EFFECT OF HIGH INTENSITY INTERVAL TRAINING COMPARED WITH MODERATE INTENSITY CONTINUOUS TRAINING ON BRACHIAL ARTERY FLOW-MEDIATED DILATION AND ARTERIAL STIFFNESS IN HUMAN AGING

By

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A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

2014
To my loving family
ACKNOWLEDGEMENTS

First of all, I would like to thank my academic advisor and supervisory committee chair Dr. Demetra Christou for giving me a great opportunity to work with her in the Integrative Cardiovascular Physiology Laboratory and for her understanding, consideration, and continuous support throughout the years at both the University of Florida and Texas A&M University. I am also deeply grateful to my dissertation committee members Drs. Michael Delp, Judy Delp, and Brad Behnke for their encouragement and support throughout my Ph.D. program. To my past and present lab fellows, I would like to express my deep appreciation for their help and support with my dissertation project. Without their help and sacrifice, it would not be possible for me to finish my dissertation project and Ph.D. degree. Lastly, from the bottom of my heart, I would like to thank my loving family members, my wife, my daughter, and my parents, for their love, sacrifice, and endless, priceless support throughout my Ph.D. program.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>ACKNOWLEDGEMENTS</th>
<th>page</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF TABLES</td>
<td>4</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>7</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>8</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>9</td>
</tr>
</tbody>
</table>

## CHAPTER

1. **INTRODUCTION** | 13

2. **LITERATURE REVIEW** | 16
   - Cardiovascular Disease Risk Factors and Aging | 16
   - Vascular Endothelium and Vasoactive Substances | 16
     - NO | 17
     - PGI₂ | 18
     - EDHF | 18
     - Endothelin-1 | 19
     - Prostaglandins | 20
     - Angiotensin II | 20
   - Vascular Endothelial Dysfunction in Aging | 21
     - NO Bioavailability and Oxidative Stress | 22
     - Inflammation | 24
   - Exercise and Age-Related Vascular Endothelial Dysfunction | 25
   - Arterial Stiffness in Aging | 26
   - Exercise and Age-Related Arterial Stiffness | 28

3. **STUDY DESIGN AND METHODS** | 31
   - Subjects | 31
   - Study Exclusion Criteria | 31
   - Study Design | 32
   - Exercise Intervention | 32
   - Study Procedures | 33
     - Brachial Artery Flow-Mediated Dilation | 33
     - Aortic Pulse Wave Velocity | 35
     - Augmentation Index | 36
     - Resting Blood Pressure | 36
     - Height, Weight and Body Composition | 36
     - Diagnostic Graded Exercise Test and Maximal Oxygen Consumption | 37
     - Blood Lipids, Glucose, Insulin and Insulin Resistance | 37
<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-1</td>
<td>Subject characteristics at pre-intervention</td>
</tr>
<tr>
<td>4-2</td>
<td>Body composition at pre- and post-intervention</td>
</tr>
<tr>
<td>4-3</td>
<td>Blood lipids, fasting glucose, and insulin at pre- and post-intervention</td>
</tr>
<tr>
<td>4-4</td>
<td>Cardiorespiratory fitness at pre- and post-intervention</td>
</tr>
<tr>
<td>4-5</td>
<td>Heart rate and blood pressure at pre- and post-intervention</td>
</tr>
<tr>
<td>4-6</td>
<td>Brachial artery flow-mediated dilation at pre- and post-intervention</td>
</tr>
<tr>
<td>4-7</td>
<td>Arterial stiffness at pre- and post-intervention</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-1</td>
<td>Endothelium-derived vasodilators and vasoconstrictors</td>
<td>30</td>
</tr>
<tr>
<td>3-1</td>
<td>Flow chart of study design</td>
<td>39</td>
</tr>
<tr>
<td>3-2</td>
<td>High intensity interval training (HIIT) protocol</td>
<td>40</td>
</tr>
<tr>
<td>4-1</td>
<td>Change in body weight in response to the intervention</td>
<td>53</td>
</tr>
<tr>
<td>4-2</td>
<td>Change in fat mass in response to the intervention</td>
<td>54</td>
</tr>
<tr>
<td>4-3</td>
<td>Change in body mass index in response to the intervention</td>
<td>55</td>
</tr>
<tr>
<td>4-4</td>
<td>Change in insulin in response to the intervention</td>
<td>56</td>
</tr>
<tr>
<td>4-5</td>
<td>Change in insulin resistance (HOMA-IR) in response to the intervention</td>
<td>57</td>
</tr>
<tr>
<td>4-6</td>
<td>Change in maximal exercise test duration in response to the intervention</td>
<td>58</td>
</tr>
<tr>
<td>4-7</td>
<td>Change in brachial artery flow-mediated dilation in response to the intervention</td>
<td>59</td>
</tr>
<tr>
<td>4-8</td>
<td>Change in aortic pulse wave velocity in response to the intervention</td>
<td>60</td>
</tr>
<tr>
<td>4-9</td>
<td>Change in augmentation index normalized to heart rate of 75 beats/min (@ HR75) in response to the intervention</td>
<td>61</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
<td></td>
</tr>
<tr>
<td>AC</td>
<td>Adenylyl cyclase</td>
<td></td>
</tr>
<tr>
<td>ACE</td>
<td>Angiotensin converting enzyme</td>
<td></td>
</tr>
<tr>
<td>ACh</td>
<td>Acetylcholine</td>
<td></td>
</tr>
<tr>
<td>ADMA</td>
<td>Asymmetric dimethylarginine</td>
<td></td>
</tr>
<tr>
<td>AGEs</td>
<td>Advanced glycation end-products</td>
<td></td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
<td></td>
</tr>
<tr>
<td>AT1</td>
<td>Angiotensin II type 1 receptor</td>
<td></td>
</tr>
<tr>
<td>BH$_2$</td>
<td>Dihydrobiopterin</td>
<td></td>
</tr>
<tr>
<td>BH$_4$</td>
<td>Tetrahydrobiopterin</td>
<td></td>
</tr>
<tr>
<td>BK$_{Ca}$</td>
<td>Big conductance calcium-activated potassium channel</td>
<td></td>
</tr>
<tr>
<td>cAMP</td>
<td>Cyclic adenosine monophosphate</td>
<td></td>
</tr>
<tr>
<td>cGMP</td>
<td>Cyclic guanosine monophosphate</td>
<td></td>
</tr>
<tr>
<td>CONT</td>
<td>Non-exercise control</td>
<td></td>
</tr>
<tr>
<td>COX</td>
<td>Cyclooxygenase</td>
<td></td>
</tr>
<tr>
<td>CuZnSOD</td>
<td>Copper-zinc superoxide dismutase</td>
<td></td>
</tr>
<tr>
<td>Cyt P450</td>
<td>Cytochrome P450</td>
<td></td>
</tr>
<tr>
<td>ecSOD</td>
<td>Extracellular superoxide dismutase</td>
<td></td>
</tr>
<tr>
<td>EDHF</td>
<td>Endothelium-derived hyperpolarizing factor</td>
<td></td>
</tr>
<tr>
<td>EDRF</td>
<td>Endothelium-derived relaxing factor</td>
<td></td>
</tr>
<tr>
<td>eNOS</td>
<td>Endothelial nitric oxide synthase</td>
<td></td>
</tr>
<tr>
<td>ET$_A$</td>
<td>Endothelin receptor type A</td>
<td></td>
</tr>
<tr>
<td>ET$_B$</td>
<td>Endothelin receptor type B</td>
<td></td>
</tr>
<tr>
<td>ET-1</td>
<td>Endothelin-1</td>
<td></td>
</tr>
<tr>
<td>FMD</td>
<td>Flow-mediated dilation</td>
<td></td>
</tr>
</tbody>
</table>
GTP  Guanosine triphosphate
HIIT  High intensity interval training
IK$_{\text{Ca}}$  Intermediate conductance calcium-activated potassium channel
K$_{\text{ATP}}$  ATP-sensitive potassium channel
L-NMMA  NG-monomethyl-L-arginine
MCP-1  Monocyte chemoattractant protein-1
MICT  Moderate intensity continuous training
MnSOD  Manganese superoxide dismutase
NADPH  Nicotinamide adenine dinucleotide phosphate
NFkB  Nuclear factor kappa B
NO  Nitric oxide
NO$_3^-$  Peroxynitrite
PGH$_2$  Prostaglandins
PGI$_2$  Prostacyclin
R  Receptor
RAAS  Renin-angiotensin-aldosterone system
sGC  Soluble guanylyl cyclase
SK$_{\text{Ca}}$  Small conductance calcium-activated potassium channel
TNF-α  Tumor necrosis factor-alpha
TxA$_2$  Thromboxane
VO$_2^{\text{max}}$  Maximal oxygen consumption
Aging is an independent risk factor for cardiovascular disease. Advancing age is associated with vascular endothelial dysfunction and large elastic artery stiffness; both of which are predictors of future cardiovascular events and death. Aerobic exercise is commonly prescribed for reducing the risk for cardiovascular disease; however the optimal exercise prescription for maximizing vascular benefits in older adults remains to be established. Recently, high intensity interval training (HIIT) has been reported to have superior effects compared with moderate intensity continuous training (MICT) in patient populations. However, it is not known if that specific HIIT protocol is feasible and whether it will result in greater improvements in vascular function compared to MICT in human aging.

Therefore, the purpose of this study was to investigate the effect of eight weeks of HIIT compared with MICT on brachial artery flow-mediated dilation (brachial artery ultrasonography) and arterial stiffness (aortic pulse wave velocity and augmentation index using SphygmoCor) in human aging. To test the hypothesis that HIIT will be superior to MICT in improving flow-mediated dilation and arterial stiffness, 23 older
adults (55 to 79 years of age) were randomly assigned to one of three groups: HIIT (n=7), MICT (n=9), or sedentary lifestyle non-exercise control (CONT, n=7).

Flow-mediated dilation increased by 2 % in MICT (P=0.09) and decreased by 1 % in HIIT (P=0.3) and 1% in CONT (P=0.1). Aortic pulse wave velocity decreased by 0.63 m/s in MICT (P=0.002), but increased by 0.16 m/s in HIIT (P=0.4) and 0.55 m/s in CONT (P=0.2). Augmentation index, normalized at heart rate of 75 beats/min, decreased by 3.2% in HIIT (P=0.2) and 0.2% in MICT (P=0.5) and increased by 0.6% in CONT (P=0.3).

In conclusion, in older adults free from cardiovascular disease, HIIT does not improve brachial artery flow-mediated dilation and arterial stiffness. MICT improves aortic pulse wave velocity, but it does not influence augmentation index or flow-mediated dilation. Contrary to our hypothesis, HIIT is not superior to MICT in improving flow-mediated dilation and arterial stiffness.
CHAPTER 1
INTRODUCTION

In the United States the aging population has been growing rapidly and is expected to double by the year 2030 (US Census, 2010). Human aging is an independent risk factor for chronic diseases including cardiovascular disease (1-4), the major cause of death in the United States (5). The principal contributor to cardiovascular disease mortality is atherosclerosis.

The pathological triggering site for atherosclerosis is the vascular endothelium, the inner-most layer of cells that lines the human vasculature (6). Vascular endothelial function measured by flow-mediated dilation decreases with age. Increased local and systemic oxidative stress and decreased nitric oxide (NO) bioavailability are considered as the main mechanisms responsible for age-related vascular endothelial dysfunction (7-9). Advancing age is also associated with stiffening of large elastic arteries (aortic pulse wave velocity and augmentation index). Arterial stiffness is due to alterations in vascular structure and function. The age-related structural alterations include increased collagen deposit and cross-linking, decreased elastin content, and smooth muscle cell hypertrophy, whereas, the age-related functional alterations include increased sympathetic nerve activity, increased vasoconstrictor, and decreased vasodilator activity (10, 11).

There are well established methodological approaches to evaluate vascular endothelial function and arterial stiffness (12-14). Brachial artery flow-mediated dilation using ultrasonography is the gold standard to assess peripheral conduit artery endothelium-dependent dilation in humans. Hyperemic blood flow in the brachial artery induces a sudden increase in shear stress on the vascular endothelium leading to the
stimulation of NO production and vascular smooth muscle cell relaxation (12). Aortic pulse wave velocity, the gold standard measure of arterial stiffness, is determined by recording the pulse waves at the carotid and femoral arteries and calculating the ratio of the distance between the recording sites to the time delay between the carotid and femoral pulse waves. Aortic augmentation index is estimated as the ratio of augmentation pressure to central pulse pressure.

Brachial artery flow-mediated dilation and aortic pulse wave velocity are independent predictors for future cardiovascular disease events and death (15-18). Thus, these measures are important therapeutic targets for prevention of cardiovascular disease in older adults. Aerobic exercise training is often prescribed for reducing the risk for cardiovascular disease, but, there is still controversy regarding the optimal exercise mode, intensity, duration and frequency to maximize the effects on vascular aging. The current exercise prescription guidelines for older adults remain generic.

Recently, high intensity interval training (HIIT) consisting of “uphill” walking on a treadmill has been reported to have superior effects compared with moderate intensity continuous training (MICT) in patients with heart failure and metabolic syndrome (19, 20). However, it is not known if this HIIT protocol is feasible in older adults and whether it will result in greater improvements in vascular function compared to MICT in human aging.

The ACSM position stand on exercise and physical activity in older adults (21) encourages exercise modalities that limit excessive orthopedic stress. Aging is associated with osteoarthritis, thus limiting the impact on the joints by implementing HIIT on a stationary cycle instead of an inclined treadmill might be better tolerated. Thus, in
the current study supervised HIIT and MICT were prescribed on Airdyne bicycles. HIIT and MICT were designed to provide an equal volume of training (i.e., isocaloric expenditure) per exercise session. Exercise intensity was based on peak heart rate (HRpeak) which was determined during maximal exercise test. MICT consisted of 47 min of continuous Airdyne exercise at 70% of HRpeak. HIIT consisted of 40 min of interval Airdyne exercise: 10-min warm-up at 70% of HRpeak, four 4-min intervals at 90% of HRpeak alternated by 3-min intervals of active recovery at 70% of HRpeak, and 5-min cool-down at 70% of HRpeak.

This randomized controlled clinical trial addressed the following aims and hypotheses:

Aim 1: To examine the effect of eight weeks of HIIT compared with MICT Airdyne exercise on brachial artery flow-mediated dilation in older sedentary men and women free from overt cardiovascular disease.

Hypothesis 1: HIIT will be more effective in improving brachial artery flow-mediated dilation compared with MICT in older adults.

Aim 2: To examine the effect of HIIT compared with MICT Airdyne exercise on arterial stiffness in healthy older sedentary men and women free from overt cardiovascular disease

Hypothesis 2: HIIT will be more effective in improving arterial stiffness compared with MICT in older adults.
CHAPTER 2
LITERATURE REVIEW

Cardiovascular Disease Risk Factors and Aging

The risk of cardiovascular disease increases with advancing age (22). Obesity, hypertension, hyperglycemia, dyslipidemia and physical inactivity are well established traditional cardiovascular disease risk factors (22, 23). Compared to young adults, older adults have increased body mass index, systolic blood pressure, fasting glucose and total cholesterol, and decreased high-density lipoprotein (HDL) and physical activity (22, 24).

Even after accounting for other traditional cardiovascular disease risk factors, there is a positive association between physical inactivity and future cardiovascular disease events (25). Increased physical activity accounts for 30 to 50% of reduction in cardiovascular disease risk (23) and is beneficial in lowering individual CVD risk factors such as blood lipids (5 to 15% of risk prevalence), blood pressure (3 to 5 mmHg), whole body adiposity (2 to 5%), and abdominal adiposity (1 to 6%) (26-29). Aerobic exercise training is a recognized strategy for reducing cardiovascular disease risk. However there is still controversy regarding the optimal exercise approach to maximize the beneficial effects.

Vascular Endothelium and Vasoactive Substances

Blood vessel walls, except for capillaries, are composed of three layers. The outmost layer is known as the tunica adventitia (or tunica externa) and is composed of mainly collagen, elastin, and other structural proteins, which connect blood vessels to the surrounding structures and protect the blood vessels from the outside environment. The middle layer is called the tunica media and is comprised of mostly vascular smooth
muscle cells, thus this layer controls the size of the blood vessels by both constriction and relaxation. The innermost layer is called the vascular endothelium and consists of a single layer of endothelial cells that line the vascular lumen. Until the 1970s, the vascular endothelium was thought to be a passive filter that allows small molecules into the vascular wall. However, in the last four decades, there has been substantial progress in unveiling the functional importance of the vascular endothelium. Currently, it is well established that the vascular endothelium plays a critical role in controlling vascular smooth muscle tone. It produces several vasoactive substances including NO, prostacyclin (PGI₂), endothelium-derived hyperpolarizing factor (EDHF), endothelin-1, other prostaglandins, and angiotensin II, thus contributing to the maintenance of vascular homeostasis (Figure 2-1).

**NO**

In response to a physiological (i.e., shear stress) and/or pharmacological (i.e., acetylcholine) stimulus, endothelial nitric oxide synthase (eNOS) produces NO and L-citrulline using L-arginine as substrate. NO is a gas molecule with a very short half-life in physiological fluids (30). Despite its short half-life in normal physiological conditions, NO has the capability to diffuse into neighboring vascular smooth muscle cells, and to facilitate the activity of soluble guanylyl cyclase (sGC) in vascular smooth muscle cells (30, 31). Stimulation of sGC increases cyclic guanosine monophosphate (cGMP) concentration, resulting in vascular smooth muscle relaxation via decreased intracellular calcium concentration in vascular smooth muscle cells (31).

Not only is NO the most prominent endothelium-derived vasodilator, but also NO plays an important role as an anti-atherosclerotic agent in the human vasculature. NO
protects the vascular endothelium and smooth muscle cells from pro-inflammatory, pro-thrombotic, and vascular adhesion molecules (32).

**PGI₂**

Cyclooxygenase (COX) produces PGI₂ using arachidonic acid as substrate (33). PGI₂ is an endogenous endothelium-derived vasoactive substance and has a half-life of 2-3 minutes in physiological blood temperature (33). Like NO, PGI₂ is another major endothelium-derived vasodilator. It combines with G-protein coupled receptors on vascular smooth muscle cell membranes (34) and upon receptor activation, PGI₂ triggers a signaling cascade in vascular smooth muscle cells, which leads to vascular smooth muscle relaxation by increasing cyclic adenosine monophosphate (cAMP) concentration via adenylyl cyclase activity (33). Not only PGI₂ is an endothelium-derived vasodilator, but also it contributes to vascular homeostasis by preventing platelet aggregation and thrombus formation (35).

**EDHF**

Even though NO and PGI₂ are the most prominent endothelium-derived relaxing factors (EDRF), other factors exist that can lead to endothelium-derived vascular smooth muscle dilation. EDHF, an alternative EDRF, is known to contribute to vascular smooth muscle relaxation (36). Since EDHF was first introduced about four decades ago, many researchers have been trying to elucidate potential pathways of EDHF-induced vascular smooth muscle dilation.

Currently, there are two potential EDHF-related pathways. The first pathway involves endothelial cell hyperpolarization. In this pathway, increased calcium concentration in the vascular endothelium by a physiological and/or pharmacological stimulus opens calcium-sensitive potassium channels (e.g., small- and intermediate-
conductance calