered the best option for determining arterial stiffness.

*Pulse Wave Analysis*

Pulse wave analysis (PWA) is often measured with the same equipment as, and measured in conjunction with, PWV. PWA is commonly taken at the radial pulse with an
applanation tonometer; while carotid measures are obtainable, they require a much higher level of expertise to extrapolate (64). After a waveform is measured at a peripheral site, a validated generalized transfer function is applied to this waveform to indirectly produce a central pressure waveform (296). From this conversion, a measure of central BP, central PP, and AIx is generated, along with various other indices that have been used to classify arterial stiffness and compliance. AIx is one of the most widely used measures that is collected during the PWA. AIx is a central hemodynamic index that is predictive of cardiovascular events and mortality (355). A measure of AIx is determined by the difference between the second and first systolic peaks expressed as a percentage of PP (186). This measure is often expressed with regard to HR of the individual (AIx_{75}), as AIx is highly influenced by HR. One limitation with AIx is that it may not be a suitable measure of cardiovascular risk in individuals over the age of 60 (214). AIx values decrease after age 60, whereas cardiovascular risk and cfPWV increase dramatically, suggesting that AIx may not be a sensitive marker for arterial stiffness after age 60 (205, 217). Another major limitation that exists with this technique is that the generalized transfer function has a range of error (232). This is due in part to assumptions that are made in calculating the generalized transfer function (186). Also, an assumption is made in determining the reflection site of the incident wave, as this site is theoretical, and this assumption has proven problematic in some instances (210). On the other hand, if AIx of the carotid artery is attainable, no transfer function is required; however, much training is required and these carotid measures are considerably more difficult to obtain (64).
Pulse Pressure

Pulse pressure (PP) is determined by subtracting diastolic blood pressure (DBP) from systolic blood pressure (SBP). As arteries become stiffer, PP has been used as a marker to indicate the rigidity of the walls of the arteries. PP is one of the easiest, most attainable, and cheapest ways to study arterial stiffness. To understand why PP increases with stiffening of the arteries, the speed at which cardiac pulse waves travel down an artery becomes very important. These arterial pressure waves are composed of two different waves: the incident wave initiated by the heart, which will travel away from the heart, and the reflected wave, which is the wave that is returned from the periphery (247). When considering a very distensible and compliant artery, cardiac pressure waves that travel down the artery travel slowly, and reflection of the pressure waves happens late, during diastole. On the other hand, a stiff artery causes cardiac pressure waves to travel quickly, triggering the reflected wave to merge with the original wave in systole, thus causing systolic pressure to increase and diastolic pressure to decrease. Therefore, PP will increase as the arteries become stiffer (235). PP is known to increase with age and serves as a good predictor of cardiovascular events in people over the age of 50 (118). Brachial PP is very practical, but it is most appropriately used as a surrogate marker in clinical settings if more sophisticated technology is unavailable (52). Central PP, as determined by the above PWA methods, is believed to be a better indicator of arterial stiffness and cardiovascular risk, as it measures PP at the aorta rather than the brachial artery (194). Thus, brachial PP serves as a surrogate marker of arterial stiffness, but central PP may be a better predictor of cardiovascular events when appropriate devices are available for measurement.
Ultrasound

Ultrasound can also be used to measure arterial compliance, a particular facet of arterial stiffness, though it is not used as frequently as the methods described above. Ultrasound measurements take into account both the distensibility and compliance of the arteries. However, only local measurements can be taken, and not all arteries are appropriate for such measurements. Due to the ability to only reach surface arteries with ultrasound equipment, only superficial arteries can be measured through ultrasound; namely, the brachial, femoral, and carotid arteries are commonly used. Diameter changes in the artery are determined by capturing images from ultrasound, and then these images are used in conjunction with a BP measurement to determine compliance (44). Compliance is defined as the change in volume over the change in pressure. Ultrasound equipment is able to provide more accurate measures of the length of the arteries than are provided with the external measures from a measuring tape, as described above with PWV. Limitations with this kind of equipment include: the expense of the ultrasound equipment, the resolution of the photos taken by the equipment, and the ability to determine volume changes based on the images taken (350). Additional concerns include: the ability of the operator to get meaningful images, reproducibility of the measurements, and lack of portability (194). Therefore, the limitations and concerns associated with this equipment may not make it the best option for determining arterial stiffness in clinics or laboratory settings.

Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) is another method for measuring local arterial stiffness, though it is also not used as widely as other methods. Like ultrasound, images
are captured of a certain artery, and then the images are used to determine distensibility and compliance of that artery. Measurements of diameter are, in turn, used to determine changes in volume over changes in pressure. Unlike ultrasound, MRI can be used to assess deep arteries, rather than simple surface measurements; thus, MRI studies have focused much attention on the aorta. MRI is advantageous in that, when compared to arterial length measurements that use a measuring tape, more accurate measurements of the artery can be determined. Instead of attaining measurements on outer surfaces of the body, MRI imaging allows for far more accurate measurements following the length of the artery (56). Nevertheless, MRI equipment is expensive and is often impractical for use in laboratory and clinical settings (194). Furthermore, other limitations make this technique somewhat less respected in practice, such as a need for: standardized software, standardization of techniques, well-determined sampling points, resolution of images, and a means to measure along vessels that are not straight (364). Though the potential for MRI equipment is clearly evident, the practical use of such equipment and need for validation limit its ability to be used more frequently at this time.

**Association Between Arterial Stiffness and Endothelial Dysfunction**

The association between endothelial function and arterial stiffness has not yet been fully elucidated, but it is believed that there is a strong association that exists between the two conditions. Mitchell describes the relationship like this: “these relations are likely to be bidirectional in that increased stiffness and excessive pressure pulsatility have been shown to impair endothelial function and the endothelium has been shown to modulate arterial properties” (214). He further suggests that there may be a detrimental feedback loop that exists between these two conditions, which would result in ongoing
decrements in both conditions over time even if only one condition is present initially. While this relationship has been explored, there is much to be discovered in the intricacies of how these two conditions function together to alter overall vascular health over time.

Defining Endothelial Function

The endothelium is the layer of tissue lining the inside of blood vessels, and it is the largest organ in the body. The location of the endothelium assists the vessels in responding to changes in the vasculature, such as BP, shear stress, and hormonal stimuli (96). In order to maintain homeostasis at a vascular level, the endothelium responds to such changes through relaxing and contracting factors (19). Some of the factors that are produced by the endothelium include NO, prostacyclin, endothelium-derived hyperpolarizing factors, and C-type natriuretic peptide (CNP) (96). Given this, the endothelium plays a critical role in the regulation of vascular tone, growth, inflammatory response, coagulation, and thrombocyte adhesion (183). Since the endothelium plays such an intricate role in all of these processes, imbalances at the endothelium leading to endothelial dysfunction can further lead to vascular exposure to vasoconstriction, leukocyte adherence, platelet activation, mitogenesis, pro-oxidation, thrombosis, impaired coagulation, vascular inflammation, and atherosclerosis (19). Therefore, proper functioning of the endothelium plays a critical role in vascular health.

Measuring Endothelial Function

There are a number of methods that have been developed to measure endothelial dysfunction, but the two most popular methods are ultrasound-measured flow-mediated dilation (FMD) and peripheral arterial tonometry (PAT), due to their non-invasive nature.
FMD is the technique used most often to measure endothelial function (173). In short, a sphygmomanometer is placed on the forearm or above the antecubital fossa of the participant, where it is inflated to occlude blood flow of the brachial artery (70). Following ischemia, deflating the cuff results in reactive hyperemia, or increased blood flow (365). Reactive hyperemia causes increased shear stress, which leads to endothelium-dependent vasodilation (265). The diameter of the brachial artery is measured via ultrasound both before testing and following deflation of the cuff. The degree of dilation produced is indicative of NO bioavailability, which is suggestive of proper functioning of the endothelium (158). FMD is advantageous in that it is reproducible, non-invasive, fast, and safe, but several problems exist as well; disadvantages include high technical skill necessary of operator, operator dependence, extreme cooperation of the participant, and poor resolution via ultrasound images (340).

Another method used for assessing endothelial function is PAT, as measured by the Endo-PAT 2000 device. This technique is newer and may have less technical difficulties than those associated with FMD (277). With this method, fingertip plethysmography is used to measure pulse volume amplitude and detect abnormalities in hyperemic response (200). Briefly, plethysmographic probes are placed on the fingertips of index fingers, where baseline measures of pulse wave are taken. A 5-minute occlusion with a sphygmomanometer is ensued and then released for post-occlusion pulse wave data collection. Post-occlusion pulse waves are compared to pre-occlusion baseline data and divided by measures of the control (non-occluded) arm to determine RHI. Advantages to this technique include its accessibility in clinical settings, reproducibility,
operator independence, and non-invasiveness; however, the values determined by PAT may not be as predictive for all populations as those determined by other methods (277).

Possible Associations Between Endothelial Dysfunction and Arterial Stiffness

While the relationship between endothelial dysfunction and arterial stiffness is not completely understood, some similarities exist in individuals presenting with both conditions. Arterial stiffness and endothelial dysfunction commonly co-exist in individuals (249). Though the two conditions are indicative of different facets of vascular health, cross-talk exists between them (19). Furthermore, measures of arterial stiffness are significantly correlated with endothelial dysfunction (236).

One commonality between arterial stiffness and endothelial dysfunction is NO. NO plays a key part in the vascular homeostatic role of the endothelium; with endothelial dysfunction, the endothelium loses the ability to generate endogenous NO (321). Furthermore, pharmacological drugs that are given to improve endothelial function in humans, such as statins and angiotensin-converting enzyme inhibitors, have also decreased arterial stiffness, which may be due to the effect on NO (19). Another possible association between endothelial dysfunction and arterial stiffness is oxidative stress, which is present in both conditions; however, the mechanisms by which arterial stiffness, endothelial function, and oxidative stress may be linked are not known (258). Further work needs to be done to advance the understanding of potential roles that NO and oxidative stress may have in both conditions and how all aspects may be linked.
Obesity and Endothelial Dysfunction

Obesity is clearly associated with endothelial dysfunction (304). Physiologically, adipose tissue can secrete hormones and pro-inflammatory cytokines, which may have detrimental effects on the endothelium (28). Endothelial dysfunction leads to the pathogenesis of cardiovascular and metabolic diseases that are associated with obesity (92). Endothelial dysfunction is an early marker for atherosclerosis and common to many CVDs (96). More specifically, endothelial dysfunction is a crucial component in the pathogenesis of atherosclerosis (47). Additionally, atherosclerosis and arterial stiffness often present together on an individual level (141). In instances of obesity, this may provide a link between endothelial dysfunction and arterial stiffness. Nevertheless, the exact relationship between obesity and endothelial dysfunction remains somewhat unclear.

Nutritional Interventions to Reduce Arterial Stiffness

Few nutritional intervention studies have been conducted to look specifically at reducing arterial stiffness in overweight and obese individuals. Three nutritional interventions have received attention in how they might impact arterial stiffness in overweight and obese individuals: omega-3, isoflavone metabolite, and conjugated-linoleic acid. In addition to the studies done on overweight and obese individuals, nutritional interventions have been studied in various other populations to modulate arterial stiffness (155, 156, 165, 167, 225, 237, 263, 306, 349, 374, 388). The mechanisms that underlie arterial stiffness associated with obesity have not been clearly elucidated, and it is unknown whether the mechanisms presenting with obesity are the same as mechanisms associated with increasing age or disease states. Therefore,
nutritional interventions that ameliorate the stiffness in one population may or may not translate to another population. The focus of this review will be the studies that have looked at nutritional interventions to improve arterial stiffness in overweight and obese individuals. However, some attention will also be given to research done on populations that were not overweight or obese.

**Omega-3**

Omega-3, a potent polyunsaturated fatty acid, has been considered in various clinical trials with regard to its potential effect on vascular health (174, 301). A study on the effects of omega-3 on the arterial compliance of overweight and obese individuals (BMI average = 31.7 kg/m²) showed promise for this population (305). Seventy-five overweight men and women were randomly placed in one of four groups: placebo (Sunola oil), low dose fish oil (520 mg docosahexaenoic acid (DHA) + 120 mg eicosapentaenoic acid (EPA) fish oil pills per day), medium dose fish oil (1040 mg DHA + 240 mg EPA fish oil pills per day), or high dose fish oil (1560 DHA + 360 mg EPA fish oil pills per day). Each group consumed capsules for 12 weeks, and capacitive arterial compliance of the large and small arteries was tested by use of a proprietary tonometer, HDI/Pulsewave CR-2000 Cardiovascular Profiling System (Hypertension Diagnostics, Inc., Eagan, MN). Briefly, a BP cuff was placed on the left arm, while the tonometer was used at the right radial artery, allowing BP and HR to be taken at the same time. The tonometer, placed on the radial artery, measured decay in diastolic pressure in the large arteries and decay in reflective waves of the small arteries; three measures were taken and averaged. They found that the compliance of the large arteries was increased only at the highest dose of the fish oil pill but not the compliance of the small arteries.
Since only this high dose fish oil pill (1560 DHA + 360 mg EPA per day) improved large arterial compliance, they suggest that high, ~5:1 DHA/EPA fish oil doses may be able to improve stiffness of the arteries due to obesity.

Similarly, comparable results were found in a group of hypertensive, overweight individuals (BMI >23 kg/m\(^2\)) from China. Fifty-two individuals were placed in a fish oil (3.0 g/day containing 540 mg EPA + 360 mg DHA) or placebo group (361). After eight weeks of intervention, the compliance of the large elastic arteries was measured similarly to the above measures (305), with a CVProfilor DO-2020 (Hypertension Diagnostics Inc., Eagan, MN). The compliance of the large elastic arteries, but not the small arteries, was improved in the fish oil group when compared to the placebo group. Interestingly, changes in BP were not seen in this study, suggesting that the fish oil had a direct effect on the compliance of the arteries independent of BP in this group of hypertensive subjects. In the presence of hypertension, a reduction in BP would be expected to reduce risk for CVD. Based on this study, even if fish oil is unable to reduce BP, it may still be able to protect against CVD by directly impacting the distensibility of the arteries, presumably through endothelial factors, as changes in inflammatory markers were not apparent in this study.

However, conflicting results were found in another study that measured the effects of omega-3 with or without an exercise intervention on cardiovascular risk factors, including large elastic artery compliance and endothelial function (140). This study considered overweight individuals (BMI >25 kg/m\(^2\)) exhibiting at least one cardiovascular risk factor (HTN, elevated plasma triacylglycerols, or elevated total cholesterol). Eighty-one men and women were randomly assigned to a placebo group.
(sunflower oil), placebo + exercise group (3 times/week of walking for 45 minutes at 75% of age-predicted HR maximum), fish oil group (6.0 grams tuna fish oil/day containing 260 mg DHA and 60 mg EPA), or fish oil + exercise group. After a 12 week intervention, large elastic artery compliance, as measured by HDI/Pulsewave CR-2000 Cardiovascular Profiler (Hypertension Diagnostics Inc., Eagan, MN) with the above methods (305), was not significantly changed in any of the four groups. However, fish oil was shown to improve endothelial function significantly over the placebo, as measured by FMD. Therefore, fish oil was still shown to have an effect on overall vascular health, though it did not seem to improve arterial compliance.

In the absence of obesity, EPA and DHA still show potential to improve arterial stiffness. In diabetics, arterial compliance was enhanced after six weeks of fish oil supplementation (1800 mg EPA + 360 mg DHA/day) (208), and arterial stiffness was reduced after a 105 week supplementation period with EPA only (1800mg/day) (212). After a 52 week supplementation period with EPA (1800 mg/day), individuals with dyslipidemia did not have an increase in arterial stiffness, as determined by PWV, but individuals treated with a placebo did (338). This suggests that omega-3 may also be able to attenuate increases in arterial stiffness that occur with time when compared to individuals consuming placebo. Furthermore, 12 weeks of EPA (1800 mg/day) supplementation in individuals with metabolic syndrome decreased PWV when compared to a placebo (283). In these studies, the populations of interest were neither obese nor overweight; however, diabetes, metabolic syndrome, and dyslipidemia are highly associated with obesity and arterial stiffness, which might suggest that these findings could translate to an obese population. Of note, two studies have looked at the
effects of omega-3 supplementation on healthy populations. Omega-3 supplementation did not have an effect on either endothelial function or arterial stiffness markers of healthy (mean BMI: 24.0) middle-aged men and women acutely (101) or endothelial function after 12 weeks of supplementation (334). While the benefits of omega-3 on vascular health may be present in other populations outside of obese populations, a healthy population does not show vascular benefit after supplementation.

These studies related to omega-3, EPA, and DHA supplementation suggest potential mechanisms for their effects on arterial health. Previously, reports have suggested that EPA can alter the arachidonic acid system (43), which can induce vasodilation and improve endothelial function (338). This vasodilatory response may also confer advantageous modifications on a structural or functional level to reduce arterial stiffness (338). A favorable modification on the functional components that determine arterial elasticity was reported following supplementation with fish oil (361). Fish oil is believed to have directly affected vasoactivity through the endothelium or smooth muscle cells (208). Furthermore, the reduction of atherogenic cytokines from EPA could potentially inhibit fibrosis of the arterial wall (3, 338). EPA can modulate oxidative lipoproteins generated by arterial inflammation, and improvements in oxidative lipoproteins correlate with improvements in arterial stiffness (283). Currently, it is unknown whether the mechanisms that improve parameters of vascular health are the same between the varied groups presented in these studies.

Based on the evidence of these randomized control trials, fish oil supplementation may be an effective intervention to reduce arterial stiffness. Strong support exists for fish oil supplementation to improve arterial stiffness in overweight individuals, with or
without hypertension (305, 361). Furthermore, fish oil supplementation might benefit individuals with diabetes (208, 212), metabolic syndrome (283), or dyslipidemia (338). Sample sizes were adequate to detect significance, and the duration of the studies ranged from 6-105 weeks of supplementation. The lowest dose that showed an effect on arterial stiffness was 540mg EPA + 360 DHA per day. However, only one study looked at the effect of fish oil on non-invasive gold standard, cfPWV (101). In this study of healthy individuals, omega-3 did not improve cfPWV. Therefore, further studies should look more specifically at the effect of fish oil on cfPWV.

*Isoflavone Metabolite*

One study has looked at the effect of isoflavone metabolite, found commonly in soy products, on arterial stiffness associated with obesity (233). Twenty-five overweight and obese men and women (mean BMI = 30.3 kg/m²) were recruited and underwent a five week intervention period consuming one gram per day of an isoflavone metabolite, *trans*-tetrahydrodaidzein, or a placebo, then completed a one week washout period and crossed over to the alternate treatment. When participants were on the isoflavone metabolite treatment, a 1.1 m/sec reduction in PWV, representing a 10% improvement in arterial stiffness after supplementation, was seen (8.80 m/s with *trans*-tetrahydrodaidzein v. 9.90 m/s with the placebo; p < 0.05). Interestingly, the treatment also caused a reduction in SBP, but changes in SBP and changes in PWV were not significantly correlated. Therefore, the reduction in arterial stiffness may be occurring separately from the reduction in SBP.

Other reports that did not target overweight or obese populations have also shown promise for isoflavones improving vascular health through both arterial stiffness and
endothelial function. Possible benefits of isoflavones on the vascular health of menopausal and perimenopausal women were considered in one study (234). They found that 5-10 weeks of supplementation with 80 mg of soy isoflavones was sufficient to improve systemic arterial compliance of these women. Additionally, another group conducted studies to look at the effects of isoflavones in men and PM women. In an initial study by this group, soy isoflavones improved peripheral PWV on an individual basis, but endothelial function was significantly worse in males after supplementing with isoflavones (330). However, further studies conducted in similar populations did not show that same negative effect on endothelial function in men; conversely, they found a reduction in central PWV without an effect on BP in the men and PM women (331). Moreover, eight weeks of soy supplementation in PM women taking tibolone for hormonal imbalances had no effect on arterial stiffness or endothelial function as a whole (339). Nevertheless, the same study found that the ability of PM women to produce equol, a gut bacterial metabolite of the isoflavone daidzein, had an overall effect on vascular health: women who were considered high equol producers had significantly lower arterial stiffness and endothelial dysfunction than the women who were not high equol producers. Nevertheless, these studies were conducted primarily in PM women, who are known to have significant hormonal changes that are specific to that population. Therefore, these results could be specific to PM women, or they could potentially be representative of responsiveness of arterial stiffness to isoflavones as a whole. Without further studies, it is difficult to speculate how isoflavones may impact arterial stiffness in the general population. However, there is reason to believe that isoflavones may have great potential to affect arterial stiffness.
The effects of the isoflavone metabolite appear to occur through functional rather than structural changes in the arteries because of the ability of isoflavone to reduce arterial stiffness after a short duration (233, 331). Isoflavones may have direct effects on the vascular tone of the arterial wall (331), as they have been previously reported to inhibit endothelium independent vasoconstriction induced by norepinephrine and stimulate vasodilation (66). Also, in an animal model, an isoflavone metabolite reduced reactive oxygen species, suggesting a potential role of the antioxidant capacity of isoflavone to reduce the stiffness of the arteries (162). Therefore, isoflavones are believed to directly affect the vasoactive properties of the arteries.

The current evidence from these randomized control trials suggests that isoflavone treatment may be effective at improving arterial stiffness in some populations. Isoflavone might be most promising for PM women (234, 330, 331), but one report did not show benefit of isoflavone on AIx in PM women (339). There is some evidence to support improvements in arterial compliance in overweight/obese men (233), healthy men (331), and menopausal and perimenopausal women (233). Supplementation periods lasted 5-12 weeks, and sample sizes were statistically adequate. Of the studies considering the impact of isoflavones on arterial stiffness, only one study has measured the effects on non-invasive gold standard, cfPWV. Thus, further studies should consider populations outside of PM women and measure cfPWV to understand the impact of isoflavones on aortic stiffness.

Conjugated Linoleic Acid

Improvements in arterial stiffness from conjugated linoleic acid have also been considered. The effect of conjugated linoleic acid in 401 overweight and obese (BMI >25
kg/m²) men and women, who were randomly assigned to a placebo or intervention group for a total of six months, was measured (309). The intervention group received 4.0 grams/day of conjugated linoleic acid. Though prior studies in mice showed benefit of the conjugated linoleic acid on atherogenesis (22, 219), no change was seen in central BP or PWV in humans. Therefore, while conjugated linoleic acid has been one of the few nutritional interventions applied to improve vascular health associated with obesity, the research in humans to date is not encouraging.

Evidence to support supplementation with conjugated linoleic acid is lacking based on this one randomized control trial considering its effect on arterial stiffness as measured by cfPWV (309). Because this study had an adequate sample size (401 subjects), was 24 weeks in duration, and measured non-invasive gold standard cfPWV, the rigor of the study design suggests strong support for a lack of change in aortic stiffness due to conjugated linoleic acid in overweight but otherwise healthy individuals; other populations have yet to be studied.

Other Interventions

Various other studies have been conducted to look at arterial stiffness in populations that were not obese or overweight, including studies on fermented milk. Fermented milk has shown some possible benefit in hypertensive subjects. After 10 weeks of supplementation with fermented milk, the ambulatory arterial stiffness index of a hypertensive population was decreased (156). Also, following 24 weeks of fermented milk supplementation, hypertensive men, but not women, saw a reduction in arterial stiffness, measured by AIx, and a decrease in overall reflection time (155).
Vitamin supplements have not shown as much potential to affect arterial stiffness as had originally been projected. Many vitamin studies have focused on antioxidants, specifically Vitamins C and E, which could theoretically affect arterial stiffness through their ability to reduce oxidative stress. Acute supplementation with Vitamin C (2000 mg) did not have an effect on the arterial stiffness of healthy individuals (165). Also, Vitamin E supplementation did not show an effect on arterial stiffness or arterial compliance when given to smokers (500 IU/day) for 152 weeks (195), to PM women (400 IU/day) for 10 weeks (269), or to type 1 diabetics (1000 IU/day) for 12 weeks (306). Furthermore, Vitamins C and E in conjunction (1000 mg/day Vitamin C + 500 mg/day Vitamin E) did not produce any favorable change on arterial stiffness of untreated hypertensive subjects after eight weeks (263). Interestingly, ascorbic acid (500 mg/day) improved the arterial stiffness in type 2 diabetics after only four weeks of treatment (225). However, no change in arterial stiffness resulted in individuals with chronic heart failure after four weeks of supplementation with ascorbic acid (4000 mg/day) (237). Folic acid, a B vitamin that has also been shown to have antioxidant effects, has revealed slightly more promise than Vitamins C and E, but studies still do not show a reduction in aortic PWV. Longer supplementation periods with folic acid have not caused changes in arterial stiffness. Folic acid supplementation was given (15 mg/day) to chronic renal failure patients for 173 weeks, and no improvement in arterial stiffness was seen (388). Also, no improvements to arterial stiffness were observed in individuals who were identified as siblings of those with arterial disease at 104 weeks of folic acid supplementation, which was combined with another B vitamin, pyridoxine (5 mg/day folic acid + 250 mg/day pyridoxine) (349). A shorter supplementation period with folic acid (400 µg/day) of 16
weeks was sufficient enough to reduce peripheral arterial stiffness, as indicated by brachial-knee PWV, in individuals who were diagnosed with peripheral arterial disease, but it did not affect central arterial stiffness (167). In men with normal or slightly elevated BP (SBP < 145 mmHg and DBP < 90 mmHg), three weeks of folic acid supplementation (5 mg/day) was sufficient to increase systemic arterial compliance, but it did not reduce PWV (374). Therefore, support for using vitamins as a treatment to improve arterial stiffness is sparse, but limited evidence exists to warrant further studies.

While there are many nutritional interventions that have yet to be explored, the various randomized controlled trials presented in this review provide insight into the potential of several nutritional interventions to impact arterial stiffness. Some evidence exists to suggest that fermented milk could improve arterial stiffness, though this evidence is limited to hypertensive subjects (155, 156). Although sample sizes were adequate in the two studies considering fermented milk, and the duration of the studies was sufficient (10 and 24 weeks), neither study considered the effects on cfPWV. Current evidence does not support improvements in arterial stiffness through antioxidant intervention from Vitamins C and E (165, 195, 269, 306). There is minimal evidence to support improvements in arterial stiffness after four weeks of ascorbic acid treatment in type 2 diabetics (225), but further work should measure cfPWV and consider other populations. Some evidence has suggested that folic acid might improve arterial stiffness when given for supplementation periods of three weeks (374) or 16 weeks (167), but longer supplementation periods of 104 (349) and 173 weeks (388) have not been effective. Folic acid treatment may only be effective for men who are pre-hypertensive or have normal BP (374) or individuals with peripheral arterial disease (167). The majority
of studies conducted on folic acid have not considered cfPWV, but one study that did measure cfPWV did not see improvements after 3 weeks of supplementation (15). The studies that have considered fermented milk and vitamins have had adequate sample sizes. Therefore, some evidence exists to suggest fermented milk or folic acid as a treatment, minimal evidence exists to suggest ascorbic acid as a treatment, and evidence does not support supplementation with Vitamins C and E for improvements in arterial stiffness.

**Curcumin**

Several studies have pointed to curcumin as a possible intervention for improving arterial stiffness, though none of these studies has focused on obese humans. Studies need to be conducted to understand the specific effect of curcumin on arterial stiffness due to obesity in a human population. To date, five main studies have looked specifically at curcumin as a possible intervention to improve vascular function. Of those, two studies were conducted in rodents, and three studies were conducted in humans. These studies and the possible role of curcumin in cardiovascular health, as well as overall health, will be discussed here.

*What is Curcumin?*

Curcumin is a naturally occurring polyphenol and the active constituent found in the spice turmeric, which is commonly found in Eastern countries, such as India and China. Within turmeric, the content of curcumin is relatively small: 3-5% (17). Though not as commonly found in the United States, it can be found in such foods as mustard and pickled radish, along with different prepared curry sauces from a grocery store or restaurant (299). Historically, the countries of Iran, China, and India have used turmeric
as an herbal remedy to treat sickness and disease (16). Though the initiation of curcumin as a nutritional intervention to help with various disease states or medical ailments may not have originally been based on research, many studies have since looked at this powerful nutritional component and found that it has beneficial effects throughout the body.

Promise in Affecting Arterial Stiffness

A study conducted in male C57BL/6 mice showed that curcumin has potential to reduce large elastic artery stiffness and attenuate endothelial dysfunction in aged mice (113). In this study, young (4-6 months) and old (26-28 months) mice were given a curcumin-supplemented chow (0.2% curcumin) or regular chow as a control for four weeks, corresponding with doses in humans of 0.23 g/kg day in the old group and 0.32 g/kg day in the young group. The mice were divided accordingly: five young control mice, seven young curcumin mice, 10 old control mice, and 10 old curcumin mice. PWV was measured with Doppler probes in mice to obtain stiffness values for the aorta, and endothelial function was measured by looking at dose-response relationships after the mice were given acetylcholine (ACh), a potent vasodilator. They found curcumin had no effect on young mice, but it reduced aortic stiffness to levels that were not significantly different from young controls when given to aged mice. Furthermore, endothelial dysfunction was reduced in aged curcumin treated mice to a level similar to young controls, but there was no effect on young mice. Also, oxidative stress, as determined by superoxide production and nitrotyrosine, was found to be reduced to levels lower than young controls in old mice treated with curcumin. Decreased superoxide, in addition to decreased p67 subunit expression, suggested that superoxide production and dismutation
result from curcumin supplementation, affecting both pro- and antioxidant enzyme expression. Nitrotyrosine is a by-product of the nitration of tyrosine due to reactive nitrogen species, such as peroxynitrite (149). Curcumin can reduce peroxynitrite formation, which further reduces the degree of tyrosine nitrination (335). Additionally, curcumin is also believed to directly scavenge free radicals (61, 113, 267). MnSOD, an antioxidant enzyme, levels were ameliorated in old curcumin mice to levels similar to young controls, though there was no effect on young mice (113). Therefore, curcumin may both directly reduce oxidative stress and indirectly reduce oxidative stress by improving antioxidant capacity of the body. Structurally, curcumin normalized collagen production in the old mice when compared to the old controls, while young mice saw no change in collagen production. Curcumin did not affect the elastin content of the aorta. AGEs were also normalized in old curcumin mice when compared to old controls, yet no effect was seen in young mice. Taken together, this study shows that curcumin was able to affect vascular health in mice, specifically by reducing aortic stiffness and decreasing endothelial dysfunction. The possible mechanisms for curcumin might be related to its antioxidant capacity, ability to reduce AGEs, and capacity to alter the structure of the vessel wall in mice.

Another study looked at the effect of tetrahydrocurcumin (THC), a metabolite of curcumin, on the vascular health of hypertensive rats (230). In this study, adult male Sprague-Dawley rats were induced with HTN through administration of L-NAME in their drinking water. Then, they were divided into three groups, receiving intragastrically administered treatment of: polyethylene glycol (control), 50 mg/kg THC per day, or 100 mg/kg THC per day for two weeks (corresponding with doses of THC in humans of 4.17
mg/kg day in the low dose group and 8.33 mg/kg day in the high dose group). Methods were as follows: BP was measured via tail cuff plethysmography, aortic elasticity and stiffness were measured with catheters inserted into the thoracic aorta to look at changes in volume of the aorta associated with induced changes in pressure, oxidative stress was measured by using an assay to assess vascular superoxide production in the carotid artery, and the antioxidant defense system was measured by an assay looking at glutathione (GSH) content in the blood of the rats. SBP was reduced with both doses of THC in these hypertensive rats when compared to control rats (Vehicle: 364.3 ± 23.5 µM, 50 mg/kg THC: 393.4 ± 25.8 µM*, 100 mg/kg THC: 449.6 ± 14.3 mmHg*; *p < 0.05). Thickness of the aorta, brought on by the induced HTN in these rats, was significantly reduced in the rats that were treated with the higher dose (100 mg/kg) but not the lower dose (50 mg/kg) of THC. Aortic stiffness, as determined by the elastic modulus from catheter readings, was lowered in the rats given the higher dose of THC but not the lower dose. Oxidative stress, as measured by superoxide production, was reduced in the hypertensive rats treated with both doses of THC: 18% less in the lower dose (counts per mg dry weight per minute: 184.6 ± 5.6 in 50 mg/kg THC v. 224.9 ± 10.3 vehicle) and 23% less in the higher dose (counts per mg dry weight per minute: 173.5 ± 9.7 in 100 mg/kg THC v. 224.9 ± 10.3 vehicle). The antioxidant defense system, as measured by an important antioxidant, GSH, was reduced in hypertensive rats but was significantly higher than control mice after being treated with both doses of THC (Vehicle: 364.3 ± 23.5 µM, 50 mg/kg THC: 393.4 ± 25.8 µM*, 100 mg/kg THC: 449.6 ± 14.3 mmHg*; *p < 0.05). To summarize, this study showed improvements in BP and aortic stiffness in hypertensive rats, possibly brought on by the antioxidative effect of the THC.
A pilot study was conducted to look at the effect of curcumin, alone and in conjunction with endurance exercise, on the central arterial hemodynamics of PM women (322). This was a randomized, double-blind, placebo-controlled study that assigned women to different groups, taking into account age and BP. The study included 45 sedentary women, randomized to each of the four groups of treatment lasting eight weeks: 11 placebo, 11 curcumin, 11 exercise + placebo, 12 exercise + curcumin. Curcumin was given as six 25 mg pills per day (150 mg total), and placebo was given as six similar looking starch pills at an equivalent dose. Exercise consisted of endurance training 3-6 days per week, initially for 25-30 minutes, 3-4 times per week but gradually increasing to 40-45 minutes, 4-5 days per week. The mode of exercise varied between supervised cycling 2-3 days per week and home-based walking. Measurements were taken to determine brachial and aortic BP as well as aortic stiffness from both PWV and AIx. Brachial SBP was decreased in the exercise + placebo group as well as the exercise + curcumin group (placebo: from 119 ± 3 to 117 ± 3 mmHg, curcumin: from 120 ± 3 to 117 ± 3 mmHg, exercise + placebo: from 117 ± 3 to 114 ± 4 mmHg*, exercise + curcumin: from 119 ± 2 to 114 ± 3 mmHg*; *p < 0.05; presented as SEM). Also, brachial DBP was reduced in the exercise + curcumin group following the eight week intervention (placebo: from 73 ± 3 to 71 ± 3 mmHg, curcumin: from 71 ± 2 to 70 ± 2 mmHg, exercise + placebo: from 71 ± 2 to 70 ± 2 mmHg, exercise + curcumin: from 72 ± 2 to 68 ± 2 mmHg*; *p < 0.05; presented as SEM). Additionally, the exercise + curcumin group was the only group that saw reductions in aortic SBP (placebo: from 114 ± 3 to 114 ± 3 mmHg, curcumin: from 112 ± 3 to 109 ± 4 mmHg, exercise + placebo: from 113 ± 2 to 111 ± 2 mmHg, exercise + curcumin: from 112 ± 2 to 107 ± 3 mmHg*; *p < 0.05;
presented as SEM) and DBP (placebo: from 73 ± 3 to 73 ± 3 mmHg, curcumin: from 72 ± 2 to 70 ± 2 mmHg, exercise + placebo: from 72 ± 2 to 70 ± 2 mmHg, exercise + curcumin: from 73 ± 2 to 69 ± 2 mmHg*; *p < 0.05; presented as SEM). No group showed differences in aortic AIx or aortic PWV. However, when AIx was corrected for HR (AIx$_{75}$), the exercise + curcumin group showed changes that were significantly different from baseline (placebo: from 25.2 ± 2.5% to 30.5 ± 2.1%, curcumin: from 26.0 ± 2.3% to 28.6 ± 1.9%, exercise + placebo: from 23.3 ± 1.9% to 23.8 ± 1.0%, exercise + curcumin: from 23.2 ± 1.3% to 17.4 ± 3.4%*; *p < 0.05; presented as SEM). They suggest that exercise and curcumin may be additive in their effects, showing greater results combined than either of the interventions alone.

A similar study done by the same laboratory examined PM women (9). In this study, 51 PM women were placed into one of four groups: 12 placebo, 12 curcumin, 13 curcumin + exercise, and 14 placebo + exercise. Like the above study, the intervention period for treatment was eight weeks, and participants were given six pills of either placebo or curcumin per day, amounting to 150 mg. The exercise intervention was the same as the previous study (322). However, in this study, different results were found as a response to the various interventions. Brachial SBPs (placebo: from 114 ± 4 to 114 ± 4 mmHg, curcumin: from 123 ± 5 to 119 ± 4 mmHg*, exercise + placebo: from 112 ± 3 to 108 ± 3 mmHg*, exercise + curcumin: from 118 ± 4 to 113 ± 4 mmHg*; *p < 0.05; presented as SEM) and carotid SBPs (placebo: from 103 ± 3 to 104 ± 3 mmHg, curcumin: from 112 ± 5 to 108 ± 4 mmHg*, exercise + placebo: from 103 ± 3 to 99 ± 3 mmHg*, exercise + curcumin: from 107 ± 4 to 102 ± 4 mmHg*; *p < 0.05; presented as SEM) were lower in the curcumin group, curcumin + exercise group, and the exercise +
placebo group after eight weeks of the intervention. In the curcumin + exercise group, brachial DBP was lower than initial values but not in any other group (placebo: from 71 ± 3 to 71 ± 3 mmHg, curcumin: from 72 ± 4 to 69 ± 3 mmHg, exercise + placebo: from 69 ± 2 to 68 ± 2 mmHg, exercise + curcumin: from 71 ± 3 to 67 ± 3 mmHg*; *p < 0.05; presented as SEM). Carotid arterial compliance was higher following eight weeks of intervention with curcumin, exercise + placebo, or exercise + curcumin, but the percentage of change was only significant in the exercise + curcumin group. The authors concluded that curcumin, as a treatment, gave similar benefits to central arterial compliance as those of aerobic exercise alone (10.1 ± 4.5% v. 10.0 ± 3.6%). Additionally, the combination of exercise and curcumin led to significantly greater increases in arterial compliance, suggesting that the combination of the two interventions could be additive.

A final study done by the same lab looked at the response of vascular endothelial function of PM women to curcumin (8). In this study, 32 PM women were placed into one of three groups: 10 placebo, 11 curcumin, and 12 exercise. The curcumin and exercise interventions were the same as those outlined in the previous studies and also lasted eight weeks (9, 322). After eight weeks, brachial SBP was lower in the curcumin and exercise groups than the placebo group (placebo: from 112 ± 12 to 113 ± 11 mmHg, curcumin: from 122 ± 17 to 116 ± 15 mmHg*, exercise: from 112 ± 10 to 107 ± 10 mmHg*; *p < 0.05). Endothelial function, as measured by FMD, was also lower in both the curcumin and exercise groups than the placebo (values not reported). They concluded that curcumin produced similar benefits on vascular endothelial function to that of aerobic exercise.
**Therapeutic Effects of Curcumin**

Curcumin shows great therapeutic promise and has been shown to improve conditions related to varying disease states (5, 11, 18, 41, 65, 161, 176, 190, 196). Most notably, curcumin has established anti-inflammatory properties (4, 11, 32, 62, 161). Because inflammation is a major component of many disease states, the ability of curcumin to impact this aspect may afford it great potential in ameliorating the conditions of many diseases. Curcumin’s effects on inflammation are due to its ability to inhibit NFκB signaling, which regulates various inflammatory pathways (333). Curcumin can modulate NFκB signaling by inhibiting the activation of NFκB and its translocation into the nucleus (126, 303). The ability to affect NFκB will specifically reduce downstream inflammatory cytokines TNF-α, IL-1β, and IL-6 in the aorta and adipocytes (122, 126). Furthermore, curcumin reduces macrophage infiltration, activation, and accumulation into adipose tissue and may thereby have a more direct influence on inflammatory mediators (362, 379).

Moreover, curcumin has documented powerful antioxidant activity (7). Oxidative stress is also a major component of various disease states, increasing the ability of curcumin to have beneficial effects in diseased individuals (241). Curcumin can both decrease oxidative stress and improve antioxidant activity within the body (103, 113, 169, 209, 227, 266). Oxidative stress is directly impacted by the ability of curcumin to scavenge free radicals (209). Additionally, curcumin can impact endogenous antioxidant systems: superoxide dismutase and glutathione peroxidase (24, 113). Gluthathione peroxidase and superoxide dismutase are considered two of the most important enzymes of the antioxidant defense system in the cell and reduce oxidative damage due to free
radicals within the cell (261). Therefore, curcumin can directly and indirectly influence oxidative stress.

The anti-carcinogenic effects of curcumin have been well-studied (18). Curcumin has been shown to inhibit the growth of tumors in the blood, brain, breast, gastrointestinal system, head, neck, liver, pancreas, colon, prostate, ovary, and skin (18). It is believed that curcumin has these potent effects based on anti-inflammatory, anti-oxidative, apoptotic, and anti-angiogenic effects in the body (41). The promise of curcumin in rodent models is present in studies that involve prevention against neurodegenerative diseases such as Alzheimer’s and Parkinson’s diseases (241). Curcumin may also have the ability to affect skin conditions, such as psoriasis (177), or even general wound healing (196). Furthermore, positive outcomes with curcumin in diabetic rodents have occurred primarily through its ability to reduce hyperglycemia (124) and hypercholesterolemia (50, 311). While evidence is not as well-established, curcumin has also shown promise in affecting the respiratory system, the urinary system, the musculoskeletal system, and the reproductive systems of both males and females (241). Few studies have even pointed to curcumin’s beneficial antimicrobial effects, enhancement of immunity, and gastrointestinal effects (10). Benefits of curcumin on the cardiovascular system will be discussed below. Undoubtedly, curcumin has been shown to have positive effects on several systems in the body to varying degrees.

Effects on Cardiovascular Disease

Very few studies have looked at the effects of curcumin on the cardiovascular health of humans, but a few have studied aspects of CVD. One study showed that curcumin increased serum HDL levels and decreased serum LDL levels of patients with
atherosclerosis after four weeks of treatment at 20 mg per day (50). Another study with a much shorter intervention period of seven days, yet a much higher dose of curcumin (500 mg), found: increases in serum HDL, decreases in total serum cholesterol, and decreases in serum lipid peroxides (311). A final study showed that curcumin reduced the proliferative activity associated with atherosclerosis in human peripheral blood mononuclear cells and vascular smooth muscle cells in vitro (148). Furthermore, rodent studies have shown that curcumin has been linked to: protecting the myocardium from ischemic insult (312), reducing cardiotoxicity (351), providing protective effects on the cardiovascular system when diabetes is present (103), showing protective effects against myocardial infarction (239), and providing preventative effects against cardiac heart failure (223). Furthermore, the anti-proliferative and anti-inflammatory nature of curcumin may contribute to curcumin’s ability to have a protective role in CVD.

Curcumin proved protective against endothelial dysfunction in porcine coronary arteries (267). Based on this evidence, there is reason to believe that curcumin could be a potent nutritional component for improving and protecting overall cardiovascular health.

Effects on Obesity

In addition to having specific effects on the above systems of the body, curcumin has been shown to have favorable outcomes on obesity. Based on curcumin’s anti-hyperlipidemic effect described above, curcumin is believed to possibly ameliorate fatty liver disease (11) and has been shown to present some benefit to fatty liver disease in hamsters (152). Also, insulin sensitivity, which is often found in obese individuals, can be improved through the anti-hyperglycemic effect of curcumin (11). Improvements in diabetes may be relevant for obese individuals, as obesity contributes to the development
of type 2 diabetes (252, 290). Inflammation plays a major role in obesity, so the ability to reduce inflammation through inflammatory mediators in obese individuals may be pivotal, especially as it relates to CVD and insulin resistance (11). Additionally, obese individuals may experience a considerable amount of oxidative stress, which has been shown to improve with curcumin (345), though not in an obese population specifically. Healthy subjects had increased postprandial plasma insulin levels as a result of curcumin (366). Therefore, curcumin could potentially protect obese individuals from diabetes through effects on oxidative stress and insulin (11). Furthermore, in a clinical trial of diabetics, curcumin improved endothelial function, reduced oxidative stress, and decreased inflammatory markers (343). One study even showed that curcumin was able to increase the basal metabolic rate and induce weight loss in high-fat-fed mice (362). Weight loss has been shown to improve arterial stiffness in overweight and obese individuals (68). Many of the studies suggesting favorable effects of curcumin on obesity have been conducted in mice (152, 248, 362), and clinical studies should consider whether these same findings would translate into human studies. Therefore, curcumin could increase function of the cardiovascular systems of overweight and obese individuals, but there is a clear need for clinical trials to examine the effects of curcumin on obesity.

Absorption of Curcumin

While curcumin has favorable therapeutic effects, curcumin has poor bioavailability in humans (241). Reduced bioavailability is believed to be attributed to poor absorption, rapid metabolism, and rapid elimination of curcumin (4). Therefore, methods to increase the bioavailability of curcumin in humans are necessary.
Fenugreek to Increase Absorption of Curcumin

One formulation that has been shown to increase the absorption of curcumin is the soluble fiber from fenugreek when infused with curcumin. Fenugreek is a naturally-occurring annual plant from India and North Africa and has been identified as one of the oldest medicinal plants (31). Fenugreek seeds are composed of 50% fiber, found in the forms of 30% soluble fiber and 20% insoluble fiber (31). Fenugreek has been shown to lower fasting serum glucose levels in type 2 diabetics (129, 291) and type 1 diabetics (292). Moreover, fenugreek is known to lower LDL and triglyceride levels in hyperlipidemic adults (293) as well as total cholesterol, LDL, and triglyceride levels while increasing HDL levels in type 2 diabetics (294, 295). While curcumin and fenugreek as a combination have been studied to look at fenugreek’s ability to improve absorption, their effects on cardiovascular health have not been studied. Fenugreek is well-tolerated, showing little to no clinically significant adverse side effects, and should be considered a safe addition to curcumin (31, 175).

In one study, the effectiveness of fenugreek as an additive to increase absorption was tested in both rats and humans (175). To enhance absorption, the soluble fiber portion of fenugreek was extracted and curcumin was added to it. Rats were divided into two groups of eight, receiving curcumin in its natural state or curcumin impregnated into the soluble fiber of fenugreek. They found that the group consuming fenugreek with curcumin absorbed the curcumin 20 times better than the group consuming curcumin alone. Furthermore, in humans, two separate doses of curcumin were added to the fenugreek fiber: 250 mg and 1500 mg. Eight healthy males aged 25-45 years were given each of these two doses as well as one gram of unformulated curcumin at different time
points, separated by one week washout periods. Thus, they served as their own controls. Compared to the unformulated curcumin, the fenugreek + curcumin supplements were absorbed 12.9 times higher in the smaller dose and 15.8 times higher in the larger dose. Therefore, they concluded that fenugreek fiber was effective at enhancing absorption, but they also noted that there was a dose dependency. This suggests that fenugreek is a novel way to increase the absorption of curcumin and could prove to be a powerful substance to increase benefits seen through the consumption of curcumin. The authors report that fenugreek was able to improve absorption of curcumin through the improved hydrophobic-hydrophilic balance, which could allow for a prolonged release of colloidal curcumin throughout the digestive tract. Whereas unformulated curcumin is rapidly metabolized and degraded by enzymes of the upper gastrointestinal tract, the combination of fenugreek fiber and curcumin creates a stable colloid that swells rather than degrades in the presence of physiological pHs simulating stomach and colonic conditions. This could increase absorption, decrease the rate of metabolism, and decrease the rate of elimination characteristic of unformulated curcumin. Studies including this combination should be conducted to look at the possible benefits in humans when absorption of curcumin is increased.

**Conclusion**

CVDs are the leading cause of death worldwide. Large elastic artery stiffness serves to be a pronounced, independent predictor of CVD and serves as a better predictor than traditional markers, such as resting BP. Therefore, interest in improving overall vascular health, as monitored by measures of large elastic artery stiffness, should be of great concern. While many studies have focused on how to reduce arterial stiffness in
aged populations, few have looked at a specific population that is prevalent and still on
the rise – obese individuals. More studies need to be done to expose novel interventions
for reducing arterial stiffness in overweight and obese individuals. Some exercise and
nutritional interventions have been studied in the past few years, but curcumin requires
further study to elucidate potential effects on the cardiovascular system. Curcumin, a
powerful nutritional constituent in reducing inflammation, oxidative stress, and even
tumors, needs to be looked at for specific effects that it can have on overall
cardiovascular health. While bioavailability can be a problem in humans, adding
fenugreek to curcumin can enhance absorption dramatically. Studies to look at curcumin
with enhanced bioavailability from fenugreek to ameliorate overall cardiovascular health
in obese individuals could be of great benefit to this increasing population.
CHAPTER III

METHODS

Experimental design

The purpose of this study was to evaluate the effectiveness of curcumin to improve arterial health in young (18-35 years old), obese men. The experimental design included a 12-week longitudinal study with four time points: 0 weeks (pre-intervention), 4 weeks, 8 weeks, and 12 weeks (post-intervention). The timeline of the visits along with the associated measures that were assessed at each visit are shown in Figure 3-1. This study was a randomized placebo-controlled study, with the investigator responsible for taking measures and the subjects unaware of the groupings of the subjects. After volunteers were screened for eligibility, qualified subjects were matched based on BMI and randomized by a random numbers table into treatment or control groups. The intervention consisted of 12 weeks of curcumin (with added fenugreek, to enhance absorption). The control intervention consisted of 12 weeks of fenugreek, serving as the placebo. To monitor changes in cardiovascular health over time, arterial stiffness, endothelial function, BP, HR, and inflammatory cytokines were measured throughout the study. All testing was performed after a minimum of a four-hour fast including no caffeine.

Subjects

Subjects were recruited via local advertisement and word of mouth at the University of Kentucky. Healthy males aged 18-35 years with BMI ≥ 30 kg/m² were recruited for this study. The subjects were pre-screened via e-mail before their initial visit to ensure that BMI, age, and medical history were in conjunction with inclusion criteria.
Figure 3-1. Timeline of visits and associated measures

1st visit: 0 weeks
- Written consent
- Screening
- Dietary History Questionnaire
- Anthropometric Measures
- Body Composition Measures
- Blood Pressure and Heart Rate
- Arterial Stiffness Measures
- Endothelial Function Measures
- Blood Draw

2nd visit: 4 weeks
- Anthropometric Measures
- Body Composition Measures
- Blood Pressure and Heart Rate
- Arterial Stiffness Measures

3rd visit: 8 weeks
- Anthropometric Measures
- Body Composition Measures
- Blood Pressure and Heart Rate
- Arterial Stiffness Measures

4th visit: 12 weeks
- Dietary History Questionnaire
- Anthropometric Measures
- Body Composition Measures
- Blood Pressure and Heart Rate
- Arterial Stiffness Measures
- Endothelial Function Measures
- Blood Draw
The subjects were continuously enrolled in the study and matched based on BMI, then randomly assigned to the curcumin (intervention) group or the placebo (control) group. When a BMI-matched counterpart was identified, he was assigned to the group opposite of his pair. Prior to participation at their first visit, written consent was obtained from each participant as required by the University of Kentucky Institutional Review Board. Following consent, all subjects filled out a health history questionnaire, self-reporting medical history for determination of eligibility, and completed a resting 12-lead EKG. Alcohol dependence and abuse was determined by the CAGE Substance Abuse Screening Tool (99), with a total score of two or higher considered clinically significant.

- **Inclusion Criteria:** Male, 18-35 years of age, BMI ≥ 30 kg/m²
- **Exclusion Criteria:** HTN ( > 140 SBP or > 90 DBP at rest), medication, CVD, diabetes mellitus, pulmonary disease, previous myocardial infarction, renal disease, HIV, alcohol dependence or abuse; smoking

While age is the predominant predictor of arterial stiffness (229), age was not the focus of this study, as the impact of curcumin on vascular health with aging has been studied previously (8, 9, 113, 322). Previous work considering arterial stiffness in young individuals has selected individuals 18-35 years of age (35, 106, 131, 147, 151, 181); thus, these cut offs were also used for this study. Also, the impact of curcumin on the arterial stiffness in women has been studied (322), and previous work has suggested that sex differences might be specific to hormonal differences between women (6). The discrepancy between the sexes may also be amplified with obesity (242). To eliminate the discrepancy that exists in arterial stiffness between the sexes and elucidate whether curcumin will have an effect on arterial stiffness with obesity, men were exclusively
chosen for this study. Inclusion and exclusion criteria were specified to eliminate other risk factors for arterial stiffness so that the current work could focus on obesity-related stiffness. It is not known whether the mechanisms that underlie arterial stiffness due to different conditions may be the same. The effects of curcumin may not uniformly impact arterial stiffness due to these varying conditions, so consideration was given to select a relatively homogenous group of young obese men. Increased arterial stiffness has been associated with HTN, CVD, diabetes, MI, renal disease, HIV, smoking, and excessive alcohol intake (40, 119, 201, 207, 211, 285, 288, 300, 324). Evidence suggests that curcumin may interact with medication (21), but specific interactions with drugs are not currently known, so individuals on medication were not accepted into this study.

Upon enrollment in the study, participants were instructed not to change any major lifestyle factors, including physical activity and nutritional habits, as these factors have been shown to influence arterial health (245, 255, 329).

Intervention

Both groups underwent a 12-week intervention period with supplementation. Previous randomized control trials to assess the efficacy of nutritional interventions to improve arterial stiffness have used a 12-week intervention (261, 283, 305, 306, 330, 334, 341). Other studies have found that a period as short as a four week intervention was sufficient to produce favorable changes in cfPWV, but it is not known whether this might have been specific to the intervention (136, 337). In randomized controlled trials specifically considering curcumin for vascular health, eight weeks was sufficient to produce favorable changes in arterial compliance, SBP, and FMD (8, 9, 322). Therefore, a 12 week intervention was hypothesized to be sufficient to detect favorable changes in
arterial stiffness, and to identify the time course of change every four weeks, subjects reported at four and eight weeks of study duration. Due to known problems with the absorption of curcumin in humans (17), the intervention group received pills that included 1.0 gram of curcumin formulated with 60% soluble fiber from fenugreek seeds, obtained from Akay Flavours & Aromatics Ltd., to enhance the absorption of curcumin. This formulation has been shown to increase bioavailability up to 15.8 times when compared to administering curcumin alone in humans (175). The placebo group was given a pill that looked identical in size and shape to the intervention pill, formulated with the same amount of fenugreek as found in the intervention pill to account for any potential benefits on arterial stiffness from the fenugreek. Placebo supplements were also obtained from Akay Flavours & Aromatics Ltd. All pills were administered in a four-week block pill box with weeks and days indicated to enhance compliance in the study. At each visit, subjects received 32 pills of the intervention or placebo (1 pill/day + 4 extra pills), ensuring contact with our research team approximately every four weeks. Each subject was encouraged to take pills at the same time every day. Additionally, subjects were required to record pill consumption dates to further enhance compliance.

Procedures

Anthropometric and Body Composition Measures

Measurements for height, body mass, hip circumference, waist circumference, and body composition were collected for each participant at each of the four visits. Subjects wore light-weight clothing and no shoes for the height, weight, hip circumference, and waist circumference measures. Height was determined using a wall-mounted stadiometer (PORTROD; Health-o-meter, Alsip, IL) and was measured to the
nearest 0.1 centimeter. Subjects stood with head, back, and heels against the wall for the height measurement. Body mass was measured to the nearest 0.01 kilogram using a calibrated electronic scale (DI-10; DIGU, Rice Lake, WI). Hip and waist circumference measures were determined using a spring-loaded fiberglass anthropometric tape (Gulick Deluxe; Baseline Evaluation Instruments, White Plains, NY). Waist and hip circumferences were taken in triplicate, alternating between the two sites, and measured to the nearest 0.1 cm for each subject. Waist circumference was measured at the level of the umbilicus, and hip circumference was measured at the largest circumference around the gluteal muscles, as indicated by the NIH in the Multi-Ethnic Study of Atherosclerosis (MESA) (45, 67, 310). The coefficient of variation for repeated measures was 0.4% for waist circumference measures and 0.8% for hip circumference measures.

**Body Composition Measures**

Body composition measures were taken with a tetrapolar bioelectrical impedance analyzer. Height and weight measurements taken in the laboratory were used to input into the Bioelectrical Impedance Analyzer (Multi-frequency Quadscan 4000 Bioelectrical Impedance Analyzer; Bodystat Ltd., Douglas, United Kingdom). Subjects laid supine on a non-conducting surface, and electrodes were placed on the right side of the body at four locations: (1) bisecting the head of the ulna of the right wrist, (2) at the base of the metacarpal-phalangeal joint on the right hand, (3) bisecting the medial and lateral malleoli of the right foot, and (4) at the base of the metacarpal-phalangeal joint of the right foot. Multiple frequencies (5, 50, 100, and 200 KHz) were used to determine impedance, and manufacturer proprietary equations from the machine determined percent
fat, fat mass, and fat-free mass. When compared to total body DXA scans, the body fat equation has a reported validity of $r = 0.88$ (323).

**Pulse Wave Velocity**

Large elastic artery stiffness was assessed by measuring cfPWV, which is considered the non-invasive gold standard (186). In trained testers, the Sphygmocor system (Sphygmocor; AtCor Medical, Sydney, Australia) shows good reproducibility within days and between days, but it should be calculated for the specific tester (332). In this study, the repeatability of measurements for the trained tester was determined as a between day coefficient of variation for repeated measures of 7.03% and a within day coefficient of variation for repeated measures of 7.08%. Before pulse wave measures could be obtained, subjects were required to lay in a rested supine position for 10 minutes in a quiet thermoneutral room. To determine the best sites for measuring arterial pressure at the carotid and femoral pulse, the two sites were identified and marked for repeated measurements. Distance from the carotid to femoral arteries was measured with a flexible fiberglass measuring tape, and the value was recorded to the nearest millimeter. Blood pressure measurements, taken at the brachial artery, were recorded for the cfPWV measures; BP measurements, taken in a rested supine position, were also assessed as a secondary outcome. Then, the subject was prepared with a 3-lead EKG. If chest hair obstructed EKG placement, hair was shaved, and the subject was prepped with an alcohol pad to remove skin oils. A non-invasive tonometer was used at the carotid and femoral arteries to consecutively record pulse waves, while the integrated EKG recorded transit times. cfPWV was determined using a simple calculation by the computer system: distance traveled / transit time of the pulse wave. Distance traveled was taken directly
from the distance measured between the carotid and femoral sites described earlier, while
the transit time was calculated as the delay in onset of the pulse wave from the carotid to
femoral sites (the device uses a foot-to-foot method from the carotid to femoral sites), as
calculated from the images of the pulse wave and EKG measurements. cfPWV
measurements were calculated automatically by the Sphygmocor system and presented in
meters per second. Three measures were taken and averaged to determine cfPWV. A
single, trained tester took all of the cfPWV measures for all subjects in the study.

*Pulse Wave Analysis*

The same Sphygmocor system (Sphygmocor; AtCor Medical, Sydney, Australia)
was used for pulse wave analysis (PWA). The tonometer was placed at the radial artery
and the signal that was produced was used in conjunction with the BP measurement in a
validated transfer function (63) to determine central BP and Alx.

*Endothelial Health*

To measure endothelial health, RHI as produced by the Endo-PAT 2000 (Itamar
Medical, Ltd., Caesarea, Israel) was determined, and all procedures were in accordance
with the manufacturer’s specifications (150). Endo-PAT 2000 has been validated as an
independent predictor of late CV adverse events (277). The device shows strong
repeatability for two PAT tests conducted on 20 healthy volunteers taken an average of
19.5 days apart (intra-class correlations for RHI = 0.74) (203). From this device, arterial
pulse waves of the peripheral arteries were used to determine endothelial function via
finger probe plethysmography. An additional resting BP measure was taken from each
participant with a standard BP cuff; BP measurements were taken from a supine position
with arms rested at the sides of the participant while in a relaxed position. Then, finger
probes were placed on the index finger on both hands of the individual, and individuals were instructed to remain still and relax. The finger cuffs of the Endo-PAT machine were inflated and five minutes of baseline data were collected from the individual after the probes were inflated. After baseline data were assessed, the BP cuff was inflated to 200 mmHg to occlude the brachial artery. Five minutes of data were collected from the finger probes during occlusion, after which the cuff was deflated. Following occlusion, an additional five minutes of post-occlusion data were collected to determine RHI. The Endo-PAT 2000 calculated RHI by determining the post- to pre-occlusion ratio of the test arm divided by the post- to pre-occlusion ratio of the control arm.

Dietary History Questionnaire

To monitor dietary changes throughout the study, a food frequency questionnaire (FFQ) known as the Dietary History Questionnaire (DHQ) (88) was given at the first and fourth visit, with a specified food database for the American population (84). The online questionnaire was administered at the time of the visit and was used in conjunction with Diet*Calc software for analysis (87). Questions provided by the DHQ were based on the one month period prior to assessment; therefore, the one month time period before the first and fourth visits were captured by the DHQ. The DHQ was chosen for its simplicity, ability to assess dietary changes over a four week period, accessibility, and validity (319, 336). Of the four versions available, DHQ-II based on dietary consumption within the past month, including questions about portion sizes, was utilized. The DHQ is freely available to researchers, clinicians, and teachers, and was developed by staff at the Risk Factor Assessment Branch (RFAB) of the National Cancer Institute (NCI) at the National Institutes of Health (NIH). The food database includes 134 food items and eight dietary
supplement questions based on national dietary data from the National Health and

Dietary intake assessment representing one month of dietary habits prior to the
visit was analyzed by the Diet*Calc software (87). Total HEI scores are based on
conformance to Dietary Guidelines for Americans (342) on a 100 point scale. Each
component of the HEI score is scored based on a 5-20 point scoring system, where higher
scores indicate higher conformance to dietary guidelines. Of the 12 categories composing
the total HEI score, six categories are on a 5-point scale: total fruit, whole fruit, total
vegetables, greens and beans, total protein food, and seafood and plant proteins. Five
categories are on a 10-point scale: whole grains, dairy, fatty acids, refined grains, and
sodium. One category is on a 20-point scale: empty calories.

**Blood Sampling**

Blood draws amounting to approximately 40 mL were taken from the antecubital
vein by trained staff at the University of Kentucky’s Center for Clinical and Translational
Sciences (CCTS). Two blood draws were taken per subject, with whole blood samples
collected at the first and fourth visits. Samples were centrifuged, processed, and frozen at
-80°C in aliquots of serum and plasma by nurses in the CCTS until all samples were
collected for all subjects. Extracted plasma was assessed by a lab technician in the CCTS
after all samples were collected using the V-PLEX Proinflammatory Panel 1 (human) Kit
by Meso Scale, a multi-spot assay system, with EDTA. Samples were analyzed for
several inflammatory cytokines from this pro-inflammatory panel, including IFN-γ, IL-
1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, and TNF-α. Briefly, this technique
employed a pre-coated plate with antibodies to capture the 10 inflammatory cytokines on specific sites. A sandwich immunoassay was used, whereby the sample and an antibody-containing solution were added to the plate. The capture antibodies on the plate were bound to the analyte of interest, and the analyte recruited detection antibodies. A buffer was added to the plate, and detection antibodies allowed for quantification of concentrations based on the intensity of the light that was emitted.

**Statistical Analysis**

To analyze the data presented in this study, we used SAS software, Version 9.4 of the SAS System Copyright © 2015 (SAS System for Windows; SAS Institute Inc., Cary, NC). A p-value of ≤ 0.05 was considered statistically significant. Descriptive characteristics of the subjects are presented as means and standard deviations. A linear regression model with mixed effects was employed to assess change scores between baseline and subsequent visits with the independent variables: treatment groups (2 levels: curcumin v. placebo) as a between subjects variable and time points (2 or 4 levels: pre v. post or 0, 4, 8, and 12 weeks) as a within subjects variable. Random effects were included to capture correlations among the observations within a pairing. A similar model was used to analyze the two levels of responsiveness (responders v. non-responders) in the curcumin-treated group, except that the random effects for pairing were no longer considered in the model. Baseline data were analyzed with paired samples t-tests to compare means between the intervention and placebo groups and independent samples t-tests for the responders and non-responders. Microsoft Excel was used to determine simple linear regression models for the primary and secondary outcomes at baseline. A
multiple regression analysis was employed using forward selection from the SAS REG procedure with SLE = 0.05 inclusion criteria for the primary outcome at baseline.
CHAPTER IV
RESULTS

Subjects

This study enrolled a total of 22 obese (BMI $\geq$ 30 kg/m$^2$) yet otherwise healthy young (18-35 year old) males. All 22 subjects completed the 12 week protocol, resulting in 100% retention in the study.

Baseline characteristics for body composition, arterial stiffness, RHI, and inflammatory cytokines of the curcumin and placebo groups can be found in Table 4-1. There were no differences in baseline subject characteristics between the curcumin and placebo groups, though concentrations of pro-inflammatory cytokine IL-2 and anti-inflammatory cytokine IL-13 in the plasma were trending ($p = 0.052$ and $p = 0.075$, respectively).

Compliance to supplementation was self-reported via pill log sheets every four weeks. Overall, compliance was 96.74% for both groups. There was no difference in compliance between the placebo and curcumin groups (98.02% v. 95.42%, respectively; $p = 0.251$). No adverse side effects from curcumin or placebo supplements were reported.
Table 4-1. Baseline characteristics of the curcumin and placebo groups

<table>
<thead>
<tr>
<th></th>
<th>Curcumin (n = 11)</th>
<th>Placebo (n = 11)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>25.91 ± 4.46</td>
<td>26.64 ± 4.06</td>
<td>0.648</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>178.26 ± 6.50</td>
<td>175.60 ± 5.15</td>
<td>0.310</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>106.13 ± 15.81</td>
<td>102.34 ± 1.60</td>
<td>0.196</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>33.29 ± 3.69</td>
<td>33.18 ± 3.38</td>
<td>0.644</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>114.47 ± 8.41</td>
<td>112.80 ± 5.87</td>
<td>0.577</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>108.93 ± 11.99</td>
<td>106.27 ± 10.37</td>
<td>0.187</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>27.22 ± 4.90</td>
<td>27.18 ± 5.46</td>
<td>0.972</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>29.46 ± 9.80</td>
<td>28.32 ± 9.22</td>
<td>0.457</td>
</tr>
<tr>
<td>Fat-free mass (kg)</td>
<td>76.66 ± 7.28</td>
<td>73.97 ± 4.07</td>
<td>0.140</td>
</tr>
<tr>
<td>Waist:hip ratio</td>
<td>0.95 ± 0.05</td>
<td>0.94 ± 0.06</td>
<td>0.442</td>
</tr>
<tr>
<td>Waist:height ratio</td>
<td>0.61 ± 0.07</td>
<td>0.61 ± 0.06</td>
<td>0.711</td>
</tr>
<tr>
<td>Brachial SBP (mmHg)</td>
<td>123.64 ± 8.48</td>
<td>124.91 ± 8.96</td>
<td>0.596</td>
</tr>
<tr>
<td>Brachial DBP (mmHg)</td>
<td>77.09 ± 9.01</td>
<td>81.82 ± 5.40</td>
<td>0.191</td>
</tr>
<tr>
<td>Brachial PP (mmHg)</td>
<td>46.55 ± 10.20</td>
<td>43.09 ± 6.41</td>
<td>0.797</td>
</tr>
<tr>
<td>Brachial MAP (mmHg)</td>
<td>92.61 ± 7.41</td>
<td>96.18 ± 6.09</td>
<td>0.221</td>
</tr>
<tr>
<td>Central SBP (mmHg)</td>
<td>105.64 ± 7.10</td>
<td>109.18 ± 6.10</td>
<td>0.197</td>
</tr>
<tr>
<td>Central DBP (mmHg)</td>
<td>77.64 ± 8.99</td>
<td>82.18 ± 5.62</td>
<td>0.201</td>
</tr>
<tr>
<td>Central PP (mmHg)</td>
<td>28.00 ± 6.18</td>
<td>27.00 ± 4.86</td>
<td>0.693</td>
</tr>
<tr>
<td>Central MAP (mmHg)</td>
<td>86.97 ± 7.89</td>
<td>91.18 ± 5.31</td>
<td>0.172</td>
</tr>
<tr>
<td>Aortic AIx (%)</td>
<td>-0.64 ± 11.91</td>
<td>7.55 ± 10.14</td>
<td>0.141</td>
</tr>
<tr>
<td>Aortic AIx$_{75}$ (%)</td>
<td>-9.45 ± 13.54</td>
<td>-5.00 ± 10.54</td>
<td>0.438</td>
</tr>
<tr>
<td>Resting HR (bpm)</td>
<td>57.90 ± 6.79</td>
<td>56.64 ± 4.82</td>
<td>0.757</td>
</tr>
<tr>
<td>cfPWV (m/s)</td>
<td>6.27 ± 0.85</td>
<td>6.31 ± 0.99</td>
<td>0.369</td>
</tr>
<tr>
<td>Reactive Hyperemia Index</td>
<td>2.02 ± 0.50</td>
<td>2.08 ± 0.53</td>
<td>0.829</td>
</tr>
<tr>
<td>IFN-γ (pg/mL)</td>
<td>2.94 ± 0.94</td>
<td>4.51 ± 0.66</td>
<td>0.144</td>
</tr>
<tr>
<td>IL-2 (pg/mL)</td>
<td>0.17 ± 0.13</td>
<td>0.45 ± 0.42</td>
<td>0.052</td>
</tr>
<tr>
<td>IL-4 (pg/mL)</td>
<td>0.07 ± 0.05</td>
<td>0.12 ± 0.10</td>
<td>0.301</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>0.77 ± 0.33</td>
<td>0.62 ± 0.27</td>
<td>0.279</td>
</tr>
<tr>
<td>IL-8 (pg/mL)</td>
<td>3.71 ± 0.72</td>
<td>3.48 ± 0.72</td>
<td>0.631</td>
</tr>
<tr>
<td>IL-10 (pg/mL)</td>
<td>0.29 ± 0.11</td>
<td>0.45 ± 0.32</td>
<td>0.217</td>
</tr>
<tr>
<td>IL-12 p70 (pg/mL)</td>
<td>0.32 ± 0.22</td>
<td>0.50 ± 0.38</td>
<td>0.359</td>
</tr>
<tr>
<td>IL-13 (pg/mL)</td>
<td>3.92 ± 3.50</td>
<td>9.00 ± 3.37</td>
<td>0.075</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>1.76 ± 0.49</td>
<td>1.71 ± 0.45</td>
<td>0.711</td>
</tr>
</tbody>
</table>

*a n = 9; b n = 10; n.d. = not detectable; *p < 0.05, between groups. Values are means ± SD. SBP = Systolic Blood Pressure; DBP = Diastolic Blood Pressure; PP = Pulse Pressure; MAP = Mean Arterial Pressure; HR = Heart Rate; cfPWV = carotid-femoral Pulse Wave Velocity; AIx = Augmentation Index; AIx$_{75}$ = Augmentation Index Corrected for Heart Rate of 75; IFN = Interferon; IL = Interleukin; TNF = Tumor Necrosis Factor
Primary Variable of Interest

Pulse Wave Velocity

There was no difference in cfPWV between groups at baseline (Table 4-1). The overall average cfPWV measures for subjects in this study was 6.29 ± 0.88 m/s at baseline, and the group averages were 6.27 ± 0.85 m/s in the curcumin group compared to 6.31 ± 0.99 m/s in the placebo group at baseline (p = 0.369, between groups comparison). Changes throughout the 12 week intervention in cfPWV can be seen in Figure 4-1. Differences after the 12 week intervention were not seen in the curcumin compared to placebo groups in cfPWV (p = 0.428, interaction of group by time).
Figure 4-1. Changes in cfPWV in curcumin and placebo groups throughout the intervention

Means ± SEM
**Responders and non-responders to the curcumin treatment**

Further exploration of the responsiveness of individuals to the curcumin treatment revealed that some individuals responded to the curcumin treatment, while others did not. Curcumin-supplemented men were grouped into “responders” and “non-responders” based on their course of response to curcumin treatment. Responders were defined as those individuals who saw reductions in cfPWV (n = 6), while non-responders were those that did not (n = 5). Characteristics of responders and non-responders to the curcumin treatment can be found in Table 4-2. It was found that individuals with higher cfPWV measures at baseline responded to the treatment whereas individuals with lower cfPWV measures at baseline did not respond to the treatment (6.81 ± 0.83 m/s v. 5.84 ± 0.41 m/s, p = 0.045). The differences between cfPWV measures at baseline in the responders and non-responders can be seen in Figure 4-2. Furthermore, differences were observed between the responders and non-responders in the waist:height ratio (0.58 ± 0.03 v. 0.65 ± 0.08, respectively; p = 0.035). Additionally, waist:hip ratio, waist circumference, and resting HR differences were trending toward significance (p = 0.067, p = 0.082, and p = 0.093, respectively), with all three measures lower in the responders than the non-responders. There were no differences in compliance to curcumin pill consumption between the responders and the non-responders (95.33 ± 0.08% v. 95.08 ± 0.04%, p = 0.950).

The responders saw a significant decrease in cfPWV (6.81 ± 0.83 m/s to 5.92 ± 0.36 m/s, p = 0.025, group by time interaction), and group comparisons between the responders and the non-responders to curcumin can be found in Table 4-3. There was a group by time interaction between the responders and non-responders (p = 0.025), and
the effect of time was significant in the responders \( (p = 0.048) \) but not in the non-responders \( (p = 0.282) \). Additionally, in Figure 4-3, the cfPWV measures from 0 to 12 weeks in the responders are depicted. Interestingly, the responders demonstrated increases in anti-inflammatory cytokine IL-13 \( (4.52 \pm 4.67 \text{ to } 4.83 \pm 4.71) \) in conjunction with decreases in cfPWV \( (p = 0.052, \text{ group by time interaction}) \). Table 4-4 shows a significant effect of time on responders \( (p = 0.018) \) but not non-responders \( (p = 0.198) \).
Table 4-2. Baseline characteristics of responders and non-responders to the curcumin intervention

<table>
<thead>
<tr>
<th></th>
<th>Responders (n = 6)</th>
<th>Non-responders (n = 5)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>25.17 ± 4.17</td>
<td>26.80 ± 5.12</td>
<td>0.670</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>179.25 ± 7.38</td>
<td>177.08 ± 5.87</td>
<td>0.169</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>102.72 ± 12.12</td>
<td>110.22 ± 20.08</td>
<td>0.592</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>31.87 ± 1.67</td>
<td>35.00 ± 4.88</td>
<td>0.162</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>111.89 ± 6.53</td>
<td>117.57 ± 10.07</td>
<td>0.261</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>103.63 ± 5.32</td>
<td>115.28 ± 15.21</td>
<td>0.082</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>25.60 ± 3.41</td>
<td>29.16 ± 6.07</td>
<td>0.115</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>26.52 ± 5.93</td>
<td>33.00 ± 12.94</td>
<td>0.273</td>
</tr>
<tr>
<td>Fat-free mass (kg)</td>
<td>76.20 ± 7.07</td>
<td>77.22 ± 8.32</td>
<td>0.808</td>
</tr>
<tr>
<td>Waist:hip ratio</td>
<td>0.93 ± 0.04</td>
<td>0.98 ± 0.05</td>
<td>0.067</td>
</tr>
<tr>
<td>Waist:height ratio</td>
<td>0.58 ± 0.03</td>
<td>0.65 ± 0.08</td>
<td>0.035*</td>
</tr>
<tr>
<td>Brachial SBP (mmHg)</td>
<td>121.00 ± 10.41</td>
<td>126.80 ± 4.60</td>
<td>0.308</td>
</tr>
<tr>
<td>Brachial DBP (mmHg)</td>
<td>76.33 ± 8.14</td>
<td>78.00 ± 10.86</td>
<td>0.474</td>
</tr>
<tr>
<td>Brachial PP (mmHg)</td>
<td>44.67 ± 11.57</td>
<td>48.80 ± 9.01</td>
<td>0.722</td>
</tr>
<tr>
<td>Brachial MAP (mmHg)</td>
<td>91.22 ± 7.11</td>
<td>94.27 ± 8.23</td>
<td>0.325</td>
</tr>
<tr>
<td>Central SBP (mmHg)</td>
<td>103.50 ± 6.72</td>
<td>108.20 ± 7.40</td>
<td>0.239</td>
</tr>
<tr>
<td>Central DBP (mmHg)</td>
<td>76.83 ± 8.33</td>
<td>78.60 ± 10.64</td>
<td>0.447</td>
</tr>
<tr>
<td>Central PP (mmHg)</td>
<td>26.67 ± 5.96</td>
<td>29.60 ± 6.73</td>
<td>0.723</td>
</tr>
<tr>
<td>Central MAP (mmHg)</td>
<td>85.72 ± 7.31</td>
<td>88.47 ± 9.15</td>
<td>0.333</td>
</tr>
<tr>
<td>Aortic AIx (%)</td>
<td>-2.67 ± 12.53</td>
<td>1.80 ± 12.03</td>
<td>0.560</td>
</tr>
<tr>
<td>Aortic AIx₇₅ (%)</td>
<td>-12.50 ± 14.52</td>
<td>-5.80 ± 12.81</td>
<td>0.384</td>
</tr>
<tr>
<td>Resting HR (bpm)</td>
<td>56.00 ± 5.48</td>
<td>59.80 ± 8.04</td>
<td>0.093</td>
</tr>
<tr>
<td>cfPWV (m/s)</td>
<td>6.81 ± 0.83</td>
<td>5.84 ± 0.41</td>
<td>0.045*</td>
</tr>
<tr>
<td>Reactive Hyperemia Index</td>
<td>2.11 ± 0.55</td>
<td>1.94 ± 0.49</td>
<td>0.204</td>
</tr>
<tr>
<td>IFN-γ (pg/mL)</td>
<td>3.00 ± 1.18 c</td>
<td>2.89 ± 0.76</td>
<td>0.670</td>
</tr>
<tr>
<td>IL-2 (pg/mL)</td>
<td>0.22 ± 0.17 c</td>
<td>0.11 ± 0.05</td>
<td>0.189</td>
</tr>
<tr>
<td>IL-4 (pg/mL)</td>
<td>0.08 ± 0.07 c</td>
<td>0.07 ± 0.02</td>
<td>0.570</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>0.60 ± 0.21 c</td>
<td>0.94 ± 0.35</td>
<td>0.317</td>
</tr>
<tr>
<td>IL-8 (pg/mL)</td>
<td>3.69 ± 0.69 c</td>
<td>3.72 ± 0.83</td>
<td>0.571</td>
</tr>
<tr>
<td>IL-10 (pg/mL)</td>
<td>0.31 ± 0.13 c</td>
<td>0.27 ± 0.09</td>
<td>0.428</td>
</tr>
<tr>
<td>IL-12 p70 (pg/mL)</td>
<td>0.38 ± 0.31 c</td>
<td>0.26 ± 0.03</td>
<td>0.434</td>
</tr>
<tr>
<td>IL-13 (pg/mL)</td>
<td>4.52 ± 4.68 c</td>
<td>3.31 ± 2.17</td>
<td>0.474</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>1.54 ± 0.18 c</td>
<td>1.97 ± 0.62</td>
<td>0.107</td>
</tr>
</tbody>
</table>

n = 5; *p < 0.05, between groups. Values are means ± SD. SBP = Systolic Blood Pressure; DBP = Diastolic Blood Pressure; PP = Pulse Pressure; MAP = Mean Arterial Pressure; HR = Heart Rate; cfPWV = carotid-femoral Pulse Wave Velocity; AIx = Augmentation Index; AIx₇₅ = Augmentation Index Corrected for Heart Rate of 75; IFN = Interferon; IL = Interleukin; TNF = Tumor Necrosis Factor
Figure 4-2. Baseline cfPWV of responders and non-responders to curcumin treatment

*p < 0.05, between groups; Means ± SEM. cfPWV = carotid-femoral Pulse Wave Velocity
<table>
<thead>
<tr>
<th></th>
<th>Visit 1</th>
<th>Visit 2</th>
<th>Visit 3</th>
<th>Visit 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Responders (n = 6)</td>
<td>6.81 ± 0.83</td>
<td>6.27 ± 0.34*</td>
<td>5.86 ± 0.49*</td>
<td>5.92 ± 0.36*</td>
</tr>
<tr>
<td>Non-responders (n = 5)</td>
<td>5.94 ± 0.41</td>
<td>6.59 ± 0.26</td>
<td>6.53 ± 0.43</td>
<td>6.75 ± 0.71</td>
</tr>
</tbody>
</table>

*p < 0.05, group by time interaction; †p < 0.05, effect of time. Values are means ± SD.
Figure 4-3. Changes in cfPWV throughout the intervention in curcumin responders (n = 6)

†p < 0.05, effect of time; Means ± SEM
Table 4-4. Changes in IL-13 pre and post intervention in curcumin responders and non-responders

<table>
<thead>
<tr>
<th></th>
<th>Visit 1</th>
<th>Visit 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Responders (n = 5)</td>
<td>4.52 ± 4.67</td>
<td>4.83 ± 4.71†</td>
</tr>
<tr>
<td>Non-responders (n = 5)</td>
<td>3.31 ± 2.17</td>
<td>2.70 ± 2.07</td>
</tr>
</tbody>
</table>

†p < 0.05, effect of time. Values are means ± SD.
Secondary Variables of Interest

Augmentation Index

Baseline differences in AIX and AIX$_{75}$ were not present between the curcumin and placebo groups, as can be seen in Table 4-1. Table 4-5 shows the change in AIX and AIX$_{75}$ from the beginning to the end of the study. After 12 weeks of supplementation, the interaction of group and time was significant for AIX (p = 0.034), indicating that the placebo treatment was more favorable on AIX than the intervention. The decrease in AIX in the placebo group compared to the curcumin group throughout the duration of the study can be seen in Figure 4-4. Nonetheless, this decrease was not apparent after adjusting the AIX for a heart rate of 75 (AIX$_{75}$). The lack of significant change between groups in AIX$_{75}$ are depicted in Figure 4-5.
Table 4-5. Effects of treatment on augmentation index

<table>
<thead>
<tr>
<th></th>
<th>Curcumin (n = 11)</th>
<th>Placebo (n = 11)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td><strong>Aortic Alx (%)</strong></td>
<td>-0.64 ± 11.91</td>
<td>3.82 ± 9.61</td>
<td>7.55 ± 10.14</td>
</tr>
<tr>
<td><strong>Aortic Alx75 (%)</strong></td>
<td>-9.45 ± 13.54</td>
<td>-4.18 ± 10.56</td>
<td>-5.00 ± 10.54</td>
</tr>
</tbody>
</table>

*p < 0.05, group by time interaction. Values are means ± SD. Alx = Augmentation Index; Alx75 = Augmentation Index Corrected for Heart Rate 75
Figure 4-4. Changes in AIx in curcumin and placebo groups throughout the intervention

* p < 0.05, group by time interaction; Means ± SEM

Figure 4-5. Changes in AIx_{75} in curcumin and placebo groups throughout the intervention

Means ± SEM
Reactive Hyperemia Index

There was no difference between the groups in baseline RHI measured via the Endo-PAT 2000, as shown in Table 4-1. Additionally, no group by time interaction occurred after the 12 week intervention (Table 4-6). RHI was unchanged throughout the study in both groups (Figure 4-6).

Inflammatory cytokines

Baseline inflammatory cytokine measures are shown in Table 4-1. There were no differences at baseline between the curcumin and placebo groups, although pro-inflammatory cytokine IL-2 and anti-inflammatory cytokine IL-13 were trending, demonstrating increased concentrations in the placebo group (p = 0.052 and p = 0.075, respectively). Changes in inflammatory cytokines throughout the study are shown in Table 4-6. There were no group by time interactions in the inflammatory markers after 12 weeks of intervention. However, a trending increase in anti-inflammatory cytokine IL-10 was found following 12 weeks of intervention with curcumin when compared to the placebo (p = 0.071). Figure 4-7 illustrates changes in IL-10 from the beginning to the end of the study in both groups.
<table>
<thead>
<tr>
<th></th>
<th>Curcumin (n = 10)</th>
<th>Placebo (n = 9)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactive Hyperemia Index</td>
<td>2.02 ± 0.50</td>
<td>1.99 ± 0.72d</td>
<td>2.08 ± 0.53d</td>
</tr>
<tr>
<td>IFN-γ (pg/mL)</td>
<td>2.94 ± 0.94</td>
<td>2.76 ± 0.69</td>
<td>4.51 ± 0.66</td>
</tr>
<tr>
<td>IL-1β (pg/mL)</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>IL-2 (pg/mL)</td>
<td>0.17 ± 0.13</td>
<td>0.17 ± 0.14</td>
<td>0.45 ± 0.42</td>
</tr>
<tr>
<td>IL-4 (pg/mL)</td>
<td>0.07 ± 0.05</td>
<td>0.07 ± 0.06</td>
<td>0.12 ± 0.10</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>0.77 ± 0.33</td>
<td>0.70 ± 0.38</td>
<td>0.62 ± 0.27</td>
</tr>
<tr>
<td>IL-8 (pg/mL)</td>
<td>3.71 ± 0.72</td>
<td>3.56 ± 1.17</td>
<td>3.48 ± 0.72</td>
</tr>
<tr>
<td>IL-10 (pg/mL)</td>
<td>0.29 ± 0.11</td>
<td>0.34 ± 0.13</td>
<td>0.45 ± 0.32</td>
</tr>
<tr>
<td>IL-12 p70 (pg/mL)</td>
<td>0.32 ± 0.22</td>
<td>0.29 ± 0.23</td>
<td>0.50 ± 0.38</td>
</tr>
<tr>
<td>IL-13 (pg/mL)</td>
<td>3.92 ± 3.50</td>
<td>3.77 ± 3.61</td>
<td>9.00 ± 3.37</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>1.76 ± 0.49</td>
<td>1.89 ± 0.63</td>
<td>1.71 ± 0.45</td>
</tr>
</tbody>
</table>

*n = 11; n.d. = not detectable; *p < 0.05, group by time interaction. Values are means ± SD. IFN= Interferon; IL = Interleukin; TNF = Tumor Necrosis Factor; n/d = not detectable
Figure 4-6. Changes in RHI in curcumin and placebo groups pre and post intervention

Means ± SEM
Figure 4-7. Changes in anti-inflammatory cytokine IL-10 in curcumin and placebo groups pre and post intervention

p value is group by time interaction. Means ± SEM

\[ p = .072 \]
There were no baseline differences in brachial SBP, DBP, PP, MAP; central SBP, DBP, PP, MAP; or resting radial HR between groups. After 12 weeks of supplementation, a significant decrease in the curcumin group in comparison to the placebo group was found in brachial PP (p = 0.038, group by time interaction) but not central PP (p = 0.256, group by time interaction). Table 4-7 shows changes in BP and HR measures from the beginning to the end of the study. The significant decrease in brachial PP in the curcumin group can be seen in Figure 4-8, while the lack of significant change in central PP can be seen in Figure 4-9. No group by time interactions were seen in radial HR; brachial SBP, DBP, or MAP; or central SBP, DBP, or MAP following 12 weeks of supplementation with curcumin or placebo. However, there was a significant effect of time on Brachial SBP (p = 0.030), indicating improvements in both groups.
Table 4-7. Effects of treatment on BP and HR measures

<table>
<thead>
<tr>
<th></th>
<th>Curcumin (n = 11)</th>
<th></th>
<th>Placebo (n = 11)</th>
<th></th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
<td></td>
</tr>
<tr>
<td>Brachial SBP (mmHg)</td>
<td>123.64 ± 8.48</td>
<td>118.73 ± 8.64</td>
<td>124.91 ± 8.96</td>
<td>122.91 ± 8.07</td>
<td>0.731†</td>
</tr>
<tr>
<td>Brachial DBP (mmHg)</td>
<td>77.09 ± 9.01</td>
<td>78.73 ± 7.60</td>
<td>81.82 ± 5.40</td>
<td>79.64 ± 5.64</td>
<td>0.350</td>
</tr>
<tr>
<td>Brachial PP (mmHg)</td>
<td>46.55 ± 10.20</td>
<td>40.00 ± 9.42</td>
<td>43.09 ± 6.41</td>
<td>43.27 ± 6.34</td>
<td>0.038*</td>
</tr>
<tr>
<td>Brachial MAP (mmHg)</td>
<td>92.61 ± 7.41</td>
<td>92.06 ± 6.61</td>
<td>96.18 ± 6.09</td>
<td>94.06 ± 5.83</td>
<td>0.538</td>
</tr>
<tr>
<td>Central SBP (mmHg)</td>
<td>105.64 ± 7.10</td>
<td>104.73 ± 7.76</td>
<td>109.18 ± 6.10</td>
<td>106.55 ± 6.04</td>
<td>0.620</td>
</tr>
<tr>
<td>Central DBP (mmHg)</td>
<td>77.64 ± 8.99</td>
<td>79.36 ± 8.20</td>
<td>82.18 ± 5.62</td>
<td>79.91 ± 5.47</td>
<td>0.383</td>
</tr>
<tr>
<td>Central PP (mmHg)</td>
<td>28.00 ± 6.18</td>
<td>25.36 ± 6.22</td>
<td>27.00 ± 4.86</td>
<td>26.64 ± 3.29</td>
<td>0.256</td>
</tr>
<tr>
<td>Central MAP (mmHg)</td>
<td>86.97 ± 7.89</td>
<td>87.82 ± 7.50</td>
<td>91.18 ± 5.31</td>
<td>88.79 ± 7.50</td>
<td>0.423</td>
</tr>
<tr>
<td>Resting HR (bpm)</td>
<td>57.90 ± 6.79</td>
<td>58.82 ± 10.44</td>
<td>56.64 ± 4.82</td>
<td>62.64 ± 8.19</td>
<td>0.163</td>
</tr>
</tbody>
</table>

*p < 0.05, group by time interaction. †p < 0.05, main effect of time. Values are means ± SD. SBP = Systolic Blood Pressure; DBP = Diastolic Blood Pressure; PP = Pulse Pressure; MAP = Mean Arterial Pressure; HR = Heart Rate
Figure 4-8. Changes in Brachial PP in curcumin and placebo groups throughout the intervention

$p < 0.05$, group by time interaction; Means ± SEM

Figure 4-9. Changes in Central PP in curcumin and placebo groups throughout the intervention

Means ± SEM
Other Physiological Measures

Body Composition

At baseline, there were no differences in body composition measures (Table 4-1). There were no significant changes in measures of body composition throughout the 12 week intervention between groups (Table 4-8).
Table 4-8. Effects of treatment on body composition

<table>
<thead>
<tr>
<th></th>
<th>Curcumin (n = 11)</th>
<th>Placebo (n = 11)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>27.22 ± 4.90</td>
<td>28.52 ± 4.42</td>
<td>27.18 ± 5.46</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>33.29 ± 3.69</td>
<td>33.99 ± 3.42</td>
<td>33.18 ± 3.38</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>106.13 ± 15.81</td>
<td>108.59 ± 15.36</td>
<td>102.34 ± 11.60</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>29.46 ± 9.80</td>
<td>31.43 ± 9.24</td>
<td>28.32 ± 9.22</td>
</tr>
<tr>
<td>Fat-free mass (kg)</td>
<td>76.66 ± 7.28</td>
<td>77.17 ± 7.62</td>
<td>73.97 ± 4.07</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>108.93 ± 11.99</td>
<td>109.32 ± 11.98</td>
<td>106.27 ± 10.37</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>114.47 ± 8.41</td>
<td>115.36 ± 6.97</td>
<td>112.80 ± 5.87</td>
</tr>
<tr>
<td>Waist:hip Ratio</td>
<td>0.95 ± 0.05</td>
<td>0.95 ± 0.06</td>
<td>0.94 ± 0.06</td>
</tr>
<tr>
<td>Waist:height Ratio</td>
<td>0.61 ± 0.07</td>
<td>0.61 ± 0.07</td>
<td>0.61 ± 0.06</td>
</tr>
</tbody>
</table>

*p < 0.05, group by time interaction. Values are means ± SD.
Dietary History Questionnaire

At baseline, the total HEI scores were not significantly different between the curcumin and placebo groups (p = 0.464). Only refined grain scores were higher in the placebo group compared to the curcumin group (p = 0.049). No other baseline differences were seen in HEI score components. Baseline scores and differences between groups can be seen in Table 4-9.

Table 4-10 contains the differences in dietary habits from the beginning to the end of the study in both groups. There was not a significant group by time interaction of the overall HEI scores. However, pre and post scores indicate differences in the HEI scores for greens and beans between the groups (p = 0.005, group by time interaction), showing a significantly higher consumption of greens and beans in the placebo group than in the intervention group at the conclusion of the study. There were no differences in other HEI dietary components revealing a group by time interaction.
<table>
<thead>
<tr>
<th></th>
<th>Curcumin (n = 11)</th>
<th>Placebo (n = 11)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total HEI Score</td>
<td>62.78 ± 12.79</td>
<td>67.32 ± 13.81</td>
<td>0.464</td>
</tr>
<tr>
<td>Total Vegetables (HEI Score)</td>
<td>3.84 ± 1.19</td>
<td>4.03 ± 1.20</td>
<td>0.705</td>
</tr>
<tr>
<td>Greens and Beans (HEI Score)</td>
<td>3.48 ± 1.81</td>
<td>3.92 ± 1.43</td>
<td>0.493</td>
</tr>
<tr>
<td>Total Fruit (HEI Score)</td>
<td>3.14 ± 1.65</td>
<td>2.71 ± 1.71</td>
<td>0.587</td>
</tr>
<tr>
<td>Whole Fruit (HEI Score)</td>
<td>3.91 ± 1.75</td>
<td>3.25 ± 1.85</td>
<td>0.434</td>
</tr>
<tr>
<td>Refined Grains (HEI Score)</td>
<td>7.42 ± 1.78</td>
<td>8.68 ± 1.93</td>
<td>0.049*</td>
</tr>
<tr>
<td>Whole Grains (HEI Score)</td>
<td>1.76 ± 0.83</td>
<td>1.67 ± 1.23</td>
<td>0.879</td>
</tr>
<tr>
<td>Dairy (HEI Score)</td>
<td>6.36 ± 2.84</td>
<td>7.56 ± 1.98</td>
<td>0.375</td>
</tr>
<tr>
<td>Total Protein Foods (HEI Score)</td>
<td>4.73 ± 0.59</td>
<td>4.79 ± 0.35</td>
<td>0.824</td>
</tr>
<tr>
<td>Seafood and Plant Proteins (HEI Score)</td>
<td>3.47 ± 1.69</td>
<td>4.09 ± 1.42</td>
<td>0.364</td>
</tr>
<tr>
<td>Fatty Acids (HEI Score)</td>
<td>5.17 ± 3.19</td>
<td>5.89 ± 3.80</td>
<td>0.658</td>
</tr>
<tr>
<td>Sodium (HEI Score)</td>
<td>4.06 ± 2.09</td>
<td>4.79 ± 2.76</td>
<td>0.522</td>
</tr>
<tr>
<td>Empty Calories (HEI Score)</td>
<td>15.4 ± 3.88</td>
<td>15.94 ± 6.24</td>
<td>0.841</td>
</tr>
</tbody>
</table>

*p < 0.05, between groups. Values are means ± SD. HEI = Healthy Eating Index
Table 4-10. Changes in dietary intake from baseline to the end of the study

<table>
<thead>
<tr>
<th></th>
<th>Curcumin (n = 11)</th>
<th>Placebo (n = 11)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>Total HEI Score</td>
<td>62.78 ± 12.79</td>
<td>60.71 ± 13.23</td>
<td>67.32 ± 13.81</td>
</tr>
<tr>
<td>Total Vegetables (HEI Score)</td>
<td>3.84 ± 1.19</td>
<td>3.67 ± 1.29</td>
<td>4.03 ± 1.20</td>
</tr>
<tr>
<td>Greens and Beans (HEI Score)</td>
<td>3.48 ± 1.81</td>
<td>2.87 ± 2.24</td>
<td>3.92 ± 1.43</td>
</tr>
<tr>
<td>Total Fruit (HEI Score)</td>
<td>3.14 ± 1.65</td>
<td>2.57 ± 1.53</td>
<td>2.71 ± 1.71</td>
</tr>
<tr>
<td>Whole Fruit (HEI Score)</td>
<td>3.91 ± 1.75</td>
<td>3.44 ± 1.65</td>
<td>3.25 ± 1.85</td>
</tr>
<tr>
<td>Refined Grains (HEI Score)</td>
<td>7.42 ± 1.78</td>
<td>8.47 ± 2.08</td>
<td>8.68 ± 1.93</td>
</tr>
<tr>
<td>Whole Grains (HEI Score)</td>
<td>1.76 ± 0.83</td>
<td>2.04 ± 0.68</td>
<td>1.67 ± 1.23</td>
</tr>
<tr>
<td>Dairy (HEI Score)</td>
<td>6.36 ± 2.84</td>
<td>4.92 ± 3.30</td>
<td>7.56 ± 1.98</td>
</tr>
<tr>
<td>Total Protein Foods (HEI Score)</td>
<td>4.73 ± 0.59</td>
<td>4.47 ± 0.89</td>
<td>4.79 ± 0.35</td>
</tr>
<tr>
<td>Seafood and Plant Proteins (HEI Score)</td>
<td>3.47 ± 1.69</td>
<td>4.01 ± 1.68</td>
<td>4.09 ± 1.42</td>
</tr>
<tr>
<td>Fatty Acids (HEI Score)</td>
<td>5.17 ± 3.19</td>
<td>5.55 ± 3.51</td>
<td>5.89 ± 3.80</td>
</tr>
<tr>
<td>Sodium (HEI Score)</td>
<td>4.06 ± 2.09</td>
<td>4.90 ± 3.05</td>
<td>4.79 ± 2.76</td>
</tr>
<tr>
<td>Empty Calories (HEI Score)</td>
<td>15.4 ± 3.88</td>
<td>13.80 ± 6.06</td>
<td>15.94 ± 6.24</td>
</tr>
</tbody>
</table>

*p < 0.05, group by time interaction. Values are means ± SD. HEI = Healthy Eating Index
The purpose of this study was to determine if 12 weeks of supplementation with curcumin (1.0 g/day) could improve arterial stiffness, endothelial dysfunction, and inflammation in young obese men. This study was the first to assess curcumin as a novel intervention for vascular health in young, obese men.

The major finding of this study was that curcumin caused a reduction in cfPWV in young obese men with baseline elevated cfPWV. Furthermore, curcumin caused significant reductions in brachial PP, a surrogate marker of arterial stiffness. The current study may provide support for improvements in vascular health with curcumin supplementation in young obese men, especially when they present with increased cfPWV.

**Arterial Stiffness as Measured by cfPWV**

Over the 12 week study, some individuals responded to the curcumin treatment with reductions in cfPWV while others did not. The curcumin group did not see significant changes in cfPWV compared to the placebo group. The overall lack of reduction in cfPWV in the curcumin group when compared to the placebo group is consistent with the previous findings in a pilot study looking at the potential role of curcumin on the vascular health of PM women (322). However, findings in another study from the same lab did show significant increases in arterial compliance, the reciprocal of arterial stiffness (9). Arterial compliance was not measured in the current study.
Exploratory statistics were employed to see if any inferences might be made about why some individuals responded to the curcumin treatment and others did not. It was shown that those individuals who responded to the curcumin had a higher baseline cfPWV than the non-responders (mean: 6.81 m/s v. 5.84 m/s, p = 0.045), showing nearly a 1.0 m/s difference in cfPWV measures at baseline. Interestingly, the responders had cfPWV values that were above reference values for their age group, while non-responders were below reference values. Established reference values show that individuals less than 30 years of age with normal blood pressure readings and no other cardiovascular risk factors had a mean PWV of 6.2 m/s, and individuals 30-40 years of age had a mean PWV of 6.5 m/s (271). This might suggest that curcumin could potentially benefit arterial stiffness in individuals who have higher than average cfPWV measures for their age. In addition to the higher cfPWV measures in the responders, waist:height ratios were significantly lower in the responders (p = 0.035), while waist:hip ratios and waist circumference measures were trending toward significance (p = 0.067 and p = 0.035, respectively). This could suggest that distribution of body fat may be playing a role in the responsiveness to the treatment, with the unexpected supposition that those individuals with a higher distribution of fat in the waist might not respond to the curcumin treatment. There is currently no evidence to suggest that distribution of body fat might determine responsiveness to curcumin, but abdominal adiposity is associated with low-grade chronic inflammation (105, 116, 168, 383). In the current work, waist circumference is positively associated with inflammation, as determined by pro-inflammatory markers TNF-α (p = 0.019) and IL-8 (p = 0.031). The known anti-inflammatory properties of curcumin (4, 11, 32, 62, 161) would indicate the potential for
curcumin to have a more pronounced impact on individuals with increased waist circumference measures. Therefore, it is unlikely that responsiveness is determined by lower waist circumference, waist:hip ratio, and waist:height ratio. Additionally, the resting HR differences at baseline were trending toward significance (p = 0.093), with the responders presenting with lower baseline HR measures. While the possibility cannot be excluded that individuals with higher HR measure might not respond to curcumin, there is little evidence to support this theory, and it is unlikely since both groups had mean HR values that were low enough to be considered bradycardic. However, the trending difference in baseline HR between responders and non-responders could suggest that the individuals identified as responders had increased cardiovascular fitness at baseline, as higher activity levels are associated with lower HRs (298). In contrast, this is not likely because the responders had cfPWV measures that were nearly 1.0 m/s higher than the non-responders, and physical activity is a strong predictor of cfPWV (20, 245, 329). Additionally, while there is little evidence to support that individuals who are more active will respond more favorably to curcumin compared to less active individuals, interventions that incorporate both curcumin and exercise have shown an interaction effect between exercise and curcumin on arterial compliance when initiated together (9). Nevertheless, intervening with exercise and curcumin concomitantly did not have an effect on cfPWV (322). Thus, while the possibility remains that lower baseline HR measures could be indicative of increased physical activity levels, cfPWV measures do not suggest that the responders had higher physical activity levels, and previous work does not indicate that increased physical activity levels will increase responsiveness to curcumin. Therefore, the most likely explanation for the responsiveness of arterial
stiffness to curcumin is that the individuals that did respond had higher cfPWV measures at baseline.

Due to the benefits of curcumin on arterial stiffness for a subset of individuals who had higher cfPWV values at baseline, insight was sought into the mechanistic role determining responsiveness. Of note, the decrease in cfPWV values corresponded with an increase in anti-inflammatory IL-13 in the responders (effect of time in responders, p = 0.018) when compared to the non-responders (p = 0.052, group by time interaction). The effects of curcumin on inflammatory cytokines are believed to occur through the ability of curcumin to inhibit NFkB signaling, and therefore, modify the downstream inflammatory pathways that it regulates (333). Curcumin inhibits the activation of NFkB (126, 303) and can affect downstream inflammatory cytokines (122, 126). A possible mechanism for reductions in arterial stiffness in the responders could be the corresponding increase in cytokine IL-13. To our knowledge, other arterial stiffness studies have not shown reductions in cfPWV that are associated with increases in IL-13. However, IL-13 has been shown to have a protective role in a murine model against atherosclerosis (55), a separate but related arterial pathology. Anti-inflammatory cytokine IL-13 modulated atherosclerotic lesions through structural changes in collagen fibers. Arterial stiffness is also modulated by structural changes in collagen fibers (58), and collagen cross-linking breakers have resulted in significant reductions in cfPWV through an effect on AGEs in experimental animals (25, 69, 344, 376) and humans (163). Furthermore, a study looking at the effect of curcumin on the large elastic artery stiffness in aging mice showed amelioration of collagen content and AGEs corresponding with reductions in PWV (113). Other possible explanations for reductions in cfPWV should
also be considered. In mice, cfPWV reductions due to curcumin were also associated with MnSOD expression attenuation and nitrotyrosine reduction, leading to amelioration of oxidative stress (113). Additionally, TEMPOL, a mimetic of antioxidant SOD, has ameliorated arterial stiffness by normalizing collagen and oxidative stress (111). The current study did not look at changes in oxidative stress associated with reductions in cfPWV, so this could be a possible mechanism by which curcumin reduces arterial stiffness in the responders.

Vascular health of young, obese individuals is a relevant clinical concern. Most studies looking at the predictive power of cfPWV in determining cardiovascular events have occurred in middle-aged and older adults; studies in younger individuals are lacking but could provide pertinent understanding in early adaptations of arterial structure and CVD risk progression (238). Furthermore, the potential to identify and alter vascular parameters at a younger age could lead to an ability to favorably reduce or prevent the development of CVD, affecting quality of life in these individuals for more substantial time periods.

Other studies have looked at associations of various parameters with cfPWV to better understand the factors that might predict arterial stiffness. In previous studies, obesity has been shown to lead to stiffer arteries, when individuals are compared to age-matched counterparts (79) at all ages from adolescence to old age (384) independent of BP, ethnicity, and age (280). Interestingly, in the current study, the cfPWV measurements were not elevated from normative data of age-matched counterparts when considering group mean. As a whole, the values for cfPWV in this study were much healthier than anticipated for an obese cohort, showing obese men presenting with a mean cfPWV of
6.29 ± 0.88 m/s at baseline. In this study, it was found that BMI was not a significant predictor of cfPWV (p = 0.376). Many other studies have shown that obesity based on BMI does not necessarily lead to higher cfPWV measures (57, 78, 279, 285, 386).

While BMI is not always the best indicator of health risk due to obesity, especially in individuals with larger than normal muscle mass (134), all subjects in this study were also considered to have elevated body fat percentages. The American College of Sports Medicine states that men aged 20-39 are considered to have a “poor” body fat percentage at 19.7-24.8% body fat or “very poor” body fat percentage at 24.9-33.4% body fat (15). The average body fat percentage of the men in this study was 27.2 ± 5.1% at baseline. Of the 22 subjects enrolled in this study, eight men (36.3%) had “poor” body fat percentages and 14 men (63.6%) had “very poor” body fat percentages. Although subjects were randomized based on BMI, body fat percentage comparisons between the groups were nearly identical (27.22 ± 4.90% v. 27.18 ± 5.46%, p = 0.972). Some studies suggest that, rather than BMI, we should determine obesity by parameters such as body fat percentage (2, 282, 381) or central adiposity measures (280) in order to identify individuals who would present with stiffer arteries. However, in this study, increased body fat percentage was not predictive of cfPWV (p = 0.550). A study in middle-aged individuals also indicated that body fat percentage was not a significant predictor of cfPWV (318).

It is believed that central adiposity may be associated with stiffer arteries due to increased levels of low-grade inflammation (281). In one study, when other obesity-related measures were not positively associated with cfPWV, waist circumference was clearly associated with cfPWV (72). In the present study, waist circumference was not
significantly associated with cfPWV (p = 0.540); this same finding was reported in a study of the general population (79). Although obesity is known to be a pro-inflammatory state (105, 383), it is believed that visceral fat depots influence inflammatory markers more than BMI or body fat percentage (117). Studies have shown that waist circumference is associated with pro-inflammatory blood markers, such as CRP, IL-6, IL-8, and TNF-α (105, 168, 383). The current study found that waist circumference was predictive of both TNF-α and IL-8 but no other pro-inflammatory markers (p = 0.031 and p = 0.019, respectively; univariate regression models can be seen in Table A-1).

Other anthropometric measures were also considered. Waist:hip circumference was trending toward significance as a predictor of cfPWV (p = 0.058) in the obese men in the current study. It is not surprising that waist:hip circumference is nearly predictive of cfPWV, as importance in predicting cfPWV based on waist:hip ratio has been reported elsewhere in 20-40 year old individuals and 41-77 year old individuals (368). Another anthropometric measure taken into consideration was waist:height ratio, an index of abdominal adiposity that has proven useful in determining cardiometabolic risk (284). One study reported that the waist:height ratio has the strongest association with aortic stiffness and that it may be the preeminent measure of excess adiposity determining cfPWV in the general population (375). The authors suggest that waist:height ratio may provide a better prediction of aortic stiffness than waist circumference, as waist circumference is a proxy measure of abdominal adiposity that does not distinguish between two individuals with the same waist circumference yet dramatically different heights; therefore, waist circumference alone is unable to account for increased abdominal fat percentage with differing heights (375). In the current study, that was not
found to be true, as the waist:height ratio was not associated with cfPWV (p = 0.472). Moreover, body weight and hip circumference have shown strong correlations with increased PWV elsewhere (368). In the current study, neither overall body weight nor hip circumference were good predictors of cfPWV (p = 0.612 and p = 0.506). Other studies have shown a lack of association between cfPWV and hip circumference or overall body weight (34, 72). Thus, obesity-associated measures did not predict higher cfPWV measures in the current study, which is consistent with some previous studies but not others.

There seems to be a lack of consensus in the literature about measures of obesity and their prediction of cfPWV. This could be due to differences in the population of interest within the studies or it could be due to the fact that other unfavorable conditions often present with obesity, leading to confounders such as metabolic syndrome, hypertension, and type 2 diabetes (34). Others have reported that measures of obesity are independently associated with higher values of cfPWV in women but not men, so sex could play a potential role in the discord that exists in the literature (242). Another possible explanation could be the age of obesity onset (1, 73, 104). Stiffer arteries have been associated with higher blood pressure and central adiposity measures in adolescents, leading to steeper increases in arterial stiffness in a 20 year follow-up period (104). Also, childhood obesity has been suggested to have a significant adverse effect on PWV in adolescents (73). The total number of risk factors in childhood and adulthood has been directly associated with PWV in adulthood, indicating that each of the following are independent predictors of PWV in adulthood: age, childhood SBP, childhood blood glucose, adulthood SBP, adulthood blood insulin, and adulthood triglycerides (1).
Furthermore, this study also indicated that reductions in the total number of risk factors from childhood to adulthood were associated with lower PWV measures in adulthood. In the current study, age of obesity onset, and thus the duration of obesity, was not determined, but it may have given insight into why cfPWV values in this obese population were not significantly higher that age-matched norms. Another important consideration could be that the men in this study were considered “fit but fat” (94). Individuals who are defined as “fit but fat” are believed to exhibit increased cardiovascular fitness that attenuates obesity-associated cardiovascular risks. It is estimated that 9% of adults fall into this category (94). Measurements to define cardiovascular fitness were not considered for this study; thus, it cannot be excluded that cardiovascular fitness levels might be responsible for the lack of association between obesity and arterial stiffness. Based on the results of this study, young obese men do not necessarily have stiffer arteries as a result of obesity.

For the current study, associations with cfPWV through univariate regression can be found in Table A-2 and through forward selection multiple regression in Table A-3. The only predictor of cfPWV that was found in this study from a simple regression model was pro-inflammatory cytokine IL-12 p70 (R$^2 = 0.258$, p = 0.031). This is consistent with previous findings in healthy individuals (382). In addition to IL-12 p70, there were a few variables that were trending toward significance in their ability to predict cfPWV: waist:hip ratio (R$^2 = 0.177$, p = 0.058), brachial SBP (R$^2 = 0.140$, p = 0.094), brachial MAP (R$^2 = 0.175$, p = 0.059), central DBP (R$^2 = 0.148$, p = 0.085), central MAP (R$^2 = 0.138$, p = 0.097), consumption of whole grains (R$^2 = 0.182$, p = 0.054), and consumption of refined grains (R$^2 = 0.148$, p = 0.085). Of note, age, the most
predominating independent predictor of cfPWV (180), was not a significant predictor of cfPWV in this sample (p = 0.268). A possible explanation for this is that, in a young, relatively homogenous group of young men, age confers no additional predictive value for cfPWV. The correlation of waist:hip ratio with cfPWV was discussed in detail above. Brachial SBP was the only predictor that prevailed in a forward selection model of multiple regression. Brachial SBP is a well-known predictor of cfPWV (1, 30, 143). Other studies have shown the association of brachial MAP (12, 48, 76, 217, 222), central DBP (262), and central MAP (199) with arterial stiffness. This is the first study to note a trending association between grain consumption (both whole and refined grains) and cfPWV. Whole grain consumption was negatively associated with cfPWV, whereas refined grain consumption was positively associated with cfPWV. Lower lifetime consumption of whole grains has been associated with stiffer carotid arteries, reportedly due to the impact of fiber content (348). Further explanations to clarify the diverging impact of whole and refined grains on these obese men could be vitamin and mineral content (specifically antioxidant content), resistant starch, oligosaccharides, phenolic compounds, and phytoestrogens (308). Further studies will need to be conducted to see if whole and refined grain consumption predicts arterial stiffness.

**Augmentation Index**

There was a significant group by time interaction for AIx. Interestingly, this interaction suggested potential benefit of the placebo rather than the intervention. However, when the augmentation index was corrected for a heart rate of 75 (AIx_{75}), this interaction did not persist. AIx_{75} is considered a more acceptable measure of augmentation index, as it takes into account HR, and AIx is highly influenced by HR
Therefore, since AIx corrected for HR (AIx75) is considered a more appropriate measure for determining arterial stiffness, fluctuations in HR might be responsible for the significant group by time interaction between the intervention and placebo groups (313, 372).

The limitations of AIx as a measure of arterial stiffness might give further elucidation as to why the placebo group saw improved augmentation indices when compared to the placebo. While the AIx has been reported to predict cardiovascular events and mortality (355), cfPWV has prevailed as a stronger predictor (216). This is due, in part, to the assumptions that underlie the general transfer function that is used to calculate the AIx (186). Not only has it been shown to produce a range of error (232), but the validation of this transfer function has been the object of criticism (63, 144, 186, 210, 257). As the assumptions underlying the general transfer function have proven problematic in some instances (210), it may follow that the assumptions made are not appropriate for a young, obese cohort of men. Furthermore, the assumed reflection site of the incident wave may not be precise, causing inaccuracy in the measurement (186). Thus, the AIx may not serve as the best determinant of arterial stiffness in young obese men.

Another possible explanation for the decrease in AIx in the placebo group could be the increased consumption of greens and beans. While all subjects were instructed to maintain current dietary habits throughout the study, a change in the greens and beans HEI score in the placebo group indicates that subjects may have incompletely adhered to these guidelines. The effect of diet on arterial stiffness has been elucidated previously (29, 53, 132, 197, 253, 255, 358), but previous studies have not considered the specific
role of greens and beans in altering arterial stiffness. (37). Dietary fiber, which tends to be high in these foods, has been reported to reduce the stiffness of the arteries (348). In one study reporting the effects of lifetime dietary intake on stiffness of the carotid artery, fiber intake was negatively associated with arterial stiffness (348). Dietary fiber can affect cardiovascular disease risk by lowering blood cholesterol concentrations and normalizing glucose and insulin (307), but whether these mechanisms are what directly affects the carotid stiffness associated with lower lifetime fiber intake is still not fully understood (348). Additionally, the dark green vegetables associated with the “greens and beans” category of the DHQ (86) are often high in dietary nitrates (145). Nitrates from green vegetables have been previously reported to affect arterial stiffness measured by AIx (160, 268). Nitrates present in vegetables will be reduced to nitrite and converted to NO, which will increase NO bioavailability in the vasculature (268). Importantly, increased NO bioavailability has been shown to reduce arterial stiffness (37, 112, 302). The potential role of greens and beans in the reduction of AIx in the placebo group cannot be eliminated. However, since cfPWV, a more robust measure of arterial stiffness, was not affected as a result of this dietary change, it is unlikely that the increased consumption of greens and beans explains the change in AIx.

It must be noted that the placebo was composed of fenugreek. Fenugreek could potentially affect arterial stiffness, but previous literature has not explored this possibility. Fenugreek, the naturally occurring medicinal plant, is composed of 50% fiber (31), and fiber can attenuate arterial stiffness (348). Additionally, fenugreek has also been shown to favorably influence glucose (129, 291), cholesterol (293), and triglyceride concentrations (294, 295). However, the curcumin intervention also contained the same
amount of fenugreek to enhance the bioavailability of curcumin, so similar changes would have been expected in the intervention group as well. Therefore, while this possibility cannot be eliminated that fenugreek alone is more beneficial to arterial health (as determined by AIx) than curcumin and fenugreek combined, it is not probable.

**Reactive Hyperemia Index**

Endothelial function was not impacted by curcumin supplementation in the current study. This was unexpected given previous findings in PM women showing improvements in endothelial function after eight weeks of supplementation with curcumin (8). A likely explanation might be that the previous study in PM women used a different method for monitoring endothelial function than the current study. The previous study used a technique for measuring FMD with ultrasound, the gold standard non-invasive technique for measuring endothelial function (95). It should be noted that PAT is not the gold-standard method of determining endothelial function, and some studies have shown that FMD and PAT are not associated (85, 133, 187, 286, 327) while others have shown association between the two methods (83, 139, 178, 250, 369). In an obese cohort specifically, FMD and PAT measures were not correlated (85). It is believed that these disparities that exist might be due in part to the population that is being tested (13, 187) or due to the fact that PAT might not be strictly measuring NO-mediated dilation (13).

Several studies suggest that PAT is measuring more complex mechanisms than strict NO-dependent vasodilation (13, 85, 95). Furthermore, it has been suggested that PAT is not a sensitive measure of endothelial function based on its lack of ability to distinguish between healthy individuals and those with known peripheral arterial disease, whereas FMD could distinguish between individuals with peripheral arterial disease (13).
Therefore, differences between the current study and the study in PM women are likely due to different techniques employed for measuring endothelial function.

The manufacturer’s guidelines for the Endo-PAT 2000 designate that the threshold for a good EndoPAT result is 1.67 (150). At baseline, the mean RHI was reported to be 2.05 ± 0.50 in the present cohort of obese men. Only four men had baseline scores that fell below the threshold value of 1.67. Differences in results between the current study and the previous study in PM women could also be due to reduced endothelial function in these women at baseline (8). It has been reported in other studies that compromised endothelial function due to obesity may be more specific to the female sex (320) or may occur in obese male populations when both increased BMI and older age are present (23, 102, 127). Therefore, when values are considered normal at baseline, there may not be a great probability of seeing improvement.

Furthermore, it has been postulated that curcumin exerts its effects on endothelial function through suppression of inflammation and oxidative stress by down-regulating TNF-α (137). Since TNF-α remained unchanged in the current study, this could be a possible explanation for why no improvements were seen in endothelial function. The study in PM did not assess plasma TNF-α levels to confirm similar mechanisms occurring in conjunction with improvements in endothelial function (8).

**Inflammatory Cytokines**

Curcumin is a well-documented anti-inflammatory agent, and its anti-inflammatory properties have been the focus of several reviews (4, 32, 62, 161, 297). The number of clinical trials focusing on curcumin is growing, and previous clinical trials
show promise for curcumin’s therapeutic potential against many diseases (130). Very few clinical trials have looked at the impact of curcumin on the cardiovascular system, but the few that have, have not looked at the impact on inflammatory cytokines (14, 311).

Arterial stiffness is associated with inflammation (204, 357), and inflammation affects structural changes within the arteries (159). Since arterial stiffness is associated with pro-inflammatory cytokines (188), the ability to impact inflammation in cases of arterial stiffness could be of great clinical importance (204, 357).

Various clinical trials have shown that inflammatory cytokines are impacted by curcumin treatment in colorectal cancer (137), pancreatic cancer (82), head and neck cancer (171), irritable bowel disease (97), osteoarthritis (36), type 2 diabetes (343), and diabetic nephropathy (166). The application of curcumin’s anti-inflammatory properties in various diseases might not be transferable from one disease state to the next. In the current study, involving obese but otherwise healthy young men, no significant plasma cytokine level differences were found, but anti-inflammatory cytokine IL-10 was trending toward an increase in the curcumin compared to placebo group. Increased IL-10 levels following curcumin treatment have been reported elsewhere (82, 97, 100, 385). A previous study has suggested curcumin may be able to increase IL-10 expression through inhibition of acetylation, but the specific mechanisms are still unknown (97). Increased plasma IL-10 levels are of importance because of the anti-inflammatory nature of IL-10 (221), whereby it can inhibit the production of pro-inflammatory cytokines (107).

No other differences in inflammatory markers were noticed in this study in the curcumin group when compared to the placebo group. Other studies have found that IL-1β (36, 97), IL-6 (36, 82, 343), IL-8 (82, 166, 171), IL-10 (82, 97), and TNF-α (137, 343)
were significantly affected after curcumin treatment. Inconsistencies between the previous clinical trials and this one include differences in age, disease state, and sex of the subjects as well as different intervention treatments; curcumin has been studied alone and in combination with quercetin, gemcitabine, piperine, docetaxel, soy isoflavones, bioperine, sulfasalazine, mesalamine, prednisone, lactoferrin, N-acetylcysteine, and pantoprazole (130). The current combination of fenugreek with curcumin has not been tested in other trials, and young obese men have not been the focus of the other studies mentioned.

**Blood Pressure and Heart Rate Measures**

Curcumin supplementation significantly reduced brachial PP when compared to the placebo (46.55 ± 10.20 mmHg to 40.00 ± 9.42 mmHg, p = 0.038, group by time interaction). The current study and the studies in PM women seem to suggest that changes in BP occur more readily with curcumin than changes in cfPWV (9, 322). In the current study, there was a significant time effect on SBP (p = 0.030), but the curcumin group did not show reductions in SBP that were significantly different than the placebo group. In PM women, brachial SBP was reduced, but brachial PP did not change significantly (9). This could likely be due to the corresponding changes that occurred in DBP in PM women, which resulted in similar PP measures after the eight week intervention period. Curcumin’s ability to impact brachial PP is, therefore, unique to the current study.

Brachial PP is accepted as a surrogate marker of arterial stiffness (185). Notably, brachial PP was reduced in the curcumin group, while cfPWV was not. Although brachial PP has been shown to correlate well with cfPWV in the general population, dissociations
have been known to occur in younger individuals (218, 347). This finding suggests that functional rather than structural differences are causing the overall reduction in brachial PP seen in the curcumin group. If structural differences in the artery were present amongst the individuals in the curcumin group, one would expect to see significant reductions in cfPWV in the group as a whole. Reductions in cfPWV in the six responders in the curcumin group could possibly be a result of structural changes in the arteries, presumably through anti-inflammatory IL-13. However, other mechanisms seem to be responsible for the overall reduction in brachial PP that is seen in the group as a whole, including the individuals who were non-responders based on cfPWV. A few explanations for this functional, rather than structural, change could be possible.

The role of vascular smooth muscle cells might be the primary action by which reductions in brachial PP were seen. Vascular smooth muscle cells, which produce different levels of active tone, can contribute to the functional ability of the arteries to vasodilate or vasoconstrict (120). With aging, the structural components of the arteries are known to work in conjunction with the vasoactive function of vascular smooth muscle cells, although the structural components have been shown to contribute to arterial stiffness more with age (49, 120). In this young group of men, curcumin could be causing relaxation of the vascular smooth muscle cells, leading to vasodilation. Animal models have demonstrated vasoactive properties of curcumin that are time- and dose-dependent (81). The ability of curcumin to affect vascular smooth muscle cells could be one possible mechanism by which curcumin affects brachial PP.

Inflammation cannot be excluded as a possible mechanism. Although none of the pro-inflammatory markers changed, anti-inflammatory cytokine IL-10 increased (p =
IL-10 is a potent anti-inflammatory cytokine (107). Inflammation, when present, can affect the functional and structural arterial wall properties through increased reactive oxygen species, which lead to further inflammatory processes (231). These inflammatory processes can directly affect the arteries, produce changes through oxidative stress, or affect the smooth muscle cells mentioned above (276).

Also, the role of endothelial function cannot be excluded as a possibility given the previously mentioned problems with measuring endothelial function using Endo-PAT 2000. Notably, there is not sufficient evidence to support that the Endo-PAT 2000 is measuring NO-dependent vasodilation, and many studies have suggested that it measures more complex mechanisms (13, 85, 95). Endothelial function can affect vascular smooth muscle cell tone through NO bioavailability (315), and the previous study in PM women found that curcumin did affect endothelial function when measured by FMD; this change in FMD corresponded with reductions in SBP (8). Further studies should use FMD to determine if endothelial function could play a role in the reduction of brachial PP by curcumin.

Lastly, any combination of vascular smooth muscle vasoactivity, inflammatory modulation via anti-inflammatory IL-10, and changes in endothelial function could have explained the significant changes in brachial PP by curcumin.

**Clinical Implications and Future Research**

The primary findings of this study demonstrated an effect of curcumin treatment on vascular stiffness. Future investigations with this curcumin compound will require a larger study population. Based on our calculations that incorporated the effect size found
in this study on the primary outcome (cfPWV), a post-hoc analysis to determine an appropriate sample size for a larger study with 80% power and significance of 0.05 would include 176 obese men, matched and randomized by BMI. Therefore, from this study, we propose a future study with a larger sample of obese men that is sufficient to detect an overall treatment effect on arterial stiffness.

In the current study, obese men with stiffer arteries at baseline had reductions in arterial stiffness determined by cfPWV, which is the first time that this has been shown. This study has also shown that curcumin can beneficially affect brachial PP, which is a new finding that could be of great benefit in a clinical setting. It also confirms previous studies, that have shown that curcumin can reduce BP measures (8, 9). Therefore, this study shows promise for curcumin to improve vascular health, and it leaves some additional questions to be answered.

The findings of this study may not be generalizable to every population, but these data seem to suggest that identifying populations with known elevated levels of cfPWV, such as an aging or diseased population, might allow the researchers to identify whether curcumin could be effective in ameliorating cfPWV. To further understand the impacts of curcumin on obesity, identifying and stratifying younger individuals with elevated cfPWV at baseline for inclusion in the study or identifying an older group of obese individuals should be considered. If individuals are stratified based on elevated cfPWV, smaller sample sizes may be sufficient to detect a treatment effect. Future studies should look at FMD to determine a potential role of endothelial function in the reduction of arterial stiffness, as measured by both cfPWV and brachial PP. Also, future studies looking at the modulation of arterial stiffness by curcumin should monitor anti-
inflammatory blood markers IL-10 and IL-13 specifically. IL-10 may play a role in modulating the inflammatory processes that lead to reductions in brachial PP, while IL-13 may play a role in modulating collagen structure that leads to reductions in cfPWV.

Finally, this study showed a trending negative association with four-week consumption of whole grains, and a trending positive association with four-week consumption of refined grains. The roles of whole grains and refined grains as they relate to cfPWV have been relatively unexplored. A previous study found that whole grain consumption throughout the lifetime is associated with decreased stiffness of the carotid artery (348). The authors report that fiber content is responsible for the negative association between whole grains and carotid stiffness. However, other potential components of whole grains cannot be excluded, such as the vitamins, minerals, resistant starches, oligosaccharides, phenolic compounds, and phytoestrogens that are present in whole grains (308). Also, it is unknown whether the four-week dietary recall is reflective of regular lifetime patterns. Further work should look at these potentially novel predictors of cfPWV.

**Limitations**

There are several limitations of the current study. First, measurements of subjects were taken at different times of day due to limitations of lab space, which can potentially alter arterial stiffness, RHI, and inflammatory measures because of post-prandial status and diurnal variation (42, 98, 191, 346, 387). While it is preferable to take measurements at the same time of day due to these potential fluctuations, post-prandial status may be a more important consideration for cardiovascular measures when abdominal adiposity is present (46, 346). Specifically, IFN-γ, TNF-α, IL-1, and IL-12 exhibit diurnal variation,
characterized by a peak in the early morning (77, 260, 387). Since we were unable to control for each subject to come into the lab at the same time for each visit, restrictions were given for meal timing: an overnight fast including caffeinated beverages was required of morning subjects (preferred timing of testing), whereas a four hour fast including caffeinated beverages was required of individuals arriving in the afternoon or evening, thereby reducing the effects of post-prandial changes in arterial stiffness, RHI, and inflammation. These standards are in accordance with what is recommended by “Task Force III: Recommendations for user procedures” given for arterial stiffness measures (346).

In addition, the 12 week duration of this study may not have been substantial enough time to see alterations in arterial stiffness. While curcumin has shown significant improvement in arterial compliance in humans after eight weeks of curcumin treatment (9), significant changes in arterial stiffness marked by cfPWV were not seen in humans after eight weeks in another study by the same group (322). After four weeks of curcumin treatment in mice, reductions were seen in cfPWV corresponding with amelioration of: structural changes in the arterial wall, endothelial dysfunction, AGEs, and oxidative stress (113). However, this study was conducted in mice, and the responsiveness in humans might require a longer time course. The current study did not find a significant change in overall cfPWV among the intervention group, though some individuals did see significant changes in arterial stiffness marked by cfPWV (responders). In this study, it is unknown whether the changes in cfPWV in the responders were due to structural changes in the arteries.
Also, the DHQ-II, a FFQ developed by the NIH, was used to account for dietary changes throughout the 12 week intervention, but FFQs are not always the most appropriate approach to detect subtle changes in dietary habits (115). In this study, dietary changes were considered for overall diet quality instead of subtle changes in dietary habits. Rather than the precise interaction of specific foods with arterial stiffness, this study was interested in an intervention that supposed a similar diet quality throughout the intervention period. Despite potential limitations, FFQs are commonly used in arterial stiffness studies (121, 157, 182, 193, 233, 270). Correlations for the DHQ, when compared to repeat 24-hour recalls collected over the course of a year, were \( r = 0.49 \) in men and \( r = 0.48 \) in women when assessing energy intake (319). Therefore, a moderate correlation exists between the DHQ and 24 hour recalls, which are frequently used in nutritional research.

Finally, the role of physical activity was not assessed throughout the current study. Due to physical activity being a predictor of cfPWV (20, 245, 329), changes in activity patterns throughout this study could have affected arterial stiffness. Although the participants were instructed to maintain regular lifestyle habits throughout the duration of the study, including physical activity and exercise habits, we cannot ensure that all participants adhered to these guidelines.

**Final Conclusions**

Curcumin ameliorates vascular function in obese men. This study provides the first evidence that curcumin ameliorates brachial PP, a clinically relevant marker of arterial stiffness. Moreover, the results of this study provide insight into a potential mechanism for the reduction in brachial PP through anti-inflammatory cytokine IL-10.
Curcumin also decreases arterial stiffness via gold standard arterial stiffness marker, cfPWV, in obese men with elevated cfPWV measures. The prospective mechanism found in this study was increased anti-inflammatory cytokine IL-13, which corresponded with the reduction in cfPWV. This study gives support for curcumin in treating arterial dysfunction associated with obesity, which could lead to a reduction in future cardiovascular events.
## APPENDIX

**Table A-1. Predicting inflammatory cytokines with waist circumference**

<table>
<thead>
<tr>
<th>Cytokine (pg/mL)</th>
<th>$\beta$</th>
<th>S.E.</th>
<th>t statistic</th>
<th>p-value</th>
<th>R square</th>
<th>Adjusted R square</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-γ</td>
<td>0.008</td>
<td>0.044</td>
<td>0.178</td>
<td>0.861</td>
<td>0.002</td>
<td>-0.057</td>
</tr>
<tr>
<td>IL-2</td>
<td>-0.002</td>
<td>0.007</td>
<td>-0.262</td>
<td>0.797</td>
<td>0.004</td>
<td>-0.055</td>
</tr>
<tr>
<td>IL-4</td>
<td>-0.001</td>
<td>0.002</td>
<td>-0.355</td>
<td>0.727</td>
<td>0.007</td>
<td>-0.051</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.008</td>
<td>0.006</td>
<td>1.261</td>
<td>0.224</td>
<td>0.086</td>
<td>0.032</td>
</tr>
<tr>
<td>IL-8</td>
<td>0.031</td>
<td>0.013</td>
<td>2.355</td>
<td>0.031*</td>
<td>0.246</td>
<td>0.202</td>
</tr>
<tr>
<td>IL-10</td>
<td>-0.001</td>
<td>0.005</td>
<td>-0.240</td>
<td>0.813</td>
<td>0.003</td>
<td>-0.055</td>
</tr>
<tr>
<td>IL-12 p70</td>
<td>-0.001</td>
<td>0.007</td>
<td>-0.182</td>
<td>0.858</td>
<td>0.002</td>
<td>-0.057</td>
</tr>
<tr>
<td>IL-13</td>
<td>0.156</td>
<td>0.124</td>
<td>1.262</td>
<td>0.224</td>
<td>0.086</td>
<td>0.032</td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.021</td>
<td>0.008</td>
<td>2.580</td>
<td>0.019*</td>
<td>0.281</td>
<td>0.239</td>
</tr>
</tbody>
</table>

*p < 0.05. IFN = Interferon; IL = Interleukin; TNF = Tumor Necrosis Factor*
Table A-2. Univariate linear regression for correlations between baseline subject characteristics and cfPWV

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>$\beta$</th>
<th>S.E.</th>
<th>t statistic</th>
<th>p-value</th>
<th>R square</th>
<th>Adjusted R square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>0.059</td>
<td>0.052</td>
<td>1.141</td>
<td>0.268</td>
<td>0.064</td>
<td>0.015</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>0.008</td>
<td>0.015</td>
<td>0.514</td>
<td>0.612</td>
<td>0.014</td>
<td>-0.038</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>0.054</td>
<td>0.059</td>
<td>0.906</td>
<td>0.376</td>
<td>0.041</td>
<td>-0.009</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>-0.021</td>
<td>0.030</td>
<td>-0.677</td>
<td>0.506</td>
<td>0.024</td>
<td>-0.028</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>0.012</td>
<td>0.020</td>
<td>0.625</td>
<td>0.540</td>
<td>0.020</td>
<td>-0.031</td>
</tr>
<tr>
<td>Waist:hip ratio</td>
<td>7.480</td>
<td>3.703</td>
<td>2.020</td>
<td>0.058</td>
<td>0.177</td>
<td>0.133</td>
</tr>
<tr>
<td>Waist:height ratio</td>
<td>2.523</td>
<td>3.434</td>
<td>0.735</td>
<td>0.472</td>
<td>0.028</td>
<td>-0.024</td>
</tr>
<tr>
<td>Body fat %</td>
<td>0.024</td>
<td>0.040</td>
<td>0.609</td>
<td>0.550</td>
<td>0.019</td>
<td>-0.032</td>
</tr>
<tr>
<td>Body fat (kg)</td>
<td>0.014</td>
<td>0.022</td>
<td>0.661</td>
<td>0.516</td>
<td>0.023</td>
<td>-0.029</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>0.005</td>
<td>0.036</td>
<td>0.127</td>
<td>0.900</td>
<td>0.001</td>
<td>-0.052</td>
</tr>
<tr>
<td>Brachial SBP (mmHg)</td>
<td>0.038</td>
<td>0.022</td>
<td>1.762</td>
<td>0.094</td>
<td>0.140</td>
<td>0.095</td>
</tr>
<tr>
<td>Brachial DBP (mmHg)</td>
<td>0.041</td>
<td>0.025</td>
<td>1.672</td>
<td>0.111</td>
<td>0.128</td>
<td>0.082</td>
</tr>
<tr>
<td>Brachial PP (mmHg)</td>
<td>0.006</td>
<td>0.024</td>
<td>0.256</td>
<td>0.801</td>
<td>0.003</td>
<td>-0.050</td>
</tr>
<tr>
<td>Brachial MAP (mmHg)</td>
<td>0.053</td>
<td>0.026</td>
<td>2.006</td>
<td>0.059</td>
<td>0.175</td>
<td>0.131</td>
</tr>
<tr>
<td>Central SBP (mmHg)</td>
<td>0.037</td>
<td>0.029</td>
<td>1.274</td>
<td>0.218</td>
<td>0.079</td>
<td>0.030</td>
</tr>
<tr>
<td>Central DBP (mmHg)</td>
<td>0.044</td>
<td>0.024</td>
<td>1.817</td>
<td>0.085</td>
<td>0.148</td>
<td>0.103</td>
</tr>
<tr>
<td>Central PP (mmHg)</td>
<td>-0.038</td>
<td>0.039</td>
<td>-0.976</td>
<td>0.341</td>
<td>0.048</td>
<td>-0.002</td>
</tr>
<tr>
<td>Central MAP (mmHg)</td>
<td>0.047</td>
<td>0.027</td>
<td>1.746</td>
<td>0.097</td>
<td>0.138</td>
<td>0.093</td>
</tr>
<tr>
<td>IFN-(\gamma) (pg/mL)</td>
<td>0.023</td>
<td>0.108</td>
<td>0.213</td>
<td>0.834</td>
<td>0.003</td>
<td>-0.060</td>
</tr>
<tr>
<td>IL-2 (pg/mL)</td>
<td>0.345</td>
<td>0.678</td>
<td>0.509</td>
<td>0.618</td>
<td>0.016</td>
<td>-0.046</td>
</tr>
<tr>
<td>IL-4 (pg/mL)</td>
<td>3.146</td>
<td>2.771</td>
<td>1.135</td>
<td>0.273</td>
<td>0.075</td>
<td>0.017</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>0.517</td>
<td>0.774</td>
<td>0.669</td>
<td>0.513</td>
<td>0.027</td>
<td>-0.034</td>
</tr>
</tbody>
</table>
Table A-2 (cont'd). Univariate linear regression for correlations between baseline subject characteristics and cfPWV

<table>
<thead>
<tr>
<th></th>
<th>β</th>
<th>S.E.</th>
<th>t statistic</th>
<th>p-value</th>
<th>R square</th>
<th>Adjusted R square</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-8 (pg/mL)</td>
<td>0.406</td>
<td>0.304</td>
<td>1.336</td>
<td>0.200</td>
<td>0.100</td>
<td>0.044</td>
</tr>
<tr>
<td>IL-10 (pg/mL)</td>
<td>0.900</td>
<td>0.904</td>
<td>0.996</td>
<td>0.334</td>
<td>0.058</td>
<td>0.000</td>
</tr>
<tr>
<td>IL-12 p70 (pg/mL)</td>
<td>1.479</td>
<td>0.626</td>
<td>2.362</td>
<td>0.031*</td>
<td>0.258</td>
<td>0.212</td>
</tr>
<tr>
<td>IL-13 (pg/mL)</td>
<td>0.056</td>
<td>0.034</td>
<td>1.624</td>
<td>0.124</td>
<td>0.141</td>
<td>0.088</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>0.350</td>
<td>0.589</td>
<td>0.595</td>
<td>0.560</td>
<td>0.022</td>
<td>-0.040</td>
</tr>
<tr>
<td>RHI</td>
<td>0.302</td>
<td>0.396</td>
<td>0.763</td>
<td>0.455</td>
<td>0.031</td>
<td>-0.022</td>
</tr>
<tr>
<td>A1x (%)</td>
<td>-0.005</td>
<td>0.018</td>
<td>-0.260</td>
<td>0.798</td>
<td>0.004</td>
<td>-0.052</td>
</tr>
<tr>
<td>A1x75 (%)</td>
<td>0.008</td>
<td>0.017</td>
<td>0.480</td>
<td>0.637</td>
<td>0.013</td>
<td>-0.042</td>
</tr>
<tr>
<td>Total HEI Score</td>
<td>0.016</td>
<td>0.015</td>
<td>1.111</td>
<td>0.281</td>
<td>0.061</td>
<td>0.012</td>
</tr>
<tr>
<td>Total Vegetables (HEI Score)</td>
<td>0.139</td>
<td>0.177</td>
<td>0.784</td>
<td>0.443</td>
<td>0.031</td>
<td>-0.020</td>
</tr>
<tr>
<td>Greens and Beans (HEI Score)</td>
<td>-0.006</td>
<td>0.125</td>
<td>-0.044</td>
<td>0.965</td>
<td>0.000</td>
<td>-0.053</td>
</tr>
<tr>
<td>Total Fruit (HEI Score)</td>
<td>0.168</td>
<td>0.114</td>
<td>1.473</td>
<td>0.157</td>
<td>0.103</td>
<td>0.055</td>
</tr>
<tr>
<td>Refined Grains (HEI Score)</td>
<td>0.177</td>
<td>0.097</td>
<td>1.819</td>
<td>0.085</td>
<td>0.148</td>
<td>0.103</td>
</tr>
<tr>
<td>Whole Grains (HEI Score)</td>
<td>-0.359</td>
<td>0.175</td>
<td>-2.058</td>
<td>0.054</td>
<td>0.182</td>
<td>0.139</td>
</tr>
<tr>
<td>Dairy (HEI Score)</td>
<td>0.039</td>
<td>0.083</td>
<td>0.465</td>
<td>0.647</td>
<td>0.011</td>
<td>-0.041</td>
</tr>
<tr>
<td>Total Protein Foods (HEI Score)</td>
<td>0.422</td>
<td>0.411</td>
<td>1.026</td>
<td>0.318</td>
<td>0.052</td>
<td>0.003</td>
</tr>
<tr>
<td>Seafood and Plant Proteins (HEI Score)</td>
<td>0.012</td>
<td>0.129</td>
<td>0.096</td>
<td>0.924</td>
<td>0.000</td>
<td>-0.052</td>
</tr>
<tr>
<td>Fatty Acids (HEI Score)</td>
<td>0.012</td>
<td>0.060</td>
<td>0.194</td>
<td>0.849</td>
<td>0.002</td>
<td>-0.051</td>
</tr>
<tr>
<td>Sodium (HEI Score)</td>
<td>0.063</td>
<td>0.085</td>
<td>0.744</td>
<td>0.466</td>
<td>0.028</td>
<td>-0.023</td>
</tr>
<tr>
<td>Empty Calories (HEI Score)</td>
<td>0.026</td>
<td>0.039</td>
<td>0.679</td>
<td>0.506</td>
<td>0.024</td>
<td>-0.028</td>
</tr>
</tbody>
</table>

*p < 0.05. BMI = Body Mass Index; SBP = Systolic Blood Pressure; DBP = Diastolic Blood Pressure; PP = Pulse Pressure; MAP = Mean Arterial Pressure; cfPWV = carotid-femoral Pulse Wave Velocity; RHI = Reactive Hyperemia Index; A1x = Augmentation Index; A1x75 = Augmentation Index Corrected for Heart Rate of 75; IFN = Interferon; IL = Interleukin; TNF = Tumor Necrosis Factor; HEI = Healthy Eating Index
<table>
<thead>
<tr>
<th></th>
<th>β</th>
<th>S.E.</th>
<th>F Value</th>
<th>p-value</th>
<th>R square</th>
<th>Adjusted R square</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP</td>
<td>0.037</td>
<td>0.016</td>
<td>5.32</td>
<td>0.035*</td>
<td>0.249</td>
<td>0.202</td>
</tr>
</tbody>
</table>

*p < 0.05. SBP = Systolic Blood Pressure
Table A-4. Univariate linear regression for correlations between baseline subject characteristics and RHI

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>β</th>
<th>S.E.</th>
<th>t statistic</th>
<th>p-value</th>
<th>R square</th>
<th>Adjusted R square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>0.006</td>
<td>0.027</td>
<td>0.208</td>
<td>0.837</td>
<td>0.002</td>
<td>-0.05</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>-0.003</td>
<td>0.008</td>
<td>-0.334</td>
<td>0.742</td>
<td>0.006</td>
<td>-0.046</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>-0.009</td>
<td>0.034</td>
<td>-0.260</td>
<td>0.797</td>
<td>0.004</td>
<td>-0.049</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>-0.018</td>
<td>0.016</td>
<td>-1.114</td>
<td>0.279</td>
<td>0.061</td>
<td>0.012</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>-0.013</td>
<td>0.010</td>
<td>-1.299</td>
<td>0.209</td>
<td>0.082</td>
<td>0.033</td>
</tr>
<tr>
<td>Waist:Hip Ratio</td>
<td>-2.432</td>
<td>2.147</td>
<td>-1.133</td>
<td>0.271</td>
<td>0.063</td>
<td>0.014</td>
</tr>
<tr>
<td>Waist:height Ratio</td>
<td>-2.190</td>
<td>1.783</td>
<td>-1.229</td>
<td>0.234</td>
<td>0.074</td>
<td>0.025</td>
</tr>
<tr>
<td>Body fat %</td>
<td>-0.030</td>
<td>0.022</td>
<td>-1.358</td>
<td>0.190</td>
<td>0.088</td>
<td>0.040</td>
</tr>
<tr>
<td>Body fat (kg)</td>
<td>-0.011</td>
<td>0.012</td>
<td>-0.904</td>
<td>0.377</td>
<td>0.041</td>
<td>-0.010</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>0.011</td>
<td>0.019</td>
<td>0.604</td>
<td>0.553</td>
<td>0.019</td>
<td>-0.033</td>
</tr>
<tr>
<td>Brachial SBP (mmHg)</td>
<td>0.009</td>
<td>0.013</td>
<td>0.684</td>
<td>0.502</td>
<td>0.024</td>
<td>-0.003</td>
</tr>
<tr>
<td>Brachial DBP (mmHg)</td>
<td>-0.024</td>
<td>0.014</td>
<td>-1.668</td>
<td>0.112</td>
<td>0.128</td>
<td>0.082</td>
</tr>
<tr>
<td>Brachial PP (mmHg)</td>
<td>0.027</td>
<td>0.012</td>
<td>2.300</td>
<td>0.033*</td>
<td>0.218</td>
<td>0.177</td>
</tr>
<tr>
<td>Brachial MAP (mmHg)</td>
<td>-0.021</td>
<td>0.016</td>
<td>-1.340</td>
<td>0.196</td>
<td>0.086</td>
<td>0.038</td>
</tr>
<tr>
<td>Central SBP (mmHg)</td>
<td>-0.007</td>
<td>0.017</td>
<td>-0.383</td>
<td>0.706</td>
<td>0.008</td>
<td>-0.045</td>
</tr>
<tr>
<td>Central DBP (mmHg)</td>
<td>-2.000</td>
<td>0.014</td>
<td>-1.664</td>
<td>0.112</td>
<td>0.127</td>
<td>0.081</td>
</tr>
<tr>
<td>Central PP (mmHg)</td>
<td>0.035</td>
<td>0.019</td>
<td>1.830</td>
<td>0.083</td>
<td>0.150</td>
<td>0.105</td>
</tr>
<tr>
<td>Central MAP (mmHg)</td>
<td>-0.021</td>
<td>0.016</td>
<td>-1.340</td>
<td>0.196</td>
<td>0.086</td>
<td>0.038</td>
</tr>
<tr>
<td>IFN-γ (pg/mL)</td>
<td>-0.014</td>
<td>0.015</td>
<td>-0.902</td>
<td>0.378</td>
<td>0.041</td>
<td>-0.009</td>
</tr>
<tr>
<td>IL-2 (pg/mL)</td>
<td>-0.035</td>
<td>0.062</td>
<td>-0.576</td>
<td>0.572</td>
<td>0.019</td>
<td>-0.039</td>
</tr>
<tr>
<td>IL-4 (pg/mL)</td>
<td>-0.103</td>
<td>0.390</td>
<td>-0.264</td>
<td>0.794</td>
<td>0.004</td>
<td>-0.054</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>0.740</td>
<td>1.640</td>
<td>0.451</td>
<td>0.658</td>
<td>0.012</td>
<td>-0.046</td>
</tr>
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</table>
Table A-4 (cont’d). Univariate linear regression for correlations between baseline subject characteristics and RHI

<table>
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<tr>
<th>Variable</th>
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<th>t statistic</th>
<th>p-value</th>
<th>R square</th>
<th>Adjusted R square</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-8 (pg/mL)</td>
<td>0.030</td>
<td>0.181</td>
<td>0.167</td>
<td>0.869</td>
<td>0.002</td>
<td>-0.057</td>
</tr>
<tr>
<td>IL-10 (pg/mL)</td>
<td>0.159</td>
<td>0.533</td>
<td>0.299</td>
<td>0.769</td>
<td>0.005</td>
<td>-0.053</td>
</tr>
<tr>
<td>IL-12 p70 (pg/mL)</td>
<td>-0.177</td>
<td>0.413</td>
<td>-0.282</td>
<td>0.781</td>
<td>0.005</td>
<td>-0.054</td>
</tr>
<tr>
<td>IL-13 (pg/mL)</td>
<td>0.011</td>
<td>0.021</td>
<td>0.547</td>
<td>0.591</td>
<td>0.017</td>
<td>-0.041</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>-0.175</td>
<td>0.277</td>
<td>-0.630</td>
<td>0.537</td>
<td>0.023</td>
<td>-0.035</td>
</tr>
<tr>
<td>cfPWV (m/s)</td>
<td>0.104</td>
<td>0.136</td>
<td>0.763</td>
<td>0.455</td>
<td>31.000</td>
<td>-0.022</td>
</tr>
<tr>
<td>A1x (%)</td>
<td>-0.017</td>
<td>0.009</td>
<td>-1.851</td>
<td>0.080</td>
<td>0.153</td>
<td>0.108</td>
</tr>
<tr>
<td>A1x75 (%)</td>
<td>-0.019</td>
<td>0.008</td>
<td>-2.225</td>
<td>0.038*</td>
<td>0.207</td>
<td>0.165</td>
</tr>
<tr>
<td>Total HEI Score</td>
<td>0.013</td>
<td>0.008</td>
<td>1.670</td>
<td>0.111</td>
<td>0.128</td>
<td>0.082</td>
</tr>
<tr>
<td>Total Vegetables (HEI Score)</td>
<td>0.023</td>
<td>0.102</td>
<td>0.224</td>
<td>0.825</td>
<td>0.003</td>
<td>-0.050</td>
</tr>
<tr>
<td>Greens and Beans (HEI Score)</td>
<td>0.038</td>
<td>0.071</td>
<td>0.538</td>
<td>0.597</td>
<td>0.015</td>
<td>-0.037</td>
</tr>
<tr>
<td>Total Fruit (HEI Score)</td>
<td>0.077</td>
<td>0.069</td>
<td>1.109</td>
<td>0.281</td>
<td>0.011</td>
<td>0.502</td>
</tr>
<tr>
<td>Whole Fruit (HEI Score)</td>
<td>0.084</td>
<td>0.060</td>
<td>1.397</td>
<td>0.179</td>
<td>0.045</td>
<td>0.493</td>
</tr>
<tr>
<td>Refined Grains (HEI Score)</td>
<td>0.016</td>
<td>0.060</td>
<td>0.261</td>
<td>0.797</td>
<td>0.004</td>
<td>-0.049</td>
</tr>
<tr>
<td>Whole Grains (HEI Score)</td>
<td>0.067</td>
<td>0.108</td>
<td>0.621</td>
<td>0.542</td>
<td>0.020</td>
<td>-0.032</td>
</tr>
<tr>
<td>Dairy (HEI Score)</td>
<td>-0.047</td>
<td>0.053</td>
<td>-0.895</td>
<td>0.382</td>
<td>0.040</td>
<td>-0.010</td>
</tr>
<tr>
<td>Total Protein Foods (HEI Score)</td>
<td>0.089</td>
<td>0.183</td>
<td>0.483</td>
<td>0.634</td>
<td>0.012</td>
<td>-0.040</td>
</tr>
<tr>
<td>Seafood and Plant Proteins (HEI Score)</td>
<td>0.066</td>
<td>0.073</td>
<td>0.905</td>
<td>0.377</td>
<td>0.041</td>
<td>-0.009</td>
</tr>
<tr>
<td>Fatty Acids (HEI Score)</td>
<td>0.047</td>
<td>0.031</td>
<td>1.524</td>
<td>0.144</td>
<td>0.109</td>
<td>0.062</td>
</tr>
<tr>
<td>Sodium (HEI Score)</td>
<td>0.053</td>
<td>0.043</td>
<td>1.239</td>
<td>0.230</td>
<td>0.075</td>
<td>0.026</td>
</tr>
<tr>
<td>Empty Calories (HEI Score)</td>
<td>0.022</td>
<td>0.018</td>
<td>1.218</td>
<td>0.238</td>
<td>0.072</td>
<td>0.024</td>
</tr>
</tbody>
</table>

*p < 0.05. RHI = Reactive Hyperemia Index; BMI = Body Mass Index; SBP = Systolic Blood Pressure; DBP = Diastolic Blood Pressure; PP = Pulse Pressure; MAP = Mean Arterial Pressure; cfPWV = carotid-femoral Pulse Wave Velocity; A1x = Augmentation Index; A1x75 = Augmentation Index Corrected for Heart Rate of 75; IFN = Interferon; IL = Interleukin; TNF = Tumor Necrosis Factor; HEI = Healthy Eating Index
Table A-5. Univariate linear regression for correlations between baseline subject characteristics and Brachial SBP

<table>
<thead>
<tr>
<th></th>
<th>β</th>
<th>S.E.</th>
<th>t statistic</th>
<th>p-value</th>
<th>R square</th>
<th>Adjusted R square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>0.137</td>
<td>0.456</td>
<td>0.301</td>
<td>0.766</td>
<td>0.005</td>
<td>-0.045</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>0.740</td>
<td>0.318</td>
<td>2.329</td>
<td>0.305</td>
<td>0.213</td>
<td>0.174</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>1.308</td>
<td>0.470</td>
<td>2.787</td>
<td>0.011*</td>
<td>0.280</td>
<td>0.244</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>0.386</td>
<td>0.253</td>
<td>1.523</td>
<td>0.143</td>
<td>0.104</td>
<td>0.059</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>0.411</td>
<td>0.146</td>
<td>2.804</td>
<td>0.011*</td>
<td>0.282</td>
<td>0.246</td>
</tr>
<tr>
<td>Waist:Hip Ratio</td>
<td>101.733</td>
<td>29.758</td>
<td>3.419</td>
<td>0.003*</td>
<td>0.369</td>
<td>0.337</td>
</tr>
<tr>
<td>Waist:height Ratio</td>
<td>70.625</td>
<td>26.505</td>
<td>2.665</td>
<td>0.015*</td>
<td>0.262</td>
<td>0.225</td>
</tr>
<tr>
<td>Body fat %</td>
<td>0.670</td>
<td>0.346</td>
<td>1.937</td>
<td>0.067</td>
<td>0.158</td>
<td>0.116</td>
</tr>
<tr>
<td>Body fat (kg)</td>
<td>0.424</td>
<td>0.182</td>
<td>2.334</td>
<td>0.030*</td>
<td>0.214</td>
<td>0.175</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>0.480</td>
<td>0.304</td>
<td>1.578</td>
<td>0.130</td>
<td>0.111</td>
<td>0.066</td>
</tr>
<tr>
<td>IFN-γ (pg/mL)</td>
<td>0.077</td>
<td>0.980</td>
<td>0.078</td>
<td>0.938</td>
<td>0.000</td>
<td>-0.058</td>
</tr>
<tr>
<td>IL-2 (pg/mL)</td>
<td>-4.490</td>
<td>6.065</td>
<td>-0.740</td>
<td>0.469</td>
<td>0.031</td>
<td>-0.026</td>
</tr>
<tr>
<td>IL-4 (pg/mL)</td>
<td>-7.656</td>
<td>25.918</td>
<td>-0.295</td>
<td>0.771</td>
<td>0.005</td>
<td>-0.053</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>6.320</td>
<td>6.482</td>
<td>0.975</td>
<td>0.343</td>
<td>0.053</td>
<td>-0.003</td>
</tr>
<tr>
<td>IL-8 (pg/mL)</td>
<td>2.318</td>
<td>2.794</td>
<td>0.830</td>
<td>0.418</td>
<td>0.039</td>
<td>-0.018</td>
</tr>
<tr>
<td>IL-10 (pg/mL)</td>
<td>-5.702</td>
<td>8.303</td>
<td>-0.687</td>
<td>0.501</td>
<td>0.027</td>
<td>-0.030</td>
</tr>
<tr>
<td>IL-12 p70 (pg/mL)</td>
<td>1.634</td>
<td>6.506</td>
<td>0.251</td>
<td>0.805</td>
<td>0.004</td>
<td>-0.055</td>
</tr>
<tr>
<td>IL-13 (pg/mL)</td>
<td>0.457</td>
<td>0.313</td>
<td>1.461</td>
<td>0.162</td>
<td>0.112</td>
<td>0.059</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>3.460</td>
<td>4.337</td>
<td>0.798</td>
<td>0.436</td>
<td>0.036</td>
<td>-0.021</td>
</tr>
</tbody>
</table>

*p < 0.05. SBP = Systolic Blood Pressure; BMI = Body Mass Index; IFN = Interferon; IL = Interleukin; TNF = Tumor Necrosis Factor
<table>
<thead>
<tr>
<th></th>
<th>β</th>
<th>S.E.</th>
<th>t statistic</th>
<th>p-value</th>
<th>R square</th>
<th>Adjusted R square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>0.134</td>
<td>0.408</td>
<td>0.330</td>
<td>0.745</td>
<td>0.005</td>
<td>-0.044</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>0.055</td>
<td>0.124</td>
<td>0.442</td>
<td>0.663</td>
<td>0.010</td>
<td>-0.040</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.530</td>
<td>0.481</td>
<td>1.103</td>
<td>0.283</td>
<td>0.057</td>
<td>0.010</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>0.067</td>
<td>0.239</td>
<td>0.281</td>
<td>0.782</td>
<td>0.004</td>
<td>-0.046</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>0.164</td>
<td>0.151</td>
<td>1.087</td>
<td>0.290</td>
<td>0.056</td>
<td>0.009</td>
</tr>
<tr>
<td>Waist:Hip Ratio</td>
<td>53.713</td>
<td>31.293</td>
<td>1.716</td>
<td>0.102</td>
<td>-11.562</td>
<td>0.085</td>
</tr>
<tr>
<td>Waist:height Ratio</td>
<td>36.543</td>
<td>26.372</td>
<td>1.386</td>
<td>0.181</td>
<td>0.088</td>
<td>0.042</td>
</tr>
<tr>
<td>Body fat %</td>
<td>0.436</td>
<td>0.323</td>
<td>1.351</td>
<td>0.192</td>
<td>0.084</td>
<td>0.038</td>
</tr>
<tr>
<td>Body fat (kg)</td>
<td>0.198</td>
<td>0.178</td>
<td>1.114</td>
<td>0.279</td>
<td>0.059</td>
<td>0.011</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>-0.204</td>
<td>0.285</td>
<td>-0.716</td>
<td>0.483</td>
<td>0.025</td>
<td>-0.024</td>
</tr>
<tr>
<td>IFN-γ (pg/mL)</td>
<td>3.922</td>
<td>1.191</td>
<td>3.293</td>
<td>0.004*</td>
<td>0.389</td>
<td>0.354</td>
</tr>
<tr>
<td>IL-2 (pg/mL)</td>
<td>-1.308</td>
<td>5.770</td>
<td>-0.227</td>
<td>0.823</td>
<td>0.003</td>
<td>-0.056</td>
</tr>
<tr>
<td>IL-4 (pg/mL)</td>
<td>0.910</td>
<td>24.367</td>
<td>0.037</td>
<td>0.971</td>
<td>0.000</td>
<td>-0.059</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>-7.198</td>
<td>5.998</td>
<td>-1.200</td>
<td>0.247</td>
<td>0.078</td>
<td>0.024</td>
</tr>
<tr>
<td>IL-8 (pg/mL)</td>
<td>2.582</td>
<td>2.598</td>
<td>0.994</td>
<td>0.334</td>
<td>0.055</td>
<td>-0.001</td>
</tr>
<tr>
<td>IL-10 (pg/mL)</td>
<td>-3.666</td>
<td>7.843</td>
<td>-0.467</td>
<td>0.646</td>
<td>0.013</td>
<td>-0.045</td>
</tr>
<tr>
<td>IL-12 p70 (pg/mL)</td>
<td>4.530</td>
<td>6.013</td>
<td>0.753</td>
<td>0.462</td>
<td>0.032</td>
<td>-0.025</td>
</tr>
<tr>
<td>IL-13 (pg/mL)</td>
<td>0.271</td>
<td>0.304</td>
<td>0.892</td>
<td>0.385</td>
<td>0.045</td>
<td>-0.011</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>0.497</td>
<td>4.141</td>
<td>0.120</td>
<td>0.906</td>
<td>-8.240</td>
<td>-0.058</td>
</tr>
</tbody>
</table>

*p < 0.05. DBP = Diastolic Blood Pressure; BMI = Body Mass Index; IFN = Interferon; IL = Interleukin; TNF = Tumor Necrosis Factor
Table A-7. Univariate linear regression for correlations between baseline subject characteristics and Brachial PP

<table>
<thead>
<tr>
<th></th>
<th>β</th>
<th>S.E.</th>
<th>t statistic</th>
<th>p-value</th>
<th>R square</th>
<th>Adjusted R square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>0.003</td>
<td>0.455</td>
<td>0.007</td>
<td>0.995</td>
<td>0.000</td>
<td>-0.050</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>0.233</td>
<td>0.129</td>
<td>1.812</td>
<td>0.085</td>
<td>0.141</td>
<td>0.098</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.778</td>
<td>0.523</td>
<td>1.489</td>
<td>0.152</td>
<td>0.100</td>
<td>0.055</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>0.319</td>
<td>0.257</td>
<td>1.241</td>
<td>0.229</td>
<td>0.072</td>
<td>0.025</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>0.248</td>
<td>0.163</td>
<td>1.517</td>
<td>0.145</td>
<td>0.103</td>
<td>0.058</td>
</tr>
<tr>
<td>Waist:Hip Ratio</td>
<td>48.020</td>
<td>35.717</td>
<td>1.344</td>
<td>0.194</td>
<td>0.083</td>
<td>0.037</td>
</tr>
<tr>
<td>Waist:height Ratio</td>
<td>34.082</td>
<td>29.761</td>
<td>1.145</td>
<td>0.266</td>
<td>0.062</td>
<td>0.015</td>
</tr>
<tr>
<td>Body fat %</td>
<td>0.234</td>
<td>0.372</td>
<td>0.628</td>
<td>0.537</td>
<td>0.019</td>
<td>-0.030</td>
</tr>
<tr>
<td>Body fat (kg)</td>
<td>0.226</td>
<td>0.198</td>
<td>1.142</td>
<td>0.267</td>
<td>0.061</td>
<td>0.014</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>0.684</td>
<td>0.282</td>
<td>2.421</td>
<td>0.025*</td>
<td>0.227</td>
<td>0.188</td>
</tr>
<tr>
<td>IFN-γ (pg/mL)</td>
<td>3.922</td>
<td>1.191</td>
<td>3.293</td>
<td>0.004*</td>
<td>0.389</td>
<td>0.354</td>
</tr>
<tr>
<td>IL-2 (pg/mL)</td>
<td>-3.182</td>
<td>6.452</td>
<td>-0.493</td>
<td>0.628</td>
<td>0.014</td>
<td>-0.044</td>
</tr>
<tr>
<td>IL-4 (pg/mL)</td>
<td>-8.567</td>
<td>27.323</td>
<td>-0.314</td>
<td>0.758</td>
<td>0.006</td>
<td>-0.053</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>13.519</td>
<td>6.212</td>
<td>2.176</td>
<td>0.044*</td>
<td>0.218</td>
<td>0.172</td>
</tr>
<tr>
<td>IL-8 (pg/mL)</td>
<td>-0.263</td>
<td>3.004</td>
<td>-0.088</td>
<td>0.931</td>
<td>0.001</td>
<td>-0.058</td>
</tr>
<tr>
<td>IL-10 (pg/mL)</td>
<td>-2.036</td>
<td>8.863</td>
<td>-0.230</td>
<td>0.821</td>
<td>0.003</td>
<td>-0.056</td>
</tr>
<tr>
<td>IL-12 p70 (pg/mL)</td>
<td>-2.896</td>
<td>6.838</td>
<td>-0.424</td>
<td>0.677</td>
<td>0.010</td>
<td>-0.048</td>
</tr>
<tr>
<td>IL-13 (pg/mL)</td>
<td>0.186</td>
<td>0.347</td>
<td>0.535</td>
<td>0.600</td>
<td>0.017</td>
<td>-0.041</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>2.962</td>
<td>4.603</td>
<td>0.644</td>
<td>0.528</td>
<td>0.024</td>
<td>-0.034</td>
</tr>
</tbody>
</table>

*p < 0.05. PP = Pulse Pressure; BMI = Body Mass Index; IFN = Interferon; IL = Interleukin; TNF = Tumor Necrosis Factor
Table A-8. Univariate linear regression for correlations between baseline subject characteristics and Brachial MAP

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>β</th>
<th>S.E.</th>
<th>t statistic</th>
<th>p-value</th>
<th>R square</th>
<th>Adjusted R square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>0.135</td>
<td>0.366</td>
<td>0.370</td>
<td>0.715</td>
<td>0.007</td>
<td>-0.043</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>0.133</td>
<td>0.108</td>
<td>1.226</td>
<td>0.234</td>
<td>0.070</td>
<td>0.023</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>0.790</td>
<td>0.408</td>
<td>1.934</td>
<td>0.067</td>
<td>0.158</td>
<td>0.115</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>0.173</td>
<td>0.212</td>
<td>0.819</td>
<td>0.422</td>
<td>0.032</td>
<td>-0.016</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>0.246</td>
<td>0.128</td>
<td>1.925</td>
<td>0.069</td>
<td>0.156</td>
<td>0.114</td>
</tr>
<tr>
<td>Waist:Hip Ratio</td>
<td>69.720</td>
<td>25.778</td>
<td>2.705</td>
<td>0.014*</td>
<td>0.268</td>
<td>0.231</td>
</tr>
<tr>
<td>Waist:height Ratio</td>
<td>47.904</td>
<td>22.383</td>
<td>2.140</td>
<td>0.045*</td>
<td>0.186</td>
<td>0.146</td>
</tr>
<tr>
<td>Body fat %</td>
<td>0.514</td>
<td>0.280</td>
<td>1.833</td>
<td>0.082</td>
<td>0.144</td>
<td>0.101</td>
</tr>
<tr>
<td>Body fat (kg)</td>
<td>0.274</td>
<td>0.153</td>
<td>1.787</td>
<td>0.089</td>
<td>0.138</td>
<td>0.095</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>0.024</td>
<td>0.260</td>
<td>0.093</td>
<td>0.927</td>
<td>0.000</td>
<td>-0.050</td>
</tr>
<tr>
<td>IFN-γ (pg/mL)</td>
<td>0.331</td>
<td>0.800</td>
<td>0.413</td>
<td>0.685</td>
<td>0.010</td>
<td>-0.048</td>
</tr>
<tr>
<td>IL-2 (pg/mL)</td>
<td>-2.369</td>
<td>5.020</td>
<td>-0.472</td>
<td>0.643</td>
<td>0.013</td>
<td>-0.045</td>
</tr>
<tr>
<td>IL-4 (pg/mL)</td>
<td>-1.945</td>
<td>21.303</td>
<td>-0.091</td>
<td>0.928</td>
<td>0.001</td>
<td>-0.058</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>-2.692</td>
<td>5.423</td>
<td>-0.496</td>
<td>0.626</td>
<td>0.015</td>
<td>-0.044</td>
</tr>
<tr>
<td>IL-8 (pg/mL)</td>
<td>2.494</td>
<td>2.257</td>
<td>1.105</td>
<td>0.285</td>
<td>0.067</td>
<td>0.012</td>
</tr>
<tr>
<td>IL-10 (pg/mL)</td>
<td>-4.345</td>
<td>6.822</td>
<td>-0.637</td>
<td>0.533</td>
<td>0.023</td>
<td>-0.034</td>
</tr>
<tr>
<td>IL-12 p70 (pg/mL)</td>
<td>3.565</td>
<td>5.275</td>
<td>0.676</td>
<td>0.508</td>
<td>0.026</td>
<td>-0.031</td>
</tr>
<tr>
<td>IL-13 (pg/mL)</td>
<td>0.333</td>
<td>0.260</td>
<td>1.283</td>
<td>0.217</td>
<td>0.088</td>
<td>0.035</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>1.484</td>
<td>3.60.</td>
<td>0.412</td>
<td>0.656</td>
<td>0.010</td>
<td>-0.048</td>
</tr>
</tbody>
</table>

*p < 0.05. MAP = Mean Arterial Pressure; BMI = Body Mass Index; IFN = Interferon; IL = Interleukin; TNF = Tumor Necrosis Factor.
Table A-9. Univariate linear regression for correlations between baseline subject characteristics and Central SBP

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>β</th>
<th>S.E.</th>
<th>t statistic</th>
<th>p-value</th>
<th>R square</th>
<th>Adjusted R square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>0.370</td>
<td>0.350</td>
<td>1.059</td>
<td>0.302</td>
<td>0.053</td>
<td>0.006</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>0.151</td>
<td>0.104</td>
<td>1.449</td>
<td>0.163</td>
<td>0.095</td>
<td>0.050</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>0.910</td>
<td>0.385</td>
<td>2.361</td>
<td>0.028*</td>
<td>0.218</td>
<td>0.179</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>0.274</td>
<td>0.201</td>
<td>1.364</td>
<td>0.188</td>
<td>0.085</td>
<td>0.040</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>0.319</td>
<td>0.116</td>
<td>2.757</td>
<td>0.012*</td>
<td>0.275</td>
<td>0.239</td>
</tr>
<tr>
<td>Waist:Hip Ratio</td>
<td>82.204</td>
<td>23.000</td>
<td>3.575</td>
<td>0.002*</td>
<td>0.390</td>
<td>0.359</td>
</tr>
<tr>
<td>Waist:height Ratio</td>
<td>61.665</td>
<td>19.947</td>
<td>3.091</td>
<td>0.006*</td>
<td>0.323</td>
<td>0.290</td>
</tr>
<tr>
<td>Body fat %</td>
<td>0.542</td>
<td>0.270</td>
<td>2.003</td>
<td>0.059</td>
<td>0.167</td>
<td>0.125</td>
</tr>
<tr>
<td>Body fat (kg)</td>
<td>0.291</td>
<td>0.148</td>
<td>1.975</td>
<td>0.062</td>
<td>0.163</td>
<td>0.121</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>0.079</td>
<td>0.253</td>
<td>0.314</td>
<td>0.757</td>
<td>0.005</td>
<td>-0.045</td>
</tr>
<tr>
<td>IFN-γ (pg/mL)</td>
<td>0.226</td>
<td>0.784</td>
<td>0.288</td>
<td>0.777</td>
<td>0.005</td>
<td>-0.054</td>
</tr>
<tr>
<td>IL-2 (pg/mL)</td>
<td>-3.497</td>
<td>4.865</td>
<td>-0.719</td>
<td>0.482</td>
<td>0.029</td>
<td>-0.028</td>
</tr>
<tr>
<td>IL-4 (pg/mL)</td>
<td>-8.368</td>
<td>20.724</td>
<td>-0.404</td>
<td>0.691</td>
<td>0.009</td>
<td>-0.049</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>-0.710</td>
<td>5.335</td>
<td>-0.133</td>
<td>0.896</td>
<td>0.001</td>
<td>-0.058</td>
</tr>
<tr>
<td>IL-8 (pg/mL)</td>
<td>2.135</td>
<td>2.224</td>
<td>0.960</td>
<td>0.351</td>
<td>0.051</td>
<td>-0.004</td>
</tr>
<tr>
<td>IL-10 (pg/mL)</td>
<td>-6.087</td>
<td>6.582</td>
<td>-0.925</td>
<td>0.368</td>
<td>0.048</td>
<td>-0.008</td>
</tr>
<tr>
<td>IL-12 p70 (pg/mL)</td>
<td>-0.118</td>
<td>6.863</td>
<td>-0.023</td>
<td>0.982</td>
<td>0.000</td>
<td>-0.059</td>
</tr>
<tr>
<td>IL-13 (pg/mL)</td>
<td>0.274</td>
<td>0.257</td>
<td>1.063</td>
<td>0.303</td>
<td>0.062</td>
<td>0.007</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>3.331</td>
<td>3.447</td>
<td>0.966</td>
<td>0.347</td>
<td>0.052</td>
<td>-0.004</td>
</tr>
</tbody>
</table>

*p < 0.05. SBP = Systolic Blood Pressure; BMI = Body Mass Index; IFN = Interferon; IL = Interleukin; TNF = Tumor Necrosis Factor
Table A-10. Univariate linear regression for correlations between baseline subject characteristics and Central DBP

<table>
<thead>
<tr>
<th></th>
<th>β</th>
<th>S.E.</th>
<th>t statistic</th>
<th>p-value</th>
<th>R square</th>
<th>Adjusted R square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>0.157</td>
<td>0.41</td>
<td>0.384</td>
<td>0.705</td>
<td>0.007</td>
<td>-0.042</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>0.041</td>
<td>0.125</td>
<td>0.328</td>
<td>0.746</td>
<td>0.005</td>
<td>-0.044</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.492</td>
<td>0.485</td>
<td>1.015</td>
<td>0.322</td>
<td>0.049</td>
<td>0.001</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>0.046</td>
<td>0.241</td>
<td>0.193</td>
<td>0.849</td>
<td>0.002</td>
<td>-0.048</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>0.158</td>
<td>0.152</td>
<td>1.041</td>
<td>0.310</td>
<td>0.051</td>
<td>0.004</td>
</tr>
<tr>
<td>Waist:Hip Ratio</td>
<td>54.832</td>
<td>31.377</td>
<td>1.748</td>
<td>0.096</td>
<td>0.132</td>
<td>0.090</td>
</tr>
<tr>
<td>Waist:height Ratio</td>
<td>36.449</td>
<td>26.524</td>
<td>1.374</td>
<td>0.185</td>
<td>0.086</td>
<td>0.041</td>
</tr>
<tr>
<td>Body fat %</td>
<td>0.409</td>
<td>0.326</td>
<td>1.254</td>
<td>0.224</td>
<td>0.073</td>
<td>0.027</td>
</tr>
<tr>
<td>Body fat (kg)</td>
<td>0.181</td>
<td>0.180</td>
<td>1.006</td>
<td>0.326</td>
<td>0.048</td>
<td>0.001</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>-0.236</td>
<td>0.285</td>
<td>-0.826</td>
<td>0.418</td>
<td>0.033</td>
<td>-0.015</td>
</tr>
<tr>
<td>IFN-γ (pg/mL)</td>
<td>0.437</td>
<td>0.918</td>
<td>0.476</td>
<td>0.640</td>
<td>0.013</td>
<td>-0.045</td>
</tr>
<tr>
<td>IL-2 (pg/mL)</td>
<td>-1.157</td>
<td>5.800</td>
<td>-0.200</td>
<td>0.844</td>
<td>0.002</td>
<td>-0.056</td>
</tr>
<tr>
<td>IL-4 (pg/mL)</td>
<td>1.571</td>
<td>24.484</td>
<td>0.064</td>
<td>0.950</td>
<td>0.000</td>
<td>-0.059</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>-6.816</td>
<td>6.056</td>
<td>-1.126</td>
<td>0.276</td>
<td>0.070</td>
<td>0.015</td>
</tr>
<tr>
<td>IL-8 (pg/mL)</td>
<td>2.706</td>
<td>2.604</td>
<td>1.039</td>
<td>0.313</td>
<td>0.060</td>
<td>0.004</td>
</tr>
<tr>
<td>IL-10 (pg/mL)</td>
<td>-3.206</td>
<td>7.894</td>
<td>-0.406</td>
<td>0.690</td>
<td>0.010</td>
<td>-0.049</td>
</tr>
<tr>
<td>IL-12 p70 (pg/mL)</td>
<td>4.814</td>
<td>6.030</td>
<td>0.798</td>
<td>0.436</td>
<td>0.036</td>
<td>-0.021</td>
</tr>
<tr>
<td>IL-13 (pg/mL)</td>
<td>0.267</td>
<td>0.306</td>
<td>0.872</td>
<td>0.396</td>
<td>0.043</td>
<td>-0.014</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>0.890</td>
<td>4.158</td>
<td>0.214</td>
<td>0.833</td>
<td>0.003</td>
<td>-0.056</td>
</tr>
</tbody>
</table>

*p < 0.05. DBP = Diastolic Blood Pressure; BMI = Body Mass Index; IFN = Interferon; IL = Interleukin; TNF = Tumor Necrosis Factor
Table A-11. Univariate linear regression for correlations between baseline subject characteristics and Central PP

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>β</th>
<th>S.E.</th>
<th>t statistic</th>
<th>p-value</th>
<th>R square</th>
<th>Adjusted R square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>0.213</td>
<td>0.288</td>
<td>0.740</td>
<td>0.468</td>
<td>0.027</td>
<td>-0.022</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>0.110</td>
<td>0.086</td>
<td>1.286</td>
<td>0.213</td>
<td>0.076</td>
<td>0.030</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.415</td>
<td>0.341</td>
<td>1.219</td>
<td>0.237</td>
<td>0.069</td>
<td>0.023</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>0.228</td>
<td>0.163</td>
<td>1.399</td>
<td>0.177</td>
<td>0.089</td>
<td>0.044</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>0.016</td>
<td>0.104</td>
<td>1.546</td>
<td>0.138</td>
<td>0.107</td>
<td>0.062</td>
</tr>
<tr>
<td>Waist:Hip Ratio</td>
<td>27.372</td>
<td>23.112</td>
<td>1.184</td>
<td>0.250</td>
<td>0.066</td>
<td>0.019</td>
</tr>
<tr>
<td>Waist:height Ratio</td>
<td>25.216</td>
<td>18.869</td>
<td>1.336</td>
<td>0.196</td>
<td>0.082</td>
<td>0.036</td>
</tr>
<tr>
<td>Body fat %</td>
<td>0.132</td>
<td>0.239</td>
<td>0.554</td>
<td>0.586</td>
<td>0.015</td>
<td>-0.034</td>
</tr>
<tr>
<td>Body fat (kg)</td>
<td>0.110</td>
<td>0.129</td>
<td>0.856</td>
<td>0.402</td>
<td>0.035</td>
<td>-0.013</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>0.315</td>
<td>0.193</td>
<td>1.630</td>
<td>0.119</td>
<td>0.117</td>
<td>0.073</td>
</tr>
<tr>
<td>IFN-γ (pg/mL)</td>
<td>-0.211</td>
<td>0.673</td>
<td>-0.314</td>
<td>0.757</td>
<td>0.006</td>
<td>-0.053</td>
</tr>
<tr>
<td>IL-2 (pg/mL)</td>
<td>-2.340</td>
<td>4.205</td>
<td>-0.556</td>
<td>0.585</td>
<td>0.018</td>
<td>-0.040</td>
</tr>
<tr>
<td>IL-4 (pg/mL)</td>
<td>-9.939</td>
<td>17.730</td>
<td>-0.561</td>
<td>0.582</td>
<td>0.018</td>
<td>-0.040</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>6.107</td>
<td>4.341</td>
<td>1.407</td>
<td>0.178</td>
<td>0.104</td>
<td>0.052</td>
</tr>
<tr>
<td>IL-8 (pg/mL)</td>
<td>-0.571</td>
<td>1.957</td>
<td>-0.292</td>
<td>0.774</td>
<td>0.005</td>
<td>-0.054</td>
</tr>
<tr>
<td>IL-10 (pg/mL)</td>
<td>-2.881</td>
<td>5.754</td>
<td>-0.501</td>
<td>0.623</td>
<td>0.015</td>
<td>-0.043</td>
</tr>
<tr>
<td>IL-12 p70 (pg/mL)</td>
<td>-4.933</td>
<td>4.326</td>
<td>-1.140</td>
<td>0.270</td>
<td>0.071</td>
<td>0.016</td>
</tr>
<tr>
<td>IL-13 (pg/mL)</td>
<td>0.007</td>
<td>0.228</td>
<td>0.031</td>
<td>0.976</td>
<td>0.000</td>
<td>-0.059</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>2.441</td>
<td>2.984</td>
<td>0.818</td>
<td>0.425</td>
<td>0.038</td>
<td>-0.019</td>
</tr>
</tbody>
</table>

*p < 0.05. PP = Pulse Pressure; BMI = Body Mass Index; IFN = Interferon; IL = Interleukin; TNF = Tumor Necrosis Factor
Table A-12. Univariate linear regression for correlations between baseline subject characteristics and Central MAP

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>β</th>
<th>S.E.</th>
<th>t statistic</th>
<th>p-value</th>
<th>R square</th>
<th>Adjusted R square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>0.228</td>
<td>0.367</td>
<td>0.623</td>
<td>0.541</td>
<td>0.019</td>
<td>-0.03</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>0.078</td>
<td>0.112</td>
<td>0.697</td>
<td>0.494</td>
<td>0.024</td>
<td>-0.025</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.631</td>
<td>0.425</td>
<td>1.485</td>
<td>0.153</td>
<td>0.099</td>
<td>0.050</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>0.123</td>
<td>0.215</td>
<td>0.570</td>
<td>0.575</td>
<td>0.016</td>
<td>-0.033</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>0.212</td>
<td>0.132</td>
<td>1.606</td>
<td>0.124</td>
<td>0.114</td>
<td>0.070</td>
</tr>
<tr>
<td>Waist:Hip Ratio</td>
<td>63.956</td>
<td>26.719</td>
<td>2.394</td>
<td>0.027*</td>
<td>0.223</td>
<td>0.184</td>
</tr>
<tr>
<td>Waist:height Ratio</td>
<td>44.854</td>
<td>22.859</td>
<td>1.962</td>
<td>0.064</td>
<td>0.161</td>
<td>0.120</td>
</tr>
<tr>
<td>Body fat %</td>
<td>0.453</td>
<td>0.288</td>
<td>1.577</td>
<td>0.131</td>
<td>0.111</td>
<td>0.066</td>
</tr>
<tr>
<td>Body fat (kg)</td>
<td>0.218</td>
<td>0.159</td>
<td>1.373</td>
<td>0.185</td>
<td>0.086</td>
<td>0.040</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>-0.131</td>
<td>0.260</td>
<td>-0.504</td>
<td>0.620</td>
<td>0.013</td>
<td>-0.037</td>
</tr>
<tr>
<td>IFN-γ (pg/mL)</td>
<td>0.367</td>
<td>0.816</td>
<td>0.449</td>
<td>0.659</td>
<td>0.012</td>
<td>-0.046</td>
</tr>
<tr>
<td>IL-2 (pg/mL)</td>
<td>-1.937</td>
<td>5.137</td>
<td>-0.377</td>
<td>0.711</td>
<td>0.008</td>
<td>-0.050</td>
</tr>
<tr>
<td>IL-4 (pg/mL)</td>
<td>-1.742</td>
<td>21.747</td>
<td>-0.080</td>
<td>0.937</td>
<td>0.000</td>
<td>-0.058</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>-4.781</td>
<td>5.454</td>
<td>-0.877</td>
<td>0.393</td>
<td>0.043</td>
<td>-0.013</td>
</tr>
<tr>
<td>IL-8 (pg/mL)</td>
<td>2.516</td>
<td>2.306</td>
<td>1.091</td>
<td>0.291</td>
<td>0.065</td>
<td>0.010</td>
</tr>
<tr>
<td>IL-10 (pg/mL)</td>
<td>-4.166</td>
<td>6.973</td>
<td>-0.597</td>
<td>0.558</td>
<td>0.021</td>
<td>-0.037</td>
</tr>
<tr>
<td>IL-12 p70 (pg/mL)</td>
<td>3.170</td>
<td>5.402</td>
<td>0.587</td>
<td>0.565</td>
<td>0.020</td>
<td>-0.038</td>
</tr>
<tr>
<td>IL-13 (pg/mL)</td>
<td>0.269</td>
<td>0.270</td>
<td>0.996</td>
<td>0.333</td>
<td>0.055</td>
<td>0.000</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>1.704</td>
<td>3.675</td>
<td>0.464</td>
<td>0.649</td>
<td>0.012</td>
<td>-0.046</td>
</tr>
</tbody>
</table>

*p < 0.05. MAP = Mean Arterial Pressure; BMI = Body Mass Index; IFN = Interferon; IL = Interleukin; TNF = Tumor Necrosis Factor
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