Effect of Nebivolol and Lifestyle Modification on Large Artery Stiffness in Middle-Aged and Older Hypertensive Adults

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ABSTRACT

For more than half a century cardiovascular disease has been the leading cause of death in the United States. Aging, hypertension, and obesity are major risk factors for cardiovascular disease and clearly associated with arterial stiffness. Arterial stiffness generates higher afterloads and diminishes coronary perfusion thereby causing ventricular hypertrophy and ischemia. Importantly, arterial stiffness is an independent predictor of cardiovascular disease risk and all-cause mortality. Current strategies such as inhibition of angiotensin II or angiotensin converting enzyme, reduction of smooth muscle tone, blood volume, or inflammatory mediators, and improving glucose homeostasis are effective destiffening options. Nebivolol, a third generation beta-blocker, has unique vasodilatory characteristics and may be particularly efficacious as a destiffening agent. Only a few studies have addressed this issue while relying on indirect, blood pressure-dependent stiffness indices precluding clear understanding of study outcomes. There remains a need to determine the potential utility of nebivolol therapy as an arterial destiffening strategy. Thus, we hypothesized that the combination of nebivolol and lifestyle modification would reduce central arterial stiffness in middle-aged and older hypertensive adults more than either intervention alone. To test this hypothesis, we randomized 45 hypertensive adults to receive lifestyle modification, nebivolol, or combination for 12 weeks. β-stiffness index, pulse wave analysis, and arterial compliance were measured at baseline and following the intervention. No baseline differences in variables of

interest were observed between groups. In contrast to our hypothesis, lifestyle modification, nebivolol, and combination groups had similar ($P>0.05$) reductions in β-stiffness index (-1.87±0.83; -2.03±0.60; and -2.51±0.90 U), respectively, while carotid-femoral pulse wave velocity declined only in the nebivolol and combination groups. Our findings suggest combination of nebivolol and lifestyle modification reduces arterial stiffness to a similar degree as either intervention alone in middle-aged and older hypertensive adults. Further studies are needed to determine if the changes in arterial stiffness continue to occur or remain clinically significant over longer durations.

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CHAPTER I

Introduction

Arterial stiffening is a physiological adaptation in response to mechanical and oxidative stress placed on the arterial system, inducing tunica media with greater tensile strength. However, the myocardial cells experience direct consequences of such vascular alterations. Central arterial stiffness accelerates with cardiovascular, inflammatory, and diabetic conditions, thus can be viewed as a time-integrated index for cardiovascular risk factors. Moreover, arterial stiffness is an independent predictor for cardiovascular diseases and categorized as an indicator of arteriosclerosis[1-3]. Therefore, it is imperative for the development of novel therapeutic strategies, providing better treatments outcomes on United States' deadliest disease.

The arteriole system functions both as a conduit, channeling oxygenated blood to metabolic tissues, and as a cushion, dissipating the pulsatile energy of ventricular ejection to maintain steady-state flow. Elastic properties of central arteries are responsible for buffering flow and perfusing the myocardium during diastole. Elastin and collagen proteins are the primary component of the artery walls, providing strength and flexibility required to maintain a constant pressure gradient throughout the arterial tree. Dynamic turnover rate for these proteins normally occurs over the course of one year in healthy individuals. Due to reasons that remain unclear, dysregulation of protein turnover will increase collagen production and elastin fragmentation. Chronic progression of this phenomenon causes central arteries to lose flexibility and become stiffer. As a result, the ability to buffer flow and perfuse the myocardium diminishes.

Arterial stiffness increases significantly with age, hypertension, inflammation, and adiposity, potentially leading to early death[4]. Hypertension, left ventricular hypertrophy,

congestive heart failure, and stroke are main pathological outcomes associated with arterial stiffness[5, 6]. Thus, arterial stiffness has gained substantial clinical importance and is now recognized as a surrogate endpoint for cardiovascular events [7].

Many treatments, mostly anti-hypertensive therapy, for arterial stiffness examined in this lab and others are efficacious strategies attenuating disease progression[8-13]. However, common side effects of many anti-hypertensives including muscle soreness and weakness, chronic cough, nausea, vertigo, erectile dysfunction, and allergic reactions discourage many patients from these treatment options. First- and second-generation β-blockers have neutral or negative influences on central arterial stiffness[14]. There is limited evidence to support nebivolol, a third-generation β-blocker, as a potential treatment option[15-17]. Nebivolol stimulates vascular nitric oxide (NO) release from the endothelial cells, augmenting vasodilatation, and reducing oxidative stress and vascular smooth muscle cell migration and growth[18-20]. Additionally, nebivolol has fewer side effects, making it a more attractive treatment option for primary care providers and patients.

Previous studies on nebivolol monotherapy utilized blood pressure-dependent indices of arterial stiffness such as pulse wave velocity and augmentation index. Additionally, these studies did not focus on older individuals with hypertension, the population with greatest risk for advanced stiffening and cardiovascular diseases. Therefore, the potential therapeutic treatment of nebivolol on arterial stiffness, particularly in older adults, remains unclear.

The cornerstone of many anti-hypertensive strategies are lifestyle modification involving changes to habitual dietary intake and physical activity. Studies measuring arterial stiffness and weight loss are limited[13, 21-23] and only two used a randomized trial to address this issue[13, 21]. Furthermore, most of these studies used small sample sizes and primarily focused on young and

middle-aged adult. Although proper lifestyle modification have been shown to reduce arterial stiffness in younger populations, there is a void in our general understanding of it on arterial stiffness in older adults. Older adults have the greatest risk of developing arterial stiffness and should be the primary focus when conducting research[5, 24]. There is currently no research addressing whether lifestyle modification or nebivolol would confer a greater impact on reduction of arterial stiffness compared to a synergistic combination. Thus, we tested the hypothesis that the combination of nebivolol and lifestyle modification reduces central arterial stiffness and biomarkers of inflammation more than either intervention alone. We recruited 45 adult hypertensives and randomized them into single or combination treatment groups. Changes in arterial stiffness indices and inflammatory markers were accessed at baseline and following the intervention. This study provides further insight on the clinical/physiological relevance of nebivolol therapy and lifestyle modification as potential arterial destiffening treatments.

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CHAPTER II

Review of Literature

A. Morbidity and Mortality of Cardiovascular Disease

Cardiovascular disease (CVD) is the leading cause of American deaths for the past 60 years[1]. In 2012, 1 in 3 deaths were attributed to CVD before the age of 75[1]. Aging is the greatest risk factor for CVD. Approximately 80% of Americans' deaths over the age of 65 were associated with CVD. In addition, the number of individuals over the age of 65 is predicted to double within the next 20 years[2].

Hypertension, hyperglycemia, hyperlipidemia, physical inactivity, obesity, and tobacco use are significant risk factors of CVD[3]. National Health and Nutrition Examination Survey (NHANES) 2008 estimated that among American adults, 33.5% have hypertension, 36.8% are pre-diabetic, 15% have total serum cholesterol levels ≥240 mg/dL, 39% are sedentary, 33.7% are obese, and 20% smoke[1]. These risk factors contribute to approximately 600,000 CVD related deaths per year[4]. Importantly, this number expected to increase given the disproportionate growth of adults over 65 years.

B. Health Care Costs of Cardiovascular Disease.

The United States spent $2.5 trillion on health care in 2010[5]. The cost of CVD for the US in 2010 was $503.2 billion, just over 19% of total health care spending. By the year 2019, health care fraction of gross domestic product is estimated to be 19.6% [5]. CVD is the highest costing diagnostic group[1]. By 2030, persons 65 years or older will increase more than two-fold and constitute 20% of the US population[2]. The disproportionate growth of older populations will have the greatest impact on the existing health care system. Current estimates forecast health

care costs climbing exponentially, placing additional stress on the nation's troubled financial system.

On average, CVD and obesity-related conditions have contributed to 9.2-11.4 workdays lost per year[6]. In addition, obese workers are more likely to chronically miss work (>2-week period) compared to their healthy coworkers[6]. This economic burden on businesses has taken a toll. There is now evidence of job discrimination for these prospective employees[7]. Developing novel treatments for CVD is of the utmost importance to preventing potentially fatal health.

C. Aging and Arterial Stiffness

The aging process in the arterial tree is heterogeneous and influenced by several factors. Aging arteries experience collagen deposition and fragmentation of elastin proteins from chronic stress and strain[8, 9]. Combined with other age-related risk factors such as obesity, hypertension, diabetes, and metabolic disorders the rate of stiffness accelerates with age. Central artery compliance declines with advancing age[10-14]. Arterial stiffness in the common carotid artery increases approximately 40-50% from age 20 to 80 years[10, 12, 13, 15]. Distal arteries show a different trend, as the muscular arterioles do not exhibit the same stiffening alterations[15]. Rather, endothelial dysfunction instigated by chronic oxidative stress and low antioxidant activity cause thickening of the intima and media tunica and contributes to vascular resistance[16]. In addition, studies have shown a linear, age-related increase in augmentation index (AIx), pulse pressure, and pulse wave velocity (PWV)[15, 17, 18]. Furthermore, central pulse pressures (PP) typically increase 200% from age 20 to 80 years[10].

Changes in PWV and PP are more prominent in older individuals (>50 years), while AIx is considered a better indicator for arterial stiffness in younger individuals (<50 years)[15, 17]. The difference is most likely driven by the age-related nature of wave reflection. In older

individuals, augmented pressure is significantly influenced by increases in PWV and early return of the reflective wave during systole due to a decline in arterial compliance. However in younger adults, augmented pressure is the result of a higher magnitude of wave reflection[17]. Therefore, PWV and PP can be viewed as a sensitive marker for arterial stiffness in older populations while AIx might be a better marker of arterial stiffness in younger adults[19, 20].

Arterial stiffness is a powerful predictor of future cardiovascular events in older adults[21]. It independently predicts stroke, coronary disease, and all-cause mortality in older individuals with essential hypertension[22, 23]. Indeed, arterial stiffness plays a significant role in disease progression and health outcomes.

D. Overweight and Obesity on Arterial Stiffness

The relationship between arterial stiffness, weight gain, and cardiovascular risk has become clearer over the past years; however, the mechanisms are not fully understood. Several factors contributing to adiposity accumulation in both genders with advancing age are hormonal, dietary, and physical activity changes[24]. Moreover, body composition changes have unfavorable effects on health outcomes and functional mobility, placing middle-aged and older adults into higher cardiovascular-related risk categories.

Overweight, defined by a BMI of 25-29.9 kg/m², and obese, defined by a BMI of >30 kg/m², individuals are at an increased risk for cardiovascular disease[25, 26]. Adults typically gain on average 1-2 lbs. per year throughout their lifetime[27, 28]. A longitudinal study showed slight sex differences in age-related weight change. Men and women 40 to 66 years of age increased weight at an average annual rate of 0.3 kg/year and 0.55 kg/year, respectively[29]. Furthermore, body mass index (BMI) tended to increase at an annual rate of 0.11 kg/m²/year in men and 0.22 kg/m²/year in women[29]. From 65-85 years of age, BMI declines in both men and women[30].

The degree to which increasing adiposity influences risk factors is currently unknown, but chronic obesity does not appear to be a prerequisite as obese children have similar risks[31].

Abdominal adiposity disproportionately affect middle-aged and older adults[32]. Approximately 70% of middle aged and older American adults are classified as overweight or obese, and 60% develop android obesity[33]. Older men were shown to have a marked redistribution of central adiposity independent of BMI[34]. Women tend to store more adiposity peripherally until menopause. Post-menopausal hormonal changes induce greater central adiposity distribution[35].

There is mounting evidence that arterial stiffness is associated with obesity[36-38] and this relationship is strengthened when comparing central versus peripheral adiposity[37, 39-42]. A study in our laboratory found intentional weight gain of 5 kg resulted in a significant increase in central adiposity depots and positively correlated with arterial stiffness[43]. In addition, changes in arterial stiffness were independent of gains in total body fat[43]. Wildman et al. found aortic PWV to be 40-90 cm/s higher in obese individuals compared to normal weight subjects regardless of age[37]. Similarly, Toto-Moukouo et al. found obesity increased PWV by 50 cm/s and 60 cm/s in hypertensive women and men, respectively[44]. When systolic blood pressure and age were adjusted for, the Health, Aging, and Body Composition study found central adiposity depots to be a very potent predictor of central arterial stiffness[14]. However, further investigation into the influences of subcutaneous fat did not reveal similar characteristics and may provide protection against cardiovascular risk[40, 45, 46]. Taken together, the deleterious effects of increasing central adiposity seem to play a substantial role in the etiology, diagnosis, and treatment of cardiovascular disease, thus stressing the importance of weight loss and management in public health.

There are several possible mechanisms linking central adiposity with arterial stiffness. First, excess central adiposity depots may increase sympathetic nervous system activity, causing greater vasoconstriction and pressure in the peripheral arteries. Obesity has been shown to increase vasomotor function[47, 48]. Additionally, intentional weight gain of 5 kg reportedly increased muscle sympathetic nerve activity by 15-20% in non-obese, young adults[49] and was positively associated with arterial stiffness, independent of other risk factors[50]. Second, central adiposity may stimulate RAAS activity and, in turn, promote factors contributing to arterial stiffening[51, 52]. Furthermore, adiposity reduction via weight loss was shown to decrease RAAS activity[53]. Finally, central adiposity may promote arterial stiffness through chronic low-grade inflammation[54, 55]. Indeed, inflammation is a major risk factor the development of arterial stiffness and cardiovascular disease. Appropriate lifestyle adjustments such as healthier dietary choices and increase physical activity can reverse obesity's deleterious effects on CVD risk[56].

E. Physiology, Pathophysiology, and Risk Factors of Arterial Stiffness

E.1 Vascular Physiology and Pathophysiology. The arterial tree can be structurally and functionally divided into two subcategories: larger, elastic arteries proximal to the heart (i.e. aorta, brachiocephalic, common carotid, and subclavian arteries) and smaller, muscular arteries located distally in the extremities (i.e. radial, superficial and deep palmer arches, and digital arteries). Elastic arteries perform both a cushioning and pulsatile function, while the muscular arteries modulate blood flow. The tunica adventitia, media, and intima are continuous throughout the entire arterial system. Local anatomical and histological differences in these layers arise from subjective forces and stressors. Additionally, there are normal variations in the tunica's elastin:collagen ratio, media (muscular) thickness, and intimal (endothelial) function along the arteries from the heart to the periphery. Pathologies developed from exposure to

multiple acute and chronic risk factors can disrupt the natural composition, causing the artery to stiffen and promoting cardiovascular-related disorders. It is also important to note alterations in the stiffening process are not uniform throughout the entire arterial system as central arteries tend to have a greater loss in compliance with advancing age[15, 57]. Aortic pressure is physiologically more relevant to the pathogenesis of heart disease as the left ventricle encounters central systolic blood pressure and coronary blood flow is largely determined by central diastolic blood pressure[58, 59].

During the systolic phase of a cardiac cycle, blood ejected from the heart is first 'seen' by elastic arteries. Due to their viscoelastic properties, a portion of the energy from ventricular ejection is transferred into the artery walls causing aortic distention as it accommodates the higher volume. Upon termination of the systolic phase, the arteries recoil, expelling more blood into the periphery during diastole. This normal phenomenon ensures a steady, continuous flow during the entire cardiac cycle. In addition, the elastic components provide a significant buffering capacity to the aorta, dissipating high pressures generated by the ventricle and protecting the fragile capillaries from exposure to stronger pulsatile forces.

Ventricular contraction generates a pulse wave that travels in and along the arterial walls. The speed at which the pulse wave travels is approximately one hundred times faster than blood flow velocity as their units of measurement denote (m/s vs. cm/s, respectively). While the pulse wave occurs independent of blood flow, it is dependent on the structural composition, mainly the elastin:collagen ratio, that compose the majority of arterial walls. In general, arteries with higher elastin:collagen ratios are more compliant and display slower PWV[60]. As the pulse wave travels into the periphery, inherent anatomical sites of impedance (i.e. bifurcations, narrowing of diameter) cause the pulse wave to reflect and travel back to the heart[61]. Wave reflection is a

normal product of the cardiac cycle and plays an important role in delivery of blood flow to metabolically active tissues.

In a healthy individual, the occurrence of a reflective wave serves two favorable purposes: 1) additional diameter augmentation of peripheral arteries propagating blood during diastole and 2) enhancement of coronary artery blood flow during diastole. The first physiological characteristic of reflective waves is referred to as the 'amplification phenomenon', or simply, the increase in pulse pressure along the length of the artery. High peripheral amplification is associated with lower afterloads, lower central blood pressure, and reduced cardiac work. The amplification phenomenon has been shown to diminish with advancing age and arterial stiffness[62], thus increasing the work of the heart to maintain tissue perfusion and systemic pressure. The second favorable property of wave reflection plays a significant role in myocardial perfusion. Due to the nature of myocardial contractile process, the myocardium receives 95% of total blood flow during diastole. Arrival of the reflective wave back to the heart should normally occur in late systole or early diastole, thus facilitating coronary perfusion. In a healthily arteries, forward PWV is naturally slower in compliant arteries and leads to proportionally slower reflective waves. However, as arteries stiffen, arrival time of the reflective wave shifts to early systole and consequently reduces coronary flow. Thus, a reduction in coronary flow, coupled with an increasing work of the myocardium to maintain tissue perfusion is suggestive of high risk for cardiovascular disease.

The etiology of arterial stiffness is a multifactor process potentially involving several pathological mechanisms acting independently and in synchrony. Stiffness is the result of hemodynamic pressure alterations and dysregulation of endothelial function, paracrine mediators, hormones, glucose homeostasis, gene expression, immune function, and lipid

metabolism. Additionally, behavioral factors such as diet composition, tobacco use, and physical activity level can also influence the process. While there are different degrees or levels of arterial stiffness severity, augmented systolic pressure and reduced diastolic pressure leading to higher pulse pressures (PP) are the primary onslaught to the cardiovascular system[63]. Indeed, PP indices are surrogate markers of arterial stiffness[64]. Stiffer arteries will not expand to normal lengths during systole, generating higher afterloads and promoting left ventricular hypertrophy[65]. Buffering capacity diminishes, exposing the fragile microvasculature to excessive pressure pulsatility as the wave travels further into the periphery and potentially injuring delicate capillaries[61]. Stiffer arteries also lose the ability to recoil during diastole, reducing coronary flow and leading to myocardial ischemia[65]. The clinical consequences of arterial stiffness are significant as 1 m/s increase in PWV corresponds to a 15% risk increase in cardiovascular disease and all-cause mortality[66]. Taken together, advancing arterial stiffness sets up a 'perfect storm' for cardiovascular disease risk and end-organ damage.

E.2 Hypertension. Effects of hypertension on arterial stiffness are substantial both in the etiology of the disease and in the structural and functional alterations within the tunicas. Several studies have shown arterial stiffness is significantly increased in hypertensive subjects compared to their aged-match, normotensive counterparts[67-69]. Arteries adapt to repeated cycles of high-pressure fluctuations through remodeling of intracellular and extracellular components. As a result, strong, rigid walls are generated and capable of sustaining and producing higher systolic blood pressures and wider pulse pressures, which further propagates the pathology as a deleterious positive feedback loop[58].

The tunica media of central arteries responds to increasing pressures by accelerating collagen synthesis, as well as elastin fragmentation and depletion[9]. The muscular, peripheral

arteries experience eutrophic or hypertrophic remodeling[70]. Hypertrophic remodeling involves growth in the number and size of VSMC with the addition of extracellular collagen deposition[49]. This type of remolding is primarily responsible for modulation of diameter and peripheral vascular resistance[71].

E.3 Endothelial Dysregulation. The endothelium undergoes different morphological changes in response to shear stress and inflammation. Under normal conditions, the monolayer of endothelial cells (EC) control plasma permeability, inhibit platelet aggregation, and regularly synthesize vasodilating and vasoconstricting factors that modulate blood flow. Altered redox status resulting in oxidative damage and accumulation of advanced glycosylated end products (AGE) impair EC ability to regulate flow and permeability[72]. Phenotypically, EC will express more cytokines and down-regulate several key enzymes involved with arterial health and function. An imbalance between the competing factors regulating growth and migration of VSMC, further impairment of EC activity, and escalation of peripheral vascular resistance occur as a result[73]. Indeed, advanced arterial stiffening is associated with increased EC production of vasoconstricting factors and a reduced production of vasodilatory factors[74].

E.4 Paracrine Mediators. Paracrine mediators associated with arterial stiffness are NO, endothelin-1 (ET-1), and C-type natriuretic peptide (CNP). Both CNP and NO are potent, local vasodilators produced in the EC and activated by endothelial NO synthase (eNOS) and furin, respectively. CNP[75] and NO[76] concentrations increase in response to shear stress and high blood volume, inducing vasodilatation by hyperpolarizing the VSMC. NO also interferes with platelet and leukocyte adhesion, and VSMC intimal migration[77-79]. CNP displays a similar effect as an anti-inflammatory agent, inhibiting vascular adhesion molecules and reducing oxidized LDL-stimulated VSMC migration[80]. NO-mediated vasomotor tone and vascular homeostasis

diminishes after mechanical and oxidative stress injures the EC, attenuating eNOS transcription[73]. Chronic inflammation, diabetes, dyslipidemia, and hypertension are known diseases linked to eNOS inhibition[81, 82]. Additionally, CNP concentrations are mitigated in the presence of advanced arterial stiffness[83]. The precise inhibitory mechanisms of CNP are not clearly understood and require further investigation.

As a potent vasoconstrictor, ET-1 has opposing effects on vasomotor tone to that of NO[83, 84] and CNP[83]. The EC naturally release ET-1 in response to adrenaline and ischemia[85]. ET-1 has been linked to arterial stiffness as its concentration increases in response to angiotensin II and oxidized-LDL, which are known agonists of the stiffness pathology[86]. McEniery et al. found infusions of ET-1 increased PWV 12% using in-vivo models[87]. Furthermore, treatments aimed to inhibit ET-1 or its receptor, ET_A, are efficacious arterial destiffening therapies[73].

E.5 Hormones. Angiotensin II, a hormone involved in the renin-angiotensin-aldosterone system (RAAS), accelerates arterial stiffness. The mechanisms by which angiotensin II contribute to the stiffening process include: blood pressure elevation[88], stimulation of collagen synthesis and elastin fragmentation[89, 90], promotion of VSMC hypertrophy and migration[91], acceleration of NADPH oxidase activity and pro-inflammatory response[92], and alteration of NO signaling patterns[89]. In addition, a hormone downstream of angiotensin II, aldosterone, has been shown to promote vascular stiffening by stimulating ET-1 concentration, VSMC hypertrophy[93], and sodium reabsorption resulting in collagen deposition and arterial wall thickening[65]. Therapeutic blockade of the RAAS system relieves many of the negative influences on vascular structure and function[94].

E.6 Enzymes. Matrix metalloproteinases (MMP) are largely involved with arterial remodeling. MMP dysregulation is associated with arterial stiffness[95]. MMP activity increase while their

endogenous inhibiters, tissue inhibitors of metalloproteinases (TIMP), decline in response to higher hemodynamic pressures, and exposure to immunological stress, advanced glycation endproducts (AGE), and paracrine vasoconstricting mediators; all of which contribute to the stiffening process[96].

MMP are produced by polymorphonuclear neutrophils and macrophages, and function to modulate the elastin:collagen ratio through collagenolytic and elastinolytic activity[97]. Asynchronies in the catalytic process generate stiffer collagen fibers and elastin fragmentation, culminating in reduced arterial compliancy[98-100]. Additionally, MMP stimulate chemotaxis of VSMC through degradation of the intimal basement membrane resulting in smooth muscle cell migration and intimal-medial thickening (IMT)[101]. Of the 23 known human MMP's, MMP-2 and MMP-9 activity are positively associated with arterial stiffness[102]. Medical treatments targeting MMP-2 and MMP-9 are complicated as their beneficial roles in angiogenesis and arterial remodeling, and similar composition with other MMP can cause vascular injury[103].

E.7 Glucose Homeostasis. Impaired glucose tolerance is strongly associated with vasculopathology. Indeed, diabetics are at a significant risk of developing arterial stiffness. Chronic hyperglycemia and hyperinsulinemia have been shown to accentuate AGE production and stimulate stress signaling, RAAS activity, and generation of reactive oxygen species (ROS)[60]. AGE activity further perpetuates the stiffening process through stimulation of VSMC growth factors, intercellular adhesion molecules (ICAM), ROS and MMP activity, and insulin resistance[104].

E.8 Genetics. Arterial stiffness appears to have a significant genetic component. Heritable contributions of PP and PWV is estimated to range from 0.21 to 0.66, suggesting distinct chromosomal region linkages[105]. Polymorphisms of elastin[106], MMP[107], RAAS[108, 109], and

eNOS[110] have moderate to substantial genetic associations with arterial stiffness. Additionally, data from the Framingham Heart Study has linked multiple genes on chromosomes 2, 7, 13 and 15 with PWV and PP, independent of age[111]. Genetic treatment options are presently unavailable as arterial stiffness is associated with multiple polymorphisms and gene abnormalities, complicating genetic therapeutic options.

E.9 Inflammation. A positive relationship between inflammation and arterial stiffness has been demonstrated in patients with acute and chronic inflammatory diseases[112, 113] . Pro-inflammatory mediators have negative influences on vasomotor tone, VSMC migration, vascular remodeling, endothelial function, and MMP activity[92]. Inflammation reduces arterial distensibility and increases afterload, placing greater stress on the myocardium independent of age[114, 115]. Indeed, vascular inflammation acts in concert with metabolic and neuro-hormonal dysregulation, playing a significant role in the pathogenesis of cardiovascular disease[116].

Vascular inflammation stimulates components of RAAS, further propagating arterial stiffness by aforementioned mechanisms. As a powerful stimulator of NADPH oxidase, angiotensin II signaling stimulates ROS and cytokine production[117, 118]. ROS is a potent inhibitor of eNOS and limits EC ability to modulate vasomotor tone. ROS also stimulates MMP activity, generating collagen crosslinking and chemotaxis of VSMC[97].

Cytokine signaling stimulates pro-inflammatory pathways triggering VSMC proliferation, endothelial destruction, and vascular fibrosis[92]. As such, tumor necrosis factor- α (TNF-α) and interleukin-6 (IL-6) have been identified as surrogate biomarkers of arterial stiffness[119]. IL-6 and its soluble receptor (sIL-6r) were recently found to be significantly correlated with markers of endothelial dysfunction (E-selectin and VCAM) and PWV[120]. TNF-α promotes dyslipidemia and pro-inflammatory pathways involved with the development of

arteriosclerosis[121]. Inhibitors of TNF- α significantly improved arterial compliance and vascular function in patients with rheumatoid arthritis and other chronic inflammatory diseases[122]. Additionally, high-sensitivity C-reactive protein (hsCRP) is a independent predictor of cardiovascular risk[123]. Produced by the liver and VSMC in response to circulating cytokines such as IL-6, hsCRP is both a product of vascular inflammation and mediator of arterial stiffness. hsCRP stimulates platelet and leukocyte adhesion, and promotes collagen synthesis and elastin degradation. Several studies have found direct relationships between hsCRP and endothelial dysfunction[124, 125], PWV[116, 126], and decreased arterial elasticity [123].

E.10 Dyslipidemia. Dyslipidemia is characterized by the reduction of high-density lipoprotein (HDL) cholesterol, and elevation of low-density lipoprotein (LDL) and total cholesterol. Through mechanisms independent of atherosclerotic plaque formation, dyslipidemia negatively impacts arterial stiffness[127]. LDL cholesterol is prone to oxidation and glycation, enabling it to penetrate the endothelial lining and modulate arterial remodeling. Epidemiological research indicates a positive relationship between BMI and oxidized-LDL cholesterol in aging adults[128]. The pro-inflammatory effects of oxidized-LDL cholesterol cause peroxynitritate formation from NO, inhibiting eNOS activity and reducing NO bioavailability[129]. Oxidized-LDL also augments IMT by stimulating VSMC hypertrophy[130]. It is unknown how other lipids influence arterial structure and function. Thus, further research is needed to fully characterize the relationship between arterial stiffness and apoproteins, triacylglycerides, diacylglycerides, and HDL cholesterol.

E.11 Lifestyle. Development of arterial stiffness is affected by many modifiable lifestyle factors. These factors include habitual dietary patterns (i.e. caffeine, sodium, saturated fatty acids (SFA), and alcohol consumption), smoking, and physical inactivity. Most of these factors

work synergistically with the pathologies previously discussed thus greatly contribute to arterial stiffening. Not surprisingly, beneficial changes to these lifestyle factors have often delayed and in many cases reversed the deleterious effects of arterial stiffness on the cardiovascular system and other disease risk.

E.12 Caffeine Consumption. Daily consumption of caffeinated products increases arterial stiffness variables both acutely[131] and chronically[132]. Studies utilizing various doses (80 to 300 mg caffeine/day) reported increases in central blood pressure[131, 133], carotid-femoral PWV (CF-PWV)[132, 134], and AIx[135]. Caffeine exerts a titration effect on arterial vasoconstriction properties causing attenuation of adenosine concentrations while at the same time augmenting catecholamine release[136]. Consequently, peripheral resistance remains the primary mechanism of action by which caffeine augments arterial stiffness. Additionally, there is a J-shaped relationship between caffeine use and coronary artery disease[137]. The beneficial effects of caffeine in moderate doses occur from the polyphenol content specifically found in coffee[138]. Further investigations into different varieties and doses of caffeinated products on vascular physiology are warranted.

E.13 Sodium Intake. Numerous studies have reported positive correlations between daily sodium intake and blood pressure[139-142]. Sodium consumption raises blood plasma osmolality and as a consequence, more water is reabsorbed at the descending limb of the Loop of Henle[143]. Thus, acute consumption of sodium causes greater plasma volumes and cardiac output resulting in higher systolic blood pressures[142]. Epidemiological and salt loading studies have provided some insight into sodium's impact on arterial stiffness. Todd et al. overloaded subjects with daily amounts of sodium for one month and found significant increases in PWV independent of blood pressure, indicating modulations in the vascular remolding process[144]. Sodium-sensitive

and sodium-resistant aged-matched hypertensives were also found to have no changes in blood pressure, cardiac output, or blood volume after chronic sodium treatment, suggesting differences in arterial compliance between groups[145]. Additionally, a study conducted on rural and urban communities reported significantly lower PWV in the rural community subjects, whose daily sodium intake is significantly lower, compared to their aged- and blood pressure-matched urban counterparts[146]. The blood pressure-independent mechanism responsible for the association between sodium consumption and arterial stiffness remain unclear. As such, further investigation is needed.

E.14 Lipid Consumption. The relationship between SFA and arterial stiffness is not well characterized. Few randomize controlled trials (RCT) have tested the dose response effect. Currently, there are no RCT on the effects of chronic consumption. Keogh et al. reported flow mediated dilation (FMD) impairment after subjects consumed a high SFA diet for three weeks[147]. FMD reportedly improved when the diet was discontinued. Participants in the maximum SFA intake quartile from the Caerphilly Prospective Study were found to have highest arterial PWV after a 17.8 year follow-up[148].

SFA are known to cause deterioration of vascular function through stimulation of pro-inflammatory pathways. In vitro studies of cultured human endothelial cells treated with palmitate showed increased expression of IL-6[149]. Additionally, SFA inhibition of NO was attenuated after administration of superoxide dismutase in rabbit aortic cells[150]. It is not known if SFA influence other arterial stiffness mediators. Further investigations are needed to elucidate differential influences of SFA on arterial stiffness.

E.15 Alcohol Consumption. There is a J-shaped association between alcohol consumption and arterial stiffness markers[151, 152]. The type of alcohol consumed (i.e. beer, wine, or spirit) does not

appear to have an additional impact on arterial pathologies[151]. While moderate consumption (10-14 beverages/week) has beneficial effects on lipid metabolism[153] and modulation of pro-inflammatory pathways[154], negative influences of vascular remodeling have been observed at higher consumption rates (22-58 beverages/week) [193]. Individuals consuming excessive amounts of alcohol were shown to have higher AIx and central blood pressures compared to control[155]. Chronic alcohol abuse lengthens ventricular contraction during systole and is positively correlated with AIx [151]. The mechanism by which this occurs is currently unknown and merits further investigation.

E.16 Smoking. Exposure to primary and second-hand smoke has deleterious effects on hemodynamic properties of the central and peripheral vasculature. Currently, 19.3% of American adults are classified as tobacco smokers[156]. Smokers were found to have higher PWV and AIx compared to nonsmokers[157]. Liang et al. showed that β-stiffness index was significantly correlated to frequency and years of smoking[158]. Similarly, Levenson et al. found smoking to increase arterial stiffness independent of blood pressure as normotensive smokers had significantly higher PWV values compared to normotensive nonsmokers[159]. Evidence suggest that smoking augments stiffness through impairment of eNOS activity[160, 161], platelet aggregation[162], and calcification of the aorta's intimal tunica[163]. Smoking cessation, regardless of total years smoked, was shown to improve vascular stiffness indices after six months[164].

E.17 Physical Inactivity. Physical inactivity is associated with changes to hemodynamic stimuli instigating vascular remodeling and phenotypic characteristics consistent with arterial stiffness. Most of the alterations appear to occur in the muscular arteries, while the effects on central arteries remain less understood[165]. Sedentary individuals have lower vascular compliance compared to aged-match, physically active counterparts[12, 166]. Inactivity was found to be

positively correlated with PWV and AIx in hypertensive adults compared to their aged-matched, normotensive counterparts[167]. Additionally, NO concentration and eNOS activity were reportedly decreased in a sedentary population[168]. Endothelial superoxide dismutase activity was also lower in this population, enhancing the potential for exposure of NO to oxidants and further perpetuating NO degradation. NO primarily influences muscular artery hemodynamics. Thus, attenuation of NO bioactivity could explain some of the discrepancies seen between central and peripheral arteries.

F. Therapeutic Destiffening Strategies

Current approaches for arterial destiffening include pharmacological treatments and therapeutic lifestyle modification. While the first step in any CVD treatment plan should focus on improving dietary and physical activity outcomes, for many individuals these are not viable options. Depending on the severity of stiffness and other CVD risk factors, individuals may require aggressive forms of treatment including pharmacological therapy and possibly surgical intervention.

Arterial stiffness is the result of many pathologies working independently or synergistically with one another, therefore, multiple pharmacological options have been examined and utilized for treatment[169-172]. These medications include β-blockers (BB), angiotensin converting enzyme inhibitors (ACEi), angiotensin receptor blockers (ARB), calcium channel blockers (CCB), diuretics, 3-hydroxy-3-methyl-glutaryl-CoA reductase inhibitors (statins), and alagebrium (ALT-711). In many cases, combinational therapies have proven to be superior treatment strategies[171, 173].

F.1 β-Blockers. Known for their negative intropic and chronotropic effects on the myocardium, BB antagonize β-adrenergic receptors limiting sympathetic drive and thus attenuate both heart

rate and strength of contractility[174]. Originally, this mechanism was thought to improve myocardial oxygen supply and ventricular demand. However, the first generation BB had limited cardio-selectivity and promoted unfavorable side effects. The CAFÉ[175], REASON[176], ASCOT[177], and LIFE[178] studies found atenolol decreased heart rate and brachial SBP but, somewhat paradoxically, increased central SBP and AIx. Furthermore, an increase in all-cause mortality and cardiovascular disease risk in patients taking first generation BB therapy when compared to other medications casted doubts on the overall effectiveness of these drugs in treating CVD[175]. Lower heart rates generate longer ejection durations, providing additional time for the reflective wave to return during systole and increasing afterload and decreasing diastolic function. To limit the impact of BB on pulmonary β-2 receptors, second generation BB appeared on the market. Similar problems with increased afterload and diminished coronary flow also occurred with this generation[179]. The role of traditional BB in the management of CVD was questioned by many, leading many to call for their dismissal from the first- and second-line of antihypertensive therapy[179-181]. Additionally, diuretics, ARB, and ACEi therapy were recommended as the primary pharmacological treatment strategy for arterial stiffness[172, 173, 182].

Nebivolol. The introduction of the third generation BB, nebivolol, corrected major issues of previous generations through cardio-selective β-1 antagonism and β-3 agonism[183]. The chronotropic effect on AIx was negligible as β-3 stimulation up-regulated NO production by eNOS causing vasodilation in the peripheral arteries and reducing the impact of reflective waves on central mechanics[184]. Nebivolol has minimal influence on heart rate reduction, thus eliminating the paradoxical cardiovascular response of pervious BB[185]. Furthermore, beneficial influences on lipid and carbohydrate metabolism, endothelial function, vascular inflammation, and patient tolerability justified nebivolol's utilization in treating uncomplicated hypertension.

Nebivolol's mechanism of action targets β-1 and β-3 receptors of the heart, as well as β-3 receptors in the endothelial cells of peripheral arteries[184]. Antagonism of β-1 receptors reduce heart rate by blocking adenocyclase from converting ATP into cAMP, thereby limiting protein kinase A from activating sodium and potassium ion gated channels, thus creating a hyperpolarized environment inside the myocardium[186]. Improvements in diastolic function, stroke volume, and cardiac output occur as a result[187]. Agonism of β-3 receptors, a G protein complex, signals inositol-3-phosphate (IP_3) activation[188] and intercellular calcium release from the endoplasmic reticulum, thus up-regulating intracellular calmodulin concentrations and directly stimulating NO synthesis from eNOS and L-arginine[189]. NO from the endothelial cells diffuse to the tunica media stimulating potassium ion channels on the sarcolemma, hyperpolarizing the smooth muscle cell and prompting vasodilatation[187].

Several RCT examining arterial stiffness with nebivolol monotherapy on indirect correlates have reported favorable outcomes and patient tolerability[190]. Nebivolol has been shown to lower blood pressure values repeatedly in clinical trials[191]. A large, multicenter trial investigating the antihypertensive efficacy of nebivolol 5mg, 10mg, and 20mg reported similar reductions in SBP and DBP from baseline[192]. However, no additional vascular benefits occurred with doses >10mg[184]. Nebivolol significantly lowered central PP compared to first generation BB atenolol[193-196] and metoprolol[197], further supporting usage of BB therapy for stiffness and cardiovascular therapy. The SENIORS study showed nebivolol reduced all-cause mortality of heart failure patients by 12%, and of those with ejection fractions ≤35%, nebivolol significantly reduced all-cause mortality by 38%[198]. Additionally, plasma glucose and lipid profiles were shown to improve after nebivolol therapy[199-202]. The mechanism of action is believed to occur through the reduction in serum triglycerides, alleviating lipotoxicity influences on pancreatic β-

cell function and improving glucose sensitivity and vascular function. Finally, nebivolol has demonstrated significant inhibition of oxidative stress factors[203-205] and VSMC proliferation[206, 207], thus improving elastic properties of the arterial wall.

Few studies have examined the influences of nebivolol on direct arterial stiffness indices. McEniery et al. reported C-F PWV significantly declined in anesthetized sheep after acute nebivolol therapy[208]. In human trials, C-F PWV was shown to decrease 1.2-1.5 m/s after 15 days[209] and 4 weeks[194] of nebivolol treatment. Conversely, a study conducted on hypertensive type 2 diabetics reported no changes in C-F PWV following 12-weeks of nebivolol therapy[210]. However, this was a pilot study with 10 subjects and therefore most likely inadequately powered to detect significant differences. Currently, there are no RCT examining the influence of nebivolol on β-stiffness index. Further investigations on local modulations with nebivolol therapy are warranted.

F.2 Renin Angiotensin Aldosterone System Blockers. Inhibitors of the RAAS have additional cardiovascular benefits beyond their antihypertensive properties. Angiotensin II blockade reduces pro-inflammatory and pro-fibrotic pathways, improving arterial compliance and central blood pressure[171]. ACEi lowered C-F PWV, central blood pressure, and AIx while enhancing arterial compliance in hypertensive adults[211-214]. Similarly, ARB therapy increased aortic compliance and distensibility in subjects with essential hypertension as determined by PWV and AI[215]. β-stiffness index and IMT significantly decreased after 24-months of valsartan treatment in 24 hypertensive adults[88]. Similar improvements in stiffness correlates occurred with hypertensive diabetics, independent of blood pressure reduction[216]. Additionally, the extent of vascular improvement does not appear to differ between ACEi and ARB therapy[173, 217].

F.3 Calcium Channel Blockers. Extensively used in the treatment of hypertension, CCB therapy target arterial stiffness indices and improve vascular function. CCB increased common carotid and aortic root diameter and distensibility after four months of treatment in hypertensive adults[218]. Additionally, the CCB, amlodipine, significantly reduced central blood pressure, CF-PWV, and AIx in hypertensive diabetics[216].

F.4 Diuretics. Thiazide-type diuretics are traditionally recommended as the first line of therapy for most hypertensive patients[219]. The diuretic hydrochlorothiazide improved carotid compliance and reduced radial IMT following nine months of treatment[220]. In addition, vascular improvements were significantly correlated with blood pressure reduction. Currently there are no RCT examining the effects of chlorthalidone on arterial stiffness. Chlorthalidone has a longer duration of action and greater potency in the treatment of hypertension compared to hydrochlorothiazide[221]. Further investigations will be required to compare the destiffening effects of chlorthalidone and hydrochlorothiazide.

F.5 3-Hydroxy-3-Methyl-Glutaryl-CoA Reductase Inhibitors. Statins are hypolipidemic agents and have significant pleiotropic destiffening effects via their anti-inflammatory properties. Statins reduce surrogate markers of arterial stiffness including: VSMC migration, MMP activity, platelet adhesion, IL-6, VCAM, ICAM, and hsCRP in human trials[222]. Few studies have examined direct indices of arterial stiffness and some have reported negative side-effects[223]. Atorvastatin therapy reduced hsCRP, aortic PWV, and β-stiffness index follow 12-weeks of treatment[224]. After one year of therapy, fluvastatin significantly decreased aortic PWV and hsCRP with no influence on blood pressure[225]. However, the same study reported pravastatin did not improve arterial stiffness indices. Due to the lack of RCT and inconsistencies in the

literature, Rizos et al. has argued for more clinical trials before sanctioning statin therapy in the treatment of arterial stiffness[223].

F.6 Alagebrium. ALT-711 nonenzymatically cleaves AGE crosslinking and improves arterial compliance. Eight weeks of ALT-711 therapy significantly reduced AIx, procollagen type I propeptide (a marker of vascular fibrosis), and MMP-9 activity in older hypertensive adults[226]. A multicenter study involving 92 subjects reported reductions in SBP, PP, and C-F PWV following 56 days of ALT-711 treatment[227]. Additionally, a study on the independent and combined influences of ALT-711 and exercise showed significant reductions in CP-PWV, with the greatest impact on arterial stiffness resulting from the combination of ALT-711 and exercise[228].

F.7 Lifestyle Modification. Lifestyle modification is a potent component of many CVD risk reduction strategies. The combined and independent influences of nutrient intake, exercise, and weight loss on vascular function are well documented in the literature[56, 136]. Several studies working with overweight and obese subjects have shown significant improvements in arterial compliance, arterial distensibility, and inflammation following the weight loss interventions[229-233]. While the precise mechanism of action is remains elusive, it does not appear to be directly associated with blood pressure attenuation. Rather, reduction in sympathovagal tone[65], RAAS influence on inflammatory pathways[65], circulating blood volume[234], MMP activity[235], and augmentation of NO bioactivity[65] are probable mechanisms for the reported improvements.

Nutrient Intake. Macro- and micronutrient intake influence vascular remodeling and pathophysiology via their interactions with inflammatory pathways. Soy isoflavones, PUFAs, and potassium chloride appear to be beneficial, while SFA and sodium chloride can exacerbate

the pathology. Soy isoflavones ranging from 80-118 mg/day increased arterial compliance and decreased PWV in healthy adults[236-238]. Clinical trials examining SFA diets consistently show deleterious effects on vascular function, while high omega-3 PUFA consumption appears to be beneficial[239, 240]. Sodium chloride loading was shown to increase C-F PWV and AIx after several weeks in hypertensive adults[144, 241]. Currently, there is insufficient evidence for vitamin supplementation including vitamins B, C, and E as practical arterial stiffness treatment options, prompting Pase et al. to withhold recommendations[138]. The American Dietetic Association recommends avoiding fast food, sugary beverages, processed foods and to incorporate more nutrient dense foods including fruits and vegetables, low-fat milk products, and fish as to attain the beneficial vascular effects from foods and associated nutrients.

Weight loss. Weight loss from hypocaloric diets are efficacious strategies for CVD risk reduction including arterial stiffness[232, 242-244]. The primary mechanism of action occurs through reductions in inflammatory mediators. IL-6[245, 246], hsCRP[247], and TNF-α[248, 249] have all been shown to decline following caloric restriction diets. Pierce et al. reported reductions in abdominal obesity and oxidized LDL were associated with improvements in endothelial function following 12-weeks of a restricted caloric diet[250]. Similarly, Miyaki et al. found hypocaloric diets significantly reduce β-Stiffness index and aortic PWV, and improved NO bioavailability following the 12-week intervention[251]. In addition, the variety of hypocaloric diets may have minimal influence on arterial stiffness outcome. After providing 36 overweight or obese women with plant- or meat-base diets for 16 weeks, Yamashita et al. concluded stiffness indice improvements were independent of diet composition[252].

Exercise. Aerobic exercise consistently improves arterial stiffness correlates[253], while the benefit of resistance training remains controversial[254]. Regular endurance-type exercise increases NO

bioavailability[255], endothelial function[256], and reduces oxidative stress[257-259]. Reductions in IMT, collagen cross-linking and deposition, and elastin fragmentation are phenotypic alterations resulting from exercise adaption. Recommendations of low-moderate (40% maximal oxygen consumption (VO_2max)) to moderate (50% VO_2max) intensities[260] utilizing walking, running, and/or cycling modalities[256] have beneficial effects on vascular remodeling.

Resistance training is not recommended for treatment[64]. A recent meta-analysis of eight RCT found resistance training increased β-stiffness index on average by 10.7% and PWV by 14.3%[254]. Physiological consequences occur from increased sympathetic nervous activity and higher blood pressures generated during the exercise bout, placing additional strain on the tunica. However, a few RCT on resistance training have shown no significant influence on arterial stiffness[261-265]. Methodological issues involving differences in subject age range, total volume, total repetitions, and muscle group trained (i.e. core, upper, and lower body muscle groups) make comparison across studies difficult. Interestingly, when combined with aerobic training, resistance training has minimal impact on arterial stiffness[266-268]. Indeed, more research on the physiological mechanisms underlying combinational training is merited.

G. Conclusions

Arterial stiffness is an independent predictor of CVD and all-cause mortality. Hypertensive middle-aged and older adults are at increased risk for developing arterial stiffness and CVD. Importantly, there is a need to develop efficacious, cost-effective arterial destiffening treatments including lifestyle modification and pharmacological therapy for middle-age and older hypertensive adults. Current lifestyle modification guidelines recommend increasing aerobic exercise and restricting caloric, sodium, and SFA intake as part of first-line therapy of hypertension. Antihypertensive, anti-inflammatory, and hypolipidemic agents are potent

pharmacological approaches for reducing advanced stiffness. One promising therapeutic option is the third-generation BB, nebivolol. Nebivolol's mild chronotropic influence, augmentation of NO bioavailability, and antioxidant properties may provide additional benefits beyond the antihypertensive actions. However, complete characterization of nebivolol's ability to reduce arterial stiffness with or without lifestyle modification requires further study. Therefore, additional investigations are required to determine the efficacy of combined strategies. As such, the findings may provide clinically significant destiffening therapies and, in turn, improve the health of individuals suffering from hypertension and other cardiovascular diseases.

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CHAPTER III

Effect of Nebivolol and Lifestyle Modification on Large Artery Stiffness in Middle-Aged and Older Hypertensive Adults

Abstract

We hypothesized that the combination of nebivolol and lifestyle modification would reduce large artery stiffness in middle-aged and older hypertensive adults more than either intervention alone. To address this, 45 men and women (age=40-75 years) were randomized to receive either nebivolol (NB) (forced titration to 10 mg OD) (n=15; age: 57.2±11.4 years; body mass index: 30.8±5.8 kg/m2), lifestyle modification (LM) (5-10% weight loss via calorie restriction and physical activity) (n=15; age: 52.7±8.5 years; body mass index: 33.9±7.2 kg/m2) or nebivolol + lifestyle modification (NBLM) (n=15; age: 58.9±9.4 years; body mass index: 32.5±4.9 kg/m2) for 12 weeks. Carotid-femoral pulse wave velocity (C-F PWV) was measured via tonometry and beta-stiffness index was measured via high resolution ultrasound and tonometry at baseline and after the 12-week intervention. There was no difference between groups in age, body weight or composition, blood pressure, or in measures of large artery stiffness at baseline (all $P>0.05$). Following the 12-week intervention period, body weight decreased ~5% ($P<0.05$) in the LM and NBLM groups but did not change from baseline in the NB group ($P>0.05$). While C-F PWV declined only in the NB and NBLM groups, β-stiffness index, a blood pressure independent measure of arterial stiffness declined similarly ($P>0.05$) in the LM, NB, and NBLM groups, respectively (-1.87±0.83; -2.03±0.60; and -2.51±0.90 U). In summary, our findings indicate that the combination of nebivolol and lifestyle modification reduce arterial stiffness to a similar degree as either intervention alone in middle-aged and older hypertensive adults.

Key Words: *Antihypertensive, Beta-blocker, Arterial distensibility*

Introduction

Large artery stiffness increases with advancing age, obesity, and hypertension[1, 2]. Indeed, arterial stiffness is an independent risk factor for a first cardiovascular event and both CVD and all-cause mortality, particularly among hypertensive adults[3, 4]. Many anti-hypertensive medications[5, 6] and lifestyle modification[7, 8] have been shown to reduce large artery stiffness. First- and second-generation beta-blockers are very effective peripheral blood pressure reducing agents, however, paradoxical increases in large artery stiffness have been observed[9, 10]. Nebivolol, a third-generation beta-blocker with potent beta-3 agonist and cardio-selective beta-1 antagonism[11, 12], has been reported to augment nitric oxide bioavailability[13], and reduce peripheral vascular resistance[14, 15] and oxidative stress[16, 17] in patients with hypertension. However, whether nebivolol reduces large artery stiffness is unclear.

There is only limited evidence to support a role for nebivolol in reducing large artery stiffness. However, the use of indirect, blood pressure dependent indices of arterial stiffness make the available studies difficult to interpret[18-20]. Furthermore, although lifestyle modification is the cornerstone of hypertension management to our knowledge there are currently no studies that have examined the independent and combined influence of nebivolol and lifestyle modification on large arterial stiffness. Therefore, we hypothesized that the combination of nebivolol and lifestyle modification would reduce central arterial stiffness in middle-aged and older hypertensive adults more than either intervention alone.

Materials and Methods

Subjects

Forty-five men (n=21) and women (n=24) 40 to 75 years of age volunteered for the study. Subjects were hypertensive (BP ≥ 140 and/or 90 mmHg and <160 and/or 100 mmHg) and free from overt chronic disease. None of the subjects were taking medications that affect variables of interest at the time of the study. Subjects receiving anti-hypertensive therapy prior to the study discontinued their medications 2-weeks before baseline testing. All subjects were weight stable (± 2 kg) and sedentary (<30 min/wk of low impact activity) for at least six months prior to joining the study. The Virginia Polytechnic and State University Institutional Review Board approval all study protocols. The nature, purpose, risks, and benefits were explained to every subject prior to obtaining informed consent.

Experimental Design and Protocol

Following the completion of baseline testing, subjects were randomly assigned to nebivolol (NB) (n=15), lifestyle modification (LM) (n=15), or the combination of nebivolol and lifestyle modification (NBLM) (n=15) groups. Subjects randomized to NB or NBLM groups began with 5 mg/day of nebivolol and dosages were increased to 10 mg/day if mean resting blood pressure was greater than 120/80 mmHg during the first two weeks of therapy. Nebivolol was provided bimonthly with a randomized extra amount of pills. Pill bottles were to be returned every two weeks to assess compliance. Participants in the NB group were instructed to maintain their current body weight, habitual dietary intake, and physical activity level. Subjects randomized to the LM and NBLM groups received weekly lifestyle counseling by a registered dietitian to ensure adequate progress and compliance. Sample menus, 14-days of meal plans, and grocery shopping lists were provided with their food preferences taken into consideration to increase adherence. They were instructed to reduce their daily caloric intake by 500-1000 calories and sustain a minimum of 150 minutes per week of moderate-intensity physical activity

or 3000 steps/day above baseline levels. The diet plan conformed to the DASH dietary guidelines emphasizing low fat dairy products, fruits and vegetable and contained 55% calories as carbohydrates, 30% calories as fat, and 15% calories as protein[21]. Sodium consumption was set at 2,400 mg/day for all subjects. All measurements were conducted in the Virginia Tech Human Integrative Physiology Laboratory between 7:00 AM and 11:00 AM after a 12-hour fast having refrained from caffeinated products for 24-hour and exercise for 48-hours prior to each testing session. All subjects reported being free of acute illness and infections at least 1-week prior to testing day.

Measurements

Body weight was measured to the nearest 0.1 kg using a digital scale (Model 5002, Scale-Tronix, Inc) at baseline and every week throughout the 12-week intervention. Height was measured to the nearest 0.1 cm using a stadiometer. Body composition was measured by dual energy x-ray absorptiometry (DEXA) (Lunar Prodigy Advance, GE Medical Systems) and using software version 8.10e.

Resting blood pressure measurements of the right brachial artery were performed every week throughout the treatment period. Subjects were instructed to avoid consuming caffeinated products 12-hours prior to the visit. The recordings were made in quiet and comfortable conditions, strictly conforming to the American Heart Association guidelines[22]. Subjects rested in a seated position for 10 minutes prior to the automated sphygmomanometry (Pilot model 9200, Colin Instruments Corp.). Measurements were continuously taken every 3 minutes until blood pressure stability (±6 mmHg difference for both systolic and diastolic blood pressure among three sequential measurements) was achieved.

To determine habitual dietary intake and compliance, all participants completed a detailed 4-day diet record (consecutive 3 weekdays and 1 weekend day). Two diet records were collected at baseline and week-12 of the intervention. Total caloric, macronutrient, and micronutrient intake were analyzed using the Nutrition Date Systems for Research (NSD-R 6.0, University of Minnesota) software. Daily step counts were measured using pedometers (Accusplit Eagle 120XL). Pedometers were return every 2-weeks to assess compliance with the daily physical activity instructions.

Plasma lipid and lipoprotein concentrations were quantified using conventional methods in a commercial laboratory. Plasma glucose concentrations were determined with a YSI Stat Plus glucose analyzer (model 2300,Yellow Springs Instruments). High sensitivity C-reactive protein (hsCRP), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) plasma concentrations were measured by enzyme-linked immunosorbent assay (ELISA) (R&D Systems). Oxidized LDL was measured with a commercially available ELISA (ALPCO). Plasma insulin concentrations were determined using a commercially available ELISA (Diagnostic System Laboratories). Insulin sensitivity was estimated by the homeostasis model assessment (HOMA) approach. The HOMA index was calculated by the product of plasma blood glucose and insulin divided by 22.5.

β-stiffness index was measured with an ultrasound unit (Sonos 7500, Philips Medical Systems) equipped with high resolution linear array transducer (3-11 MHz) and applanation tonometry carotid blood pressure acquisition (NIHem, Cardiovascular Engineering, Inc.) after 20 minutes of quite rest in a supine position. Longitudinal B-mode images of left common carotid artery diameter 1-2 cm proximal to the carotid bulb were obtained over the course of 15 consecutive cardiac cycles. The transducer was placed at a 90^0 angle directly over the artery for

clear visibility of the near and far walls. The images were stored on optical disks for offline quantifications of systolic and diastolic carotid artery diameters using commercially available software (Vascular Research Tools 5, Medical Imaging Applications, LLC). A high-fidelity, non-invasive pulse tonometer measured left common carotid, brachial, radial, and femoral arterial pressure waveforms and amplitudes. The average of three stable left brachial blood pressure recordings (within ±5 mmHg for both systolic and diastolic blood pressure among three sequential measurements) were taken every 2 minutes by a sphygmomanometer (NIHem, Cardiovascular Engineering, Inc) and were used to calibrate peak and trough single-averaged waveforms. Electrocardiographic R-waves were recorded simultaneously and served as the fiducial point for signal-averaging tonometry waveforms.

Pulse wave velocity for carotid-femoral recordings were measured using applanation tonometry (NIHem, Cardiovascular Engineering, Inc.). Correct hold down pressure and waveform amplitude simultaneously guided the analyzer for optimal recordings. Body surface sites were measured as the distance from the suprasternal notch (SSN) to the recorded site using a measuring tape for carotid site and an enlarged caliper for the femoral site to the nearest 0.5cm. β-stiffness index was calculated as: $\beta=(\log P_1/P_0) / ((D_1-D_0)/D_0)$, where D_0 was the minimal diameter recorded during diastole, D_1 was the maximal diameter recorded during systole, P_0 was the minimal pressure measured during diastole, and P_1 was the maximal pressure measured during systole.

Statistical Analysis.

Repeated measures analysis of variance was used to examine the between and within groups differences on arterial stiffness and other dependent variables from baseline to follow-up

testing. If interactions were significant, a post-hoc comparison utilizing the Tukey procedure was conducted to make pairwise comparisons between groups. It should be noted that the present study was not powered to detect significant gender or racial differences between the random variables of interest. All statistical analyses were conducted using SPSS version 12 software. Data is expressed as means±SE. The significance level was set *a priori* at $P<0.05$.

Results

Subjects' characteristics before and after the intervention are shown in Table 1. There were no baseline anthropometric differences in age, weight, body mass index (BMI), body fat percentage, fat mass, and fat free mass (all P>0.05). There were no differences in supine resting heart rate, brachial SBP and DBP, and carotid SBP and DBP between groups (all P>0.05). In addition, baseline daily physical activity was not different in the LM and NBLM groups (P>0.05).

Following the 12-week intervention, there was a significant decreased in body weight (-5.55±0.59 and -5.35±0.97 kg), corresponding to -6.1 and -5.6% of initial body weight in the LM and NBLM groups, respectively. BMI (-1.92±0.20 and -1.77±0.34 kg/m2), body fat percent (-1.83±0.32 and -0.87±0.59 %), fat mass (-3.79±0.36 and -2.67±0.72 kg), and fat free mass (-1.76±0.35 and -2.69±0.62 kg) decreased in the LM and NBLM groups, respectively. There was a significant increase in step count (1701±550 and 2032±669 steps/day) in the LM and NBLM groups, respectively. There were no changes in body weight, BMI, fat mass, and fat free mass (all P>0.05) in the NB group following the 12-week intervention. Supine resting heart rate decreased (-5±2, -10±1, and -12±2 bpm) in the LM, NB, and NBLM groups, respectively. Supine brachial SBP and DBP decreased (-5±2/-2±1, -9±3/-6±2, and -9±3/-7±2 mmHg; all P<0.05) in the LM, NB, and NBLM groups, respectively. Additionally, supine carotid SBP and

DBP decreased (-4±2/-2±1, -5±4/-7±2, and -7±4/-7±2 mmHg; all $P<0.05$) in the LM, NB, and NBLM groups, respectively. The magnitude of change was not significant between groups. Compliance to nebivolol was 98.2% overall with no individual missing more than three daily doses. Five subjects (2 from the NBLM group) remained on the 5mg/day dose while 25 participants had dosages increased to 10mg/day for the duration of the study. There was no difference in dosage received in the NB and NBLM group.

Habitual dietary intake before and after the intervention are shown in Table 2. Baseline carbohydrate intake (% of total) was higher ($P>0.05$) in the NBLM compared to LM group. There were no other baseline differences in macro- or micro-nutrient intake (all $P>0.05$).

Following the 12-week intervention, the total calorie intake declined (-442±144 and -399±132 Kcal; $P<0.05$) in the LM and NBLM groups, respectively. Total caloric intake did not change ($P>0.05$) in the NB group during the intervention. There were no changes in macronutrient composition in the three groups following the treatment period (all $P>0.05$). Cholesterol intake decreased (-107±47 and -47±47 g; $P<0.05$) in the LM and NBLM groups, respectively. Cholesterol intake increased (40±46 g; $P<0.05$) in the NB group. Saturated fatty acid intake significantly decreased (-11±3 and -7±3 g) in the LM and NBLM groups, respectively, while it increased (10±4 g) in the NB group. Monounsaturated fatty acid consumption decreased (-10±3 and -8±3 g; $P<0.05$) in the LM and NBLM groups, respectively. Trans fatty acids intake decreased (-2±1, 1±1, and -2±1 g; $P=0.04$) in the LM, NB, and NBLM groups, respectively. Daily sodium intake changed (-516±230, 373±306, and -577±303 g; $P=0.049$) over the treatment period in the LM, NB, and NBLM groups, respectively. There were no other differences in dietary intake between groups or following the intervention.

Circulating metabolic and cardiovascular disease risk factors before and after the intervention are shown in Table 3. There were no baseline metabolic and cardiovascular disease risk factor differences (all $P>0.05$).

Plasma triglycerides, total cholesterol, plasma HDL-cholesterol, and fasting blood glucose concentration showed a significant time effect following the intervention. However, there were no significant differences between the groups. Following the intervention, HOMA index decreased (-1.98±1.43, -0.36±0.33, and -2.12±0.73; $P=0.04$) in the LM, NB, and NBLM groups, respectively. Oxidized LDL changed (4.7±4.2, -2.5±6.2, and -13.4±5.1 ng/dL; $P=0.048$) in the LM, NB, and NBLM groups, respectively. LDL-cholesterol, insulin, and other markers of inflammation did not change following the 12-week intervention (all $P>0.05$).

Arterial stiffness variables before and after the intervention are shown in Table 4. Ultrasonography was not completed during baseline measurements for one male subject in the LM group due to mechanical issues. No baseline differences in stiffness variables were observed in the groups (all $P>0.05$).

Following the 12-week intervention, C-F PWV decreased (-134±35 and -79±49 cm/s; $P=0.03$) in the NB and NBLM groups, respectively, while C-F PWV did not change from baseline ($P>0.05$) in the LM group. β-stiffness index decreased (Figure 1) (-1.87±0.83; -2.03±0.60; and -2.51±0.90 U; $P<0.01$) representing an arterial destiffening effect of 13.8, 14.1, and 19.0% in the LM, NB, and NBLM groups, respectively. Arterial compliance increased (Figure 2) (0.200±0.060, 0.140±0.040, and 0.180±0.060 mm2/mmHg x 10-1; $P=0.02$) in the LM, NB, and NBLM groups, respectively. The magnitude of changes in β-stiffness index and arterial compliance were not significant between groups. No other stiffness indice changes were observed within or between the groups.

Discussion

The major finding of the present study was that, in contrast to our primary hypothesis, the combination of nebivolol and lifestyle modification reduced large arterial stiffness to a similar degree as either intervention alone in middle-aged and older adult hypertensives. The reduction in arterial stiffness occurred with modest weight loss (5-6% of initial body weight). Importantly, our observation that of β-stiffness index declined similarly in the combined and singular interventions suggests that the reduction in large arterial stiffness was independent of blood pressure lowering. Additionally, in accordance with our secondary hypothesis, the NBLM group experienced greater improvement in insulin sensitivity and greater reduction in oxidized LDL. We did not observe any obvious additive benefit of the combined intervention on oxidative stress or inflammatory biomarkers.

C-F PWV declined only in the NB and NBLM groups, while β-stiffness index declined similarly in all three groups. This might have occurred from an abnormal distribution during the randomization process. The LM group, while not significant at the P=0.05 level, had the lowest baseline and follow-up mean C-F PWV values. It is possible that the reduction in C-P PWV did not occur because the baseline velocities were already in the normal range, thus less room for improvement. However, this may also be due to the hemodynamic influences of nebivolol on central and peripheral blood pressure. C-F PWV is a blood pressure dependent indice of arterial stiffness; however, β-stiffness index is not directly influenced by acute blood pressure fluctuations. Nebivolol's antioxidant, negative chronotropic, and vasodilating properties may have an additional impact on endothelial dysfunction and structural alterations producing the present findings. Additionally, β-stiffness is a time-sensitive index of arterial stiffness and

extending the duration of the intervention may cause significant interactions between the groups. Further long-term investigations are warranted.

Nebivolol's ability to reduce arterial stiffness has been reported in previous studies[19, 23-25]. A plausible explanation for these findings is that nebivolol augments NO bioavailability in the coronary and peripheral arteries, distinguishing it from first- and second-generation beta-blockers. This characteristic becomes apparent when evaluating nebivolol alongside previous generation beta-blockers. Nebivolol was shown to have greater reductions on central arterial pressure[19, 20, 26] and augmentation index[18, 19], and greater improvements on endothelial function[27, 28] and coronary flow reserve[29] when compared to atenolol or metoprolol. Nebivolol therapy may hold distinct advantages over other beta-blockers that can translate into better destiffening strategies and cardiovascular risk reduction.

We recently reported that weight loss via a hypocaloric diet reduces arterial stiffness in overweight and obese middle-aged and older adults[8]. However, there remained a "residual" level of arterial stiffness which was higher in these individuals compared with healthy younger (i.e., 18-40 years) individuals[30]. Whether the combination of weight loss with a second arterial destiffening intervention would influence the residual level of arterial stiffness is unclear. In contrast with our hypothesis, the combination of lifestyle modification and nebivolol did not reduce arterial stiffness more than either of the singular interventions. Future studies are needed to identify the most efficacious arterial destiffening therapies.

There are some limitations of our study that should be considered. First, our sample size was small, and the age range of our subjects was confined to 40 to 75 years. Consequently, we are not able to generalize these results beyond this group.

Second, our study was not powered to detect gender and racial differences in arterial destiffening response. Whether nebivolol therapy reduces large arterial stiffness similarly in men and women or between different racial/ethnic groups is currently unknown. Further studies are needed to address these issues.

Third, the intervention period was limited to 12 weeks in duration. Whether the reductions in large artery stiffness are sustained over a longer period of time is not known. Finally, our study was not designed to determine the dose-response effects or potential mechanisms mediating the large artery destiffening effects of nebivolol. Additional studies are needed to discern the possible dose-response effects of nebivolol therapy on arterial stiffness.

In summary, these results support the combination of nebivolol and lifestyle modification is as efficacious as either intervention alone in reducing arterial stiffness in middle-aged and older adult hypertensives. There does not appear to be an additive effect when combining nebivolol therapy with lifestyle modification. Future studies utilizing larger sample size, longer duration, and different racial/ethnic populations are necessary to determine the efficacy of nebivolol in reducing large arterial stiffness.

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Disclosures

None.

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Table 1. Subject characteristics before and after the intervention.

	LM (N=15)		NB (N=15)		NBLM (N=15)	
Variable	PRE	POST	PRE	POST	PRE	POST
Age, yr	52.7±2.2		57.7±3.1		58.4±2.2	
Weight, kg	95.6±4.9	90.1±5.1	89.2±6.3	89.1±6.3	93.5±3.4	88.2±3.1*‡
BMI, kg/m^2	33.9±1.9	32.0±1.9	30.6±1.5	30.6±1.5	32.6±1.3	30.9±1.3*‡
Body Fat, %	42.5±2.5	40.7±2.7	37.9±2.8	38.6±2.8	39.6±2.1	38.7±2.4*‡
Fat Mass, kg	41.0±3.6	37.2±3.8	34.1±3.5	34.7±3.4	37.1±2.5	34.4±2.8*‡
Fat Free Mass, kg	54.6±3.1	52.8±3.1	55.0±4.3	54.4±4.3	56.4±2.7	53.7±2.4*‡
Supine HR, bpm	67±3	61±3	64±1	53±1	66±2	53±2*‡
Seated Brachial SBP, mmHg	145±1.4	135±2	148±1.4	136±3.0	149±1.6	130±3.1*‡
Seated Brachial DBP, mmHg	83±1.4	79±1.6	83±1.7	76±2.4	87±1.5	77±1.9*‡
Supine Brachial SBP, mmHg	138±3.1	133±2.7	146±4.1	136±3.8	142±2.6	133±3.9*
Supine Brachial DBP, mmHg	79±1.8	77±1.5	80±2.1	74±2.2	83±2.3	76±2.2*
Supine Carotid SBP, mmHg	139±4	135±4	144±4	139±5	142±3	134±4*
Supine Carotid DBP, mmHg	80±2	78±1	82±2	75±2	84±2	78±2*
PA, steps/day	6838±859	8539±672	NA	NA	5740±813	7873±1006*

Values expressed as mean ± SE. BMI=Body Mass Index; HR=Heart Rate; SBP=Systolic Blood Pressure; DBP=Diastolic Blood Pressure; PA=Physical Activity; NA=Not Applicable.
* P<0.05 Time Effect
‡ P< 0.05 Interaction Effect

Table 2. Dietary Intake at baseline and following intervention.

	LM (N=13)		NB (N=13)		NBLM (N=14)	
Variable	PRE	POST	PRE	POST	PRE	POST
Energy, kcal	1944±117	1488±127	2000±154	2314±230	2227±141	1793±95*‡
Fat, %	40±1.4	35±2.0	36±1.9	38±1.8	36±1.2	34±1.2
CHO, %	40±1.5[a]	43±2.5	45±2.2	42±2.0	46±1.0	46±1.5
Protein, %	17±0.9	20±1.0	17±0.9	18±1.0	17±0.6	19±1.0
Alcohol, %	2.9±1.2	2.3±0.9	2.2±0.9	2.6±1.1	1.4±0.7	0.4±0.4
Cholesterol, mg	385±36	270±27	363±52	444±64	375±35	303±21‡
SFA, g	29±3	18±2	27±3	37±5	30±3	22±2‡
PUFA, g	18±1	13±1	19±2	20±2	20±2	16±1*
MUFA, g	32±3	22±2	30±3	35±4	32±3	24±2*‡
TFA, g	4±1	2±1	3±1	4±1	5±1	3±1‡
Sodium, mg	3357±195	2722±220	3553±272	3943±428	3873±284	3268±153‡
Potassium, mg	2283±170	2095±195	2602±215	2823±246	2867±218	2662±187
Magnesium, mg	267±25	260±28	303±27	324±26	332±24	305±19
Fiber, g	15±2	16±1	18±2	18±1	21±2	20±1

Values expressed as mean ± SE. CHO=Carbohydrate; SFA=Saturated Fatty Acids; PUFA=Polyunsaturated Fatty Acids; MUFA=Monounsaturated Fatty Acids; TFA=Trans Fatty Acid.

[a] Baseline difference with NBLM (P=0.04).

* P<0.05 Time Effect

‡ P< 0.05 Interaction Effect

Table 3: Blood variables before and following intervention.

	LM (N=15)		NB (N=15)		NBLM (N=15)	
Variable	PRE	POST	PRE	POST	PRE	POST
Triglycerides, mg/dL	155.1±20.0	126.5±11.7	120.9±18.0	123.7±21.9	170.3±26.2	136.5±16.9*
Total Cholesterol, mg/dL	203.9±8.3	198.8±6.7	206.9±8.3	197.7±7.9	217.2±11.0	207.3±13.5*
HDL, mg/dL	52.3±2.9	50.2±2.0	59.5±4.4	55.0±4.9	49.8±3.1	50.1±3.8*
LDL, mg/dL	120.4±9.5	123.3±7.8	126.4±5.2	121.3±5.9	133.3±8.0	129.9±10.9
Glucose, mg/dL	96.6±3.8	86.2±2.8	95.7±1.8	91.9±2.9	97.8±1.7	83.9±2.8*
Insulin, pg/dL	337.4±40.8	316.7±36.4	307.6±41.9	282.1±49.7	366.0±49.6	284.2±74.9
HOMA Index	3.31±0.43	2.72±0.33	2.99±0.41	2.64±0.47	4.73±0.84	2.61±0.51*‡
Oxidized LDL, ng/dL	46.1±3.3	50.8±6.4	78.9±12.8	72.3±9.8	71.2±12.1	57.8±9.8‡
hsCRP, mg/dL	4.8±0.9	6.6±1.7	4.1±1.2	4.0±1.5	5.4±1.5	6.6±2.1
IL-6, pg/dL	15.0±0.7	20.1±5.6	14.7±0.4	13.9±0.7	15.7±1.2	15.2±1.4
TNF-α, pg/dL	12.9±3.7	11.3±1.4	9.2±0.4	8.9±0.3	13.0±3.7	11.4±1.4

Values expressed as ± SE of mean; HDL=High density lipoprotein cholesterol; LDL=Low density lipoprotein cholesterol; HOMA=Homeostatic Model Assessment**;** hsCRP=High Sensitivity C-Reactive Protein; IL-6=Interleukin 6; TNF-α=Tumor Necrosis Factor Alpha.
* P<0.05 Time Effect
‡ P< 0.05 Interaction Effect

Table 4. Stiffness variables before and following intervention.

	LM (N=14)		NB (N=15)		NBLM (N=15)	
Variable	PRE	POST	PRE	POST	PRE	POST
C-F PWV, cm/s	850±28	865±42	1001±72	867±56	991±61	912±45*‡
β-SI, *U*	13.32±1.16	11.48±0.80	14.40±1.16	12.37±1.07	13.15±1.28	10.65±0.88*
AC, $mm^2/mmHg \times 10^{-1}$	0.710±0.046	0.920±0.081	0.741±0.061	0.885±0.072	0.827±0.094	1.002±0.094*

Values expressed as ± SE of mean; C-F PWV=Carotid Femoral Pulse Wave Velocity; β-SI=β Stiffness Index; AC=Arterial Compliance.
* P<0.05 Time Effect
‡ P< 0.05 Interaction Effect

FIGURE LEGEND

Figure 1. Changes in Beta Stiffness after the intervention. Values are mean±SE.

Figure 2. Changes in Arterial Compliance after the intervention. Values are mean±SE.

Figure 1

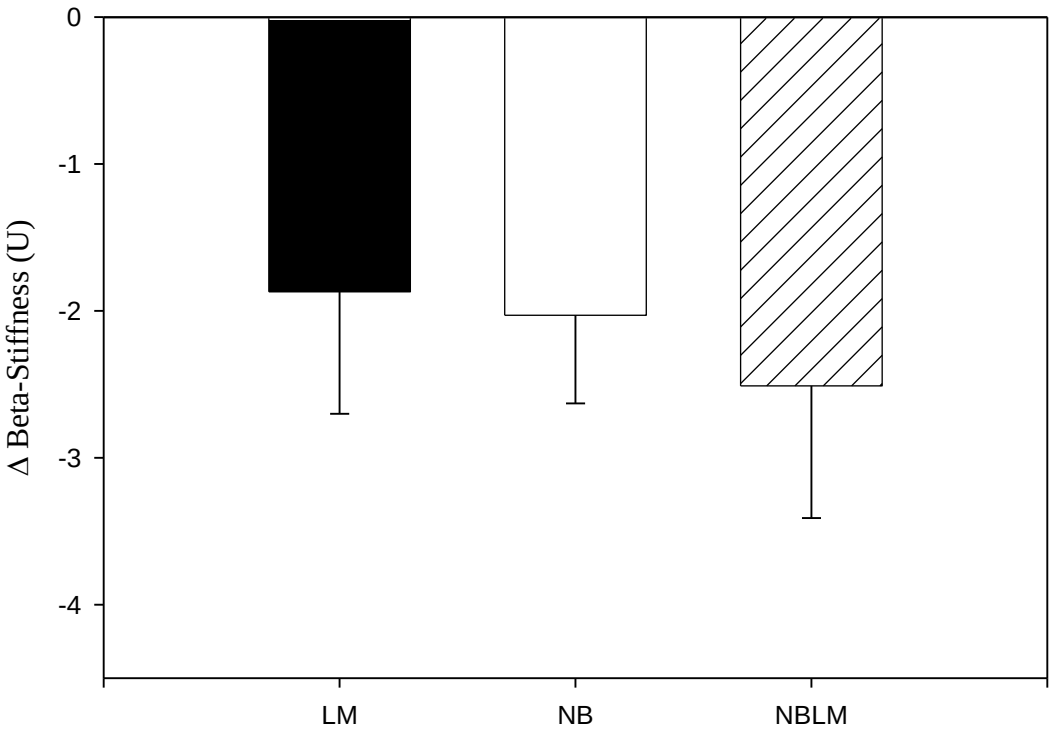
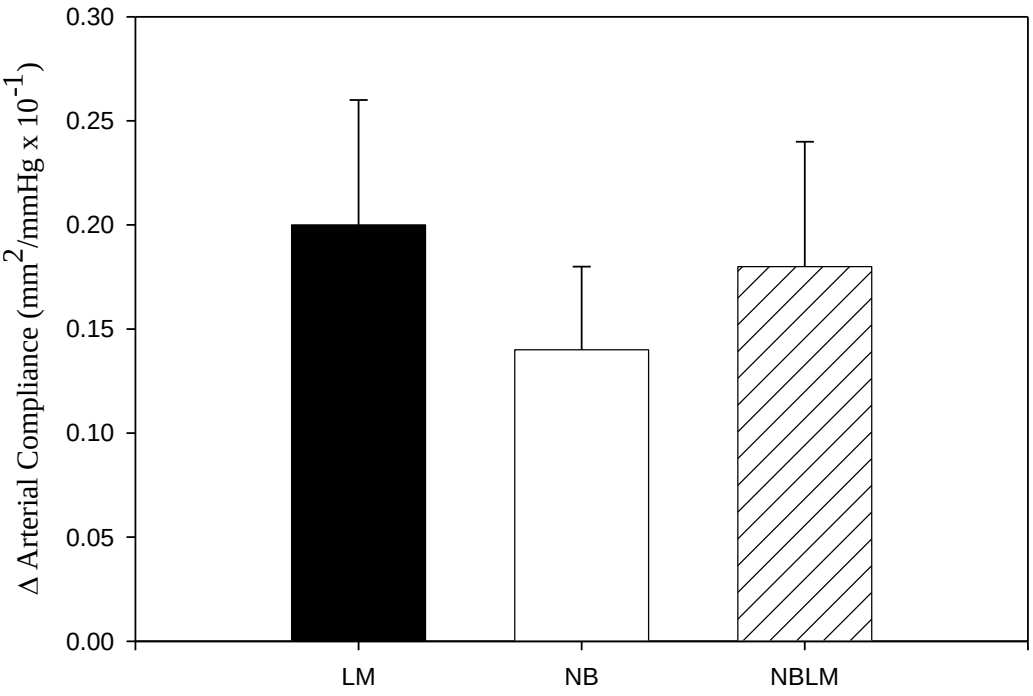


Figure 2


0.30
0.25
0.20
0.15
0.10
0.05
0.00
Δ Arterial Compliance (mm2/mmHg x 10-1)
LM
NB
NBLM

CHAPTER IV

Conclusions and Future Directions

Aging and hypertension are major factors in the pathogenesis of cardiovascular disease (CVD). Currently, a disproportionate growth of US aging population with a higher rate of hypertension (approximately 1 in 3 middle-aged and older adults) will undoubtedly increase the clinical and financial burden of CVD on America's health care system. As such, there is an overwhelming need for the characterization and development of novel, cost-effective therapeutic strategies to address this crisis. Such investigations may lead to breakthroughs in modern medicine, affording better prognosis and treatment outcomes.

Arterial stiffness is an independent predictor of CVD morbidity and mortality, especially in individuals with hypertensive, most of whom are older and overweight. It is an evolving pathophysiological process caused by pleotropic mechanisms with multiple risk factors including aging, hypertension, central adiposity, inflammation, and genetics functioning independently or synergistically. These factors stimulate vascular remodeling, resulting in stronger, rigid arterial walls capable of resisting such assaults. However, this vascular adaptation occurs at a detriment to the myocardium as the heart experiences higher afterloads and diminish coronary diastolic flow.

Measurement and expression of arterial stiffness indices are conducted with several clinical devices and mathematical formulas, all of which have strengths and weaknesses. Many non-invasive techniques can easily be incorporated into medical offices, providing additional insight into the diagnosis and therapeutic strategy. Such treatment strategies are usually analogous in nature to other CVD risk factors as many share a commonality in the pathogenesis. These treatments generally antagonize pro-hypertensive and pro-inflammatory pathways.

Initially, lifestyle modification (e.g., diet and exercise) is recommended as safe and effective means to improve cardiovascular health. However, due to low rates of adherence and adoption to lifestyle changes, alternative strategies including pharmacological therapies are typically prescribed. Multiple medications including angiotensin converting enzyme inhibitors, angiotensin receptor blockers, calcium channel blockers, diuretics, 3-hydroxy-3-methyl-glutaryl-CoA reductase inhibitors, and alagebrium reduce arterial stiffness in older individuals. First- and second-generation beta-blockers (BB) are potent brachial antihypertensives but paradoxically increase central blood pressure. Importantly, there is insufficient evidence on the role of third-generation BB as potential destiffening agents. Third-generation BB have a distinct ability to augment peripheral vasodilation and may attenuate deleterious effects of reflective waves on central hemodynamics.

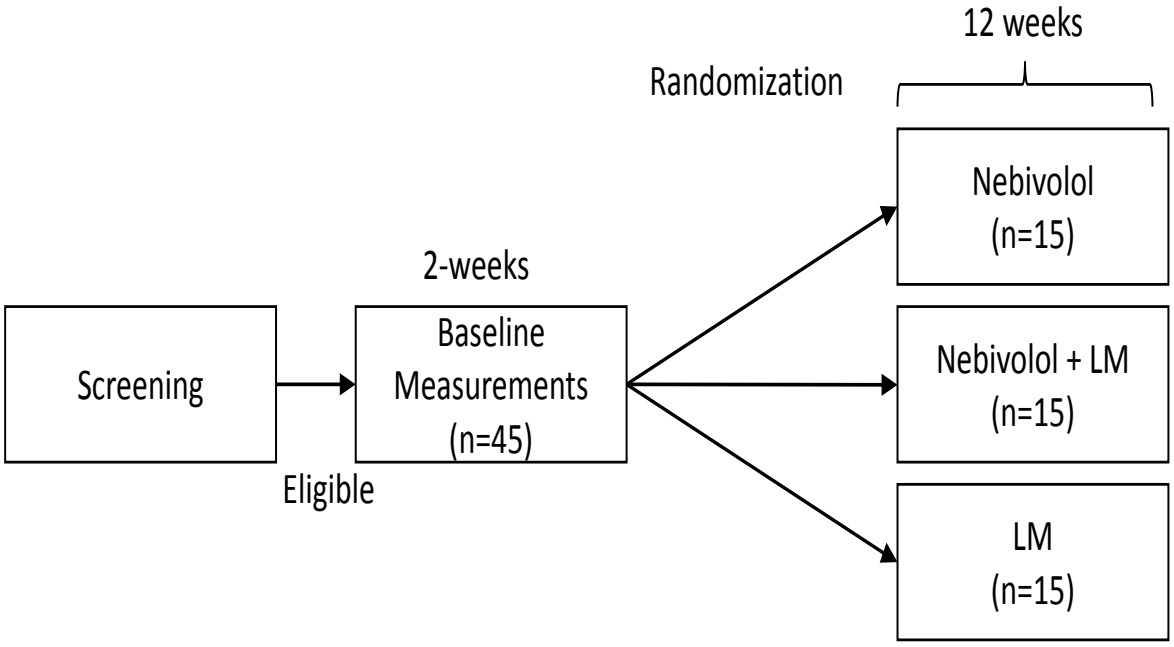
The purpose of this study was to determine if nebivolol, a third-generation BB with cardio-selective β-1 antagonism and β-3 agonism, and lifestyle modification would reduce arterial stiffness in middle-aged and older hypertensive adults. In contrast to our hypothesis, reduction in arterial stiffness was similar with singular and combination treatments. Additionally, post-treatment arterial stiffness indices remained elevated when compared to healthy, young adults. Our study may have been limited by intervention duration, insufficient dosage, or underpowered to detect significant changes between treatments. As such, further investigations on the combined and independent effects of nebivolol therapy and lifestyle modification are warranted.

Our findings are positive in the sense that arterial stiffness can be reduced in middle-aged and older adults with moderate weight loss (~5%) and nebivolol therapy, translating into lower CVD risk. Additionally, none of the interventions reportedly had tolerability issues, a potential

problem with many other therapeutic options. In light of this, nebivolol in combination with healthier dietary choices and increase physical activity may prove to be a superior destiffening strategy. Future investigations will ultimately determine the efficacy of prescribing such treatment.

APPENDICES

Appendix A: Study Design



Appendix B: Health History Questionnaire

Virginia Tech
Department of Human Nutrition, Foods, and Exercise

HEALTH HISTORY QUESTIONNAIRE

STUDY_____ **DATE**_____

SUBJECT ID #_____

PLEASE PRINT

1. Address:_____

   City:_____ State:_____ Zip Code_____

   Home Phone: _____ Work Phone:_____

   E-mail address:_____

Emergency Contact:_____ Phone:_____

Relation to you:_____

2. Employer:_____ Occupation:_____

3. Date of Birth:_____ Age:_____ Sex:___

Race and/or Ethnic Origin

☐ American Indian or Alaskan Native ☐ Asian or Pacific Islander ☐ Black, not of Hispanic Origin

☐ Hispanic ☐ White, not of Hispanic Origin

☐ Other

4. **GENERAL MEDICAL HISTORY**

Do you have any current medical conditions? YES ☐ NO ☐
If Yes, please explain:

Are you allergic to any medications? YES ☐ NO ☐
If Yes, please explain:

Have you had any major illnesses in the past? YES ☐ NO ☐
If Yes, please explain:

Have you ever been hospitalized or had surgery? YES ☐ NO ☐
If Yes, please explain: (include date and type of surgery, if possible)

Are you currently taking any medications or supplements, including aspirin, hormone replacement therapy, or other over-the-counter products?
YES ☐ NO ☐
If Yes, please explain:

Medication/Supplement	**Reason**	**Times taken per Day**	**Taken for how long?**

Have you ever had an EKG? YES ☐ NO ☐
If Yes, please explain:

Have you been diagnosed with diabetes? YES ☐ NO ☐
If Yes, please explain:

Age at diagnosis_____

5. **FAMILY HISTORY**

	Age (if alive)	Age of Death	Cause of Death
Father			
Mother			
Brothers/Sisters			

Do you have a family history of any of the following: (Blood relatives only, please give age at diagnosis if possible)

		YES	NO	Relation	Age at Diagnosis
a.	High blood pressure	☐	☐		
b.	Heart Attack	☐	☐		
c.	Coronary bypass surgery	☐	☐		
d.	Stroke	☐	☐		
e.	Diabetes	☐	☐		
f.	Obesity	☐	☐		

6. **TOBACCO/ALCOHOL HISTORY** (check one) (if applicable)

None ☐
Quit ☐ (when)_____
Cigarette ☐
Cigar ☐
Pipe ☐
Chew Tobacco ☐
Snuff ☐

CURRENT TOBACCO USE

	# per day
Cigarette	
Cigar	
Pipe	
Chew Tobacco	
Snuff	

Total years of tobacco use_____

Do you consume alcohol? Drinks per day ____ Drinks per week ____

7. **CARDIORESPIRATORY/METABOLIC HISTORY**

	YES	NO
Are you presently diagnosed with heart disease?	☐	☐
Do you have any history of heart disease?	☐	☐
Do you have a heart murmur?	☐	☐
Occasional chest pain or pressure?	☐	☐
Chest pain or pressure on exertion?	☐	☐
Episodes of fainting?	☐	☐
Daily coughing?	☐	☐
High blood pressure?	☐	☐
Shortness of breath?		
At rest?	☐	☐
lying down?	☐	☐
After 2 flights of stairs?	☐	☐
Do you have asthma?	☐	☐
Do you have a history of bleeding disorders?	☐	☐

	YES	NO
Do you have a history of problems with blood clotting?	☐	☐
Do you have high cholesterol? Or, low good (HDL) cholesterol?	☐	☐
Do you have thyroid problems?	☐	☐

If you checked YES to any of the above, you will be asked to clarify your response by an investigator so we can be sure to safely determine your ability to participate.

8. **MUSCULOSKELETAL HISTORY**

	YES	NO
Any current muscle injury or illness?	☐	☐
Any muscle injuries in the past?	☐	☐
Do you experience muscle pain at rest?	☐	☐
Do you experience muscle pain on exertion?	☐	☐
Any current bone or joint (including spinal) injuries?	☐	☐
Any previous bone or joint (including spinal) injuries?	☐	☐
Do you ever experience painful joints?	☐	☐
Do you ever experience swollen joints?	☐	☐
Do you ever experience edema (fluid build up)?	☐	☐
Do you have pain in your legs when you walk?	☐	☐

If you checked YES to any of the above, you will be asked to clarify your response by an investigator so we can be sure to safely determine your ability to participate.

9. **NUTRITIONAL HABITS**

Have you ever dieted? YES ☐ NO ☐

If YES, have you dieted within the past 12 months or are you currently on a diet?

YES ☐ NO ☐

If YES, please describe the diet:

a). Name (if applicable):_____

b). Prescribed by a Physician/nutritionist? YES ☐ NO ☐

c). Have you lost weight? YES ☐ NO ☐

d). Duration of diet_____

What was your weight 24 months ago? _____ 12 months ago? _____ 6 months ago? _____

Have you dieted other than in the past 12 months? YES ☐ NO ☐

If YES, please answer the following:

a). How many times have you dieted?

b). How old were you?

c). Weight loss (amount)?

You may be asked to complete a more detailed diet survey if you are volunteering for a research study.

10. **PHYSICAL ACTIVITY SURVEY**

Compared to a year ago, how much regular physical activity do you get? (Check one)

Much less ☐

Somewhat less ☐

About the same ☐

Somewhat more ☐

Much more ☐

Have you been exercising regularly for the past three months? YES ☐ NO ☐

If YES, what type of exercise do you regularly participate in? (check those that apply)

		Days per week	Minutes per session	Intensity (1=easy, 10=very hard)
Walking	☐	_____	_____	_____
Running	☐	_____	_____	_____
Cycling	☐	_____	_____	_____
Swimming	☐	_____	_____	_____
Aerobics	☐	_____	_____	_____
Weight Training	☐	_____	_____	_____
Martial Arts	☐	_____	_____	_____
Other (describe)		_____	_____	_____

You may be asked to complete a more detailed diet survey if you are volunteering for a research study.

11. **OBSTETRIC/GYNECOLOGICAL HISTORY**

	YES	NO
Do you have a normal menstrual cycle (1 menses each ~1 month)?	☐	☐

If no, please indicate frequency_____

Do you take any kind of contraceptive (oral, injectable, implant)? ☐ ☐

If yes, please indicate type and name_____

How many full term pregnancies have you had? _____ How long ago was your more recent pregnancy?_____

Have long since you have last breast fed?_____

12. **SLEEP HISTORY**

	YES	NO
Do you snore?	☐	☐

Don't Know ☐

Snoring loudness

- ☐ Loud as breathing
- ☐ Loud as talking
- ☐ Louder than talking
- ☐ Very loud. Can be heard in nearby rooms.

Snoring frequency

- ☐ Almost every day
- ☐ 3-4 times per week
- ☐ 1-2 times per week
- ☐ 1-2 times per month
- ☐ Never or almost never

Does your snoring bother other people? ☐ ☐

Has anyone told you that you quit breathing during your sleep? ☐ ☐

How often have your breathing pauses been noticed?

- ☐ Almost every day
- ☐ 3-4 times per week
- ☐ 1-2 times per week
- ☐ 1-2 times per month
- ☐ Never or almost never

Are you tired after sleeping?

- ☐ Almost every day
- ☐ 3-4 times per week
- ☐ 1-2 times per week
- ☐ 1-2 times per month
- ☐ Never or almost never

Are you tired during waketime?

- ☐ Almost every day
- ☐ 3-4 times per week
- ☐ 1-2 times per week
- ☐ 1-2 times per month
- ☐ Never or almost never

Have you ever fallen asleep while driving?

- ☐ Almost every day
- ☐ 3-4 times per week
- ☐ 1-2 times per week
- ☐ 1-2 times per month
- ☐ Never or almost never

Sleepiness Assessment

0 (zero) = would never doze off
1 (one) = slight chance of dozing
2 (two) = moderate chance of dozing
3 (three) = high chance of dozing

Situation	**Chance of Dozing**
Sitting and reading	___
Watching TV	___
Sitting, inactive in a public place (e.g., a theatre or meeting)	___
As a passenger in a car for an hour without a break	___
Lying down to rest in the afternoon when circumstances permit	___
Sitting quitely after lunch without alcohol	___
Sitting and talking to someone	___
In a car, while stopped for a few minutes in traffic	___

13. **EDUCATION**

Please check the highest degree obtained:

Grade School	☐
Junior High	☐
High School	☐
College Degree	☐
Master's Degree	☐
Doctorate	☐

14. **FAMILY PHYSICIAN**

Name:_____

Address:_____

_____

Phone: _____

Reviewer:_____ Date:_____
Print Name Signature

Appendix C: Carotid Ultrasound & Tonometry

Carotid Ultrasound & Tonometry

Subject ID#: _____ **Date:** _____ **Time:** _____

Intervention: Baseline or Post Test

Subject Fasted (≥ 12 hrs)?: Y N

Meds in the past 48 hrs? Y N

If yes, specify:

_____

Any illnesses in the past week? Y N

If yes, specify:

_____

Exercise in the past 24 hrs: Y N

If yes, specify:

_____

Carotid Imaging & Tonometry:

- Locate and mark SSN, brachial, radial, femoral, and carotid recording sites.
- Measure and record distances using measuring tape and caliper.

Site	Distance
SSN-Carotid	_____mm
SSN-Brachial	_____mm
SSN-Radial	_____mm
SSN-Femoral (use caliper)	_____inches _____mm

- Record brachial blood pressure 3 times. BP must be within +/-5mmHg. If out of range repeat no more than twice.
- Record brachial and radial waveforms for 10 seconds.
- Record femoral and carotid waveforms for 20 seconds.
- Save, analyze, save, and print.

Appendix D: Informed Consent for Subjects

Informed Consent for Participants of Investigative Projects
Department of Human Nutrition, Foods and Exercise
Virginia Tech

TITLE: Weight Loss and Medication for Blood Pressure Control

INVESTIGATORS: **Kevin P. Davy, Ph.D.**
Kris Osterberg, M.S.

MEDICAL DIRECTOR: Jose Rivero, M.D.

SPONSOR: Forest Research Institute

PURPOSE:
Nebivolol is a relatively new beta blocker which may be more effective in reducing cardiovascular disease risk than older beta blockers. The purpose of the present study is to determine the effect of nebivolol and lifestyle changes alone and together on blood vessel function. Forty five people will be included in this study.

METHODS:
You are being asked to participate in all of the sessions described below. If you agree to participate in this study, you will first be required to complete a personal health history questionnaire and undergo blood and urine tests. The results of your medical history and blood and urine tests may be discussed with the study medical director to determine your eligibility. Based on our evaluation of the questionnaire and your current health, you may then be eligible to become a study subject. Eligible candidates will be males or females between 40 and 75 years of age with high blood pressure. You must have a blood pressure greater than or equal to 140/80 mmHg to be included. You will not be eligible to participate in the study if you have diabetes, secondary hypertension (hypertension with a known cause), a history of stroke, myocardial infarction or chronic kidney disease (or renal artery stenosis), or cardiovascular (e.g., chronic heart failure), respiratory (e.g., chronic obstructive pulmonary disease), neurological (e.g., Parkinson's), metabolic (e.g., hyperthyroidism), oncological (e.g., active cancer) diseases or other diseases that would make participation unsafe. The medical director of the study must approve your participation. You will not be able to participate if you take medications (other blood pressure lowering medicines) that might influence the study variables or make participation unsafe. You will not be able to participate if you exercise three or more times a week at a moderate to hard level (e.g., exercise that causes you to breathe hard and sweat).

You may be eligible to participate if you are currently taking blood pressure medications. However, your blood pressure prior to starting medications must have been greater than or equal to 140/90 mmHg but less than 160/100 mmHg. Your own doctor must also approve your participation and agree to safely stop your current blood pressure medication for at least 2 weeks prior to starting the study as well as while you are participating in the study. You must also agree to have your blood pressure measured every other day for the 2-week period. If you are taking vitamins or nutritional supplements, you may be asked to stop taking these for at least 2 weeks before beginning the study. If your doctor has prescribed or recommended these supplements, we will need his/her approval for you to stop taking the vitamins or supplements. You will need to discuss the best way to start your medication with your doctor after completing the study. If your doctor does not approve or you are unwilling to stop your medication for the study, then you will not be allowed to participate.

You are being asked to participate in one of three groups: a group that receives nebivolol alone, a group that receives lifestyle modification (diet and exercise) alone or a group that receives both nebivolol and lifestyle modification. Each will require approximately 14 weeks of your time. The actual amount of time may differ by as much as one to two weeks depending on your schedule for testing. You will be assigned to participate in one of these groups. The order will be determined by a process

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similar to a coin toss. If you are assigned to one of the groups receiving nebivolol, you will take one 5 mg Benicar pill every day for 2 weeks and then 10 mg every day for another 10 weeks. You will be provided with 1-2 weeks of medication at a time to take home with you. You will need to come back to the laboratory every 1-2 weeks to return unused medication as well as to obtain more medication for the next 1-2 week period. If your blood pressure falls below 110/70 mmHg during the first two weeks, then you will continue to take only one pill each day for the remainder of the study. You will continue to take nebivolol during all of the follow-up measurements which takes approximately 2 weeks. You will be asked to not change your daily diet and physical activity throughout the entire study if you are assigned to the group which receives nebivolol alone. However, you will be offered diet and exercise counseling at the completion of the study.

If you are assigned to one of the groups that includes lifestyle changes, you will meet with one of our professional staff at least weekly during the study when you visit the laboratory to have your blood pressure measured. You will be given instructions for making a number of lifestyle changes including eating a healthy diet, increasing physical activity and reducing your weight if weight loss is necessary. Our professional staff will talk with you in person or by phone or email with you and give you feedback on your progress to help you make these lifestyle changes. You will be asked to keep a diary of your physical activity and diet over the course of the study. In addition, you will be provided a pedometer (a small computer-type device that counts your steps) to help you keep track of your physical activity. We ask that you return the pedometer each week so that we can monitor your progress.

At the completion of the study, you will be instructed to see your own physician for follow-up care as soon as possible. Individuals who do not have a primary care physician will see Dr. Rivero for further evaluation. Dr. Rivero, the medical director of the study, is a board certified cardiologist specializing in the treatment of high blood pressure. If you withdraw from the study before it is completed, you will be referred back to your physician (or Dr. Rivero) for follow-up care so that your ongoing treatment can be evaluated.

You are being asked to participate in all of the testing sessions two times (except the medical history and Session 2), once at baseline and again after each study period. There will be approximately 20-30 visits if you participate in this study. The actual number and order of visits may depend on your schedule and the availability of the study staff. The session order may differ from the order of appearance in this document.

Session 1

- **Overnight Fast:** You will be asked to avoid eating or drinking for 12 hours prior to your visit. This is to make sure that the test results will not be influenced by the food you eat or by the normal digestion process.
- **Urine Test:** You will be asked to urinate in a small cup that we provide to you. We will measure the amount of sodium and other electrolytes, glucose, protein, pH and whether there are blood cells present to determine whether it is safe for you to participate in the study.
- **Pregnancy Test:** If you are female, you will be required to have a pregnancy test. This will require collection of 2-3 teaspoons of your urine. If you are pregnant or the test indicates that you are pregnant, you will not be able to participate in this study.
- **Medical History:** You will be asked to complete a medical history questionnaire. This procedure is used to screen for health problems or reasons you should not participate in this study.
- **Body Mass and Height:** Your height and weight will also be measured at this time. Your body weight will be measured on a standard digital scale and will include the weight of light indoor clothing or hospital gown without your shoes. Your waist, hip, and neck circumference will be measured using a measuring tape.
- **Body Composition:** This test is to measure your body fat. You will lie on a hospital-type bed and a small amount of x-ray will be passed through your body to determine the amount of bone, muscle and fat in your body. This unit is called a DEXA scan. This test takes approximately 5

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minutes and there is no pain associated with the procedure. This procedure will be performed once at the beginning and a second time at the end of each treatment.

- **Blood Pressure:** You will be asked to sit quietly for 15 minutes. We will then measure your resting blood pressure using a stethoscope and standard blood pressure cuff and an automated blood pressure monitor.
- **Blood Draw:** A small venipuncture needle will be inserted into a large forearm or hand vein to draw blood (approximately 3 tablespoons). The blood collected will be used to measure your blood sugar, cholesterol and other hormones that influence your health.

Approximately time required: 1.5 hours

Session 2

- **Health and Physical Exam:** The medical director (Jose Rivero, M.D.) will listen to your heart and lungs with a stethoscope, measure your blood pressure, review your blood and urine tests and ask you basic questions about your health history. This test will take place at Dr. Jose Rivero's medical office in Christiansburg. Directions will be provided to you.

Approximate time required: 15 minutes. There may be up to one hour of waiting time depending on the number of patients in Dr. Rivero's office.

Session 3

- **Overnight Fast:** You will be asked to avoid eating or drinking for 12 hours prior to your visit. This is to make sure that the test results will not be influenced by the food you eat or by the normal digestion process.
- **Blood Pressure:** You will be asked to sit quietly for 15 minutes. We will then measure your resting blood pressure using a stethoscope and standard blood pressure cuff and an automated blood pressure monitor.
- **Diet Records:** You will be asked to write down everything you eat for a 4-day period (3 consecutive weekdays and 1 weekend day) four times, at the beginning and end of each treatment period. This will be used to determine what and how much you eat on a daily basis.
- **Physical Activity Monitor and Questionnaire:** You will be asked a series of questions to estimate your usual physical activity level, which will require about 15 minutes to complete. You will also be asked to wear a small monitor to measure your physical activity performed during 3 consecutive weekdays (72 hrs) and 1 weekend day (24 hrs). The monitor is slightly larger than a watch and will clip to your belt or waistband and will not interfere with your normal daily activity.

Approximate time required: 60 minutes.

Session 4

- **Overnight Fast:** You will be asked to avoid eating or drinking for 12 hours prior to your visit. This is to make sure that the test results will not be influenced by the food you eat or by the normal digestion process.
- **Blood Pressure:** You will be asked to sit quietly for 15 minutes. We will then measure your resting blood pressure using a stethoscope and standard blood pressure cuff and an automated blood pressure monitor.
- **Arterial Stiffness**: To measure arterial stiffness, the blood flow and diameter in the arteries in your chest, neck and leg will be measured with an ultrasound machine. An ultrasonic machine is sort of like radar – a low frequency radio wave that bounces off the tissues and sends a picture back to a "TV-like" screen. A mobile hand unit used will be pressed gently against an artery in your neck and leg.

Approximate time required: 1 hour.

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Session 5: This visit will take place at Carilion New River Valley Medical Center in Christiansburg, VA. Subjects will be asked to avoid eating for 4 hours prior to the visit. Directions will be provided to each individual.

Cardiovascular Magnetic Resonance Imaging: You will be asked to remove all jewelry and metal objects on your body and change into a hospital gown. The diameter of the aorta will be measured at several locations. These measurements will take 15-30 minutes. Blood pressure and applanation tonometric measurements will be made before and following the diameter measurements. A measure of the stiffness of the aorta will be calculated from these measurements. The size and function of your heart will also be measured during this session. The approximate time for the entire session is one hour. A longer time may be required due to heavy scheduling and/or in the event of an emergency requiring the equipment at Carilion New River Valley Medical Center.

Approximate time required: 1 hour.

In addition to the above visits, you will be asked to return up to 10-20 additional times to have your blood pressure measured. We will measure your blood pressure at least once every week during the study. We will also use this time to discuss and give you feedback on your progress. These will be scheduled at a time that is convenient for you. Each of these visits may take up to 30 minutes.

SUMMARY OF SUBJECT RESPONSIBILITIES

- Provide an accurate history of any health problems or medications you use before the study begins.
- Inform the experimenters of any discomfort or unusual feelings including dizziness.
- Be on time and attend all of the scheduled testing sessions.
- Follow all participant instructions for each session.
- Following diet and exercise instructions if assigned to one of the lifestyle modification groups
- Take only the number of pills of the medicine each day and return the unused portion at scheduled visits if assigned to one of the nebivolol groups.
- Inform the study investigators if you are pregnant or intend on becoming pregnant.

RISKS OF PARTICIPATION

- Nebivolol: Nebivolol is approved by the Food and Drug Administration (FDA) for the treatment of high blood pressure. Nebivolol is considered a very safe and effective medication. However, you should not take Nebivolol if you are pregnant, intend to become pregnant, or are nursing. If you are hypersensitive or allergic to this medication or any of its ingredients, you will not be permitted to participate in this study. The most common side effects of this medication are headache, fatigue and dizziness. Diarrhea, trouble sleeping and nausea are other less common side effects. You should report anything new or different to the investigator regardless of whether you think it might be related to the study medicine. If you feel dizzy, faint, lightheaded or have an increased heart rate, you should sit or if possible lie down immediately. If these feeling do not go away soon after sitting or lying down, you should call 911 or have someone take you to the nearest emergency room. If you experience signs of severe allergic reaction (including difficulty breathing, tightness in the chest, or swelling of the mouth, face, lips, or tongue), you should call 911 or have someone take you to the nearest hospital emergency room. Do not take any more medication until this issue is discussed with the medical director. If you have an allergic response to the medication, you will not be able to continue. However, you may be able to continue if you have experienced dizziness but this will need to be determined by the medical director of the study. If you have any concerns while participating in the study, you can contact the investigator immediately (phone numbers are at the end of the document). He will notify Dr. Rivero immediately and get instructions for what you should do next.

- Stopping Medicines: You will not receive any health benefit from stopping your current medications. In fact, there is the potential for you to experience an increased risk of health complications when you stop taking your current blood pressure medicine particularly. However,

you should know that this risk of health complications is extremely small given your blood pressure level and the short length of the study. We will measure your blood pressure every week during the study. We will let you and your doctor know immediately if your blood pressure increases too much. You should tell the study investigators immediately if have any concerns or experience anything new or different while participating in the study.

- Catheter and Blood Draw: Some pain or discomfort may be experienced when the catheter is inserted in the vein, but this persists for only a short time. During the blood draws, you may have pain and/or bruising at the place on your arm where the blood is taken. In about 1 in 10 or 10% of the cases, a small amount of bleeding under the skin will cause bruising. The risk of a blood clot forming in the vein is about 1 in 200 (less than 0.5%), while the risk of infection or significant blood loss is 1 in 1000 (less than 0.1%). There is a small risk of the vein becoming inflamed and/or painful in the hours or days after the catheter is removed. If you feel faint during or after a blood draw, you should notify the study doctor or study staff immediately and lie down right away to avoid falling down. Having staff who are experienced in catheter placement and blood draws will minimize these risks.

- HIV/AIDS: Your blood will be tested for the presence of HIV if one of the study investigators is exposed to your blood. There will not be any cost to you for this test. The results will be sent to your primary care physician or the study medical director, Dr. Jose Rivero, if you do not have a primary care physician. He/she will discuss them with you and provide you with the necessary referral for further evaluation and/or counseling if your results are positive. The results of your test will remain confidential.

- DEXA Scan: The amount of radiation that you will receive in the DEXA exam is less than the amount permitted by the Food and Drug Administration (FDA) per year. The amount you will receive is equal to 1/20 of a chest x-ray. The more radiation you receive over the course of your lifetime, the more likely your risk increases in developing cancerous tumors. The radiation in this study is not expected to greatly increase these risks; however, the exact increase in such risk is not known.

- Pregnancy: You should not become pregnant during this study because of the exposure to x-rays and study drugs. If you are capable of having a child, you must have a negative pregnancy test before each session that may pose a risk to an embryo or fetus (x-ray exposure or medication injection). You must agree to use an effective method of birth control, such as abstinence, condom use, or use of an intrauterine device, to ensure that you will not get pregnant. If you become pregnant during this study, you must notify your study investigator immediately. There may be unforeseen risks to the embryo or fetus in the event that you become pregnant.

- Arterial Stiffness: There are no known risks associated with this procedure.

- Weight Gain: Weight gain is common following weight loss programs. You may gain some or all of any weight you lost during the study. You should consider this before you agree to participate.

- Cardiovascular Magnetic Resonance Imaging: There are no known risks associated with this procedure. However, you will not be permitted to have this procedure performed if they have any metal implants in your body (e.g., artificial joint).

It is not possible to identify all potential risks in an experimental study; however, the study investigators and staff will take all possible safeguards to minimize any known and potential risks to your well-being.

Side effects are possible in any research study despite high standards of care and could occur through no fault of your own or the study doctors or the study staff.

BENEFITS OF PARTICIPATION
You will receive the following as part of your participation:
- Health and physical examination by a physician.
- Information on your blood pressure, cholesterol and other risk factors for cardiovascular disease.
- Information on healthy lifestyle habits including increased physical activity, weight loss and reducing sodium intake.
- Improve general medical knowledge

COMPENSATION
You will receive $100 for completing the baseline testing and another $100 for completing the follow-up testing at the end of the study. You will receive another $200 if you complete all aspects of the study. You are being asked to complete all of the sessions two times. The total amount of compensation you can receive is $400. If you do not complete the study, you will be compensated $25 for each session you have completed.

CONFIDENTIALITY
The data from this study will be kept strictly confidential. In the event that any of your tests indicate you may have a health problem, Dr. Rivero and the investigators may need to discuss this information with your doctor. The Food and Drug Administration may require that your identifying information be released to them in the event that you are injured as a result of participating in this study. For all other situations, your study information will be identified only by a code of numbers and letters, without anything to identify you by name.

FREEDOM TO WITHDRAW
You are free to withdraw from the study at any time for any reason. Simply inform the experimenters of your intention to cease participation. You should understand that circumstances may come up that the researcher will determine that you should not be a subject in the study. For example, lack of compliance to instructions, failure to take the medication, and illness could be reasons for the researchers to stop your participation in the study. If you withdraw, it is important that you consult your personal physician on how to change from the study medication back to your original prescription.

INJURY DURING PARTICIPATION IN THIS STUDY
Neither the researchers nor the University have money set aside to pay for medical treatment that would be necessary if you are injured as a result of your participation in this study. Any expenses that you incur including emergencies and long term expenses would be your own responsibility. You should consider this limitation before you consider participating in this study.

REVIEW OF RESEARCH
This research protocol has been submitted to and reviewed by the Virginia Tech Institutional Review Board For Research Involving Human Subjects, and was found to meet the requirements set forth in federal laws and regulations governing the protection of human subjects. You will receive a copy of this form to take with you.

SUBJECT PERMISSION
I have read the informed consent and fully understand the procedures and conditions of the project. I have had all my questions answered, and I hereby give my voluntary consent to be a participant in this research study. I agree to abide by the rules of the project. I understand that I may withdraw from the study at any time.

If you have questions, you may contact:

- Principal Investigator: Kevin Davy, Associate Professor, Department of Human Nutrition, Foods, and Exercise. (540) 231-3487; After hours: 540-230-0486

Virginia Tech Institutional Review Board: Project No. 10-008
Approved March 4, 2011 to January 13, 2012

- Chairman, Institutional Review Board for Research Involving Human Subjects: David Moore, (540) 231-4991

Name of Subject (please print)_____

Signature of Subject_____ Date_____

Appendix E: Institutional Review Board Approval

VirginiaTech

Office of Research Compliance
Institutational Review Board
2000 Kraft Drive, Suite 2000 (0497)
Blacksburg, VA 24060
540/231-4606 Fax 540/231-0959
email irb@vt.edu
website http://www.irb.vt.edu

MEMORANDUM

DATE: January 10, 2013

TO: Kevin Davy, Kris Osterberg, Jose Rivero M.D., Tim Jason Werner

FROM: Virginia Tech Institutional Review Board (FWA00000572, expires May 31, 2014)

PROTOCOL TITLE: Weight loss and medication for blood pressure control

IRB NUMBER: **10-008**

Effective January 9, 2013, the Virginia Tech Institution Review Board (IRB) Chair, David M Moore, approved the Continuing Review request for the above-mentioned research protocol.

This approval provides permission to begin the human subject activities outlined in the IRB-approved protocol and supporting documents.

Plans to deviate from the approved protocol and/or supporting documents must be submitted to the IRB as an amendment request and approved by the IRB prior to the implementation of any changes, regardless of how minor, except where necessary to eliminate apparent immediate hazards to the subjects. Report within 5 business days to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.

All investigators (listed above) are required to comply with the researcher requirements outlined at:

http://www.irb.vt.edu/pages/responsibilities.htm

(Please review responsibilities before the commencement of your research.)

PROTOCOL INFORMATION:

Approved As:	**Full Review**
Protocol Approval Date:	**January 11, 2013**
Protocol Expiration Date:	**January 10, 2014**
Continuing Review Due Date*:	**December 27, 2013**

*Date a Continuing Review application is due to the IRB office if human subject activities covered under this protocol, including data analysis, are to continue beyond the Protocol Expiration Date.

FEDERALLY FUNDED RESEARCH REQUIREMENTS:

Per federal regulations, 45 CFR 46.103(f), the IRB is required to compare all federally funded grant proposals/work statements to the IRB protocol(s) which cover the human research activities included in the proposal / work statement before funds are released. Note that this requirement does not apply to Exempt and Interim IRB protocols, or grants for which VT is not the primary awardee.

The table on the following page indicates whether grant proposals are related to this IRB protocol, and which of the listed proposals, if any, have been compared to this IRB protocol, if required.

Date*	OSP Number	Sponsor	Grant Comparison Conducted?
09/04/2012	10148301	Forest Research Institute	Not required (Not federally funded)

* Date this proposal number was compared, assessed as not requiring comparison, or comparison information was revised.

If this IRB protocol is to cover any other grant proposals, please contact the IRB office (irbadmin@vt.edu) immediately.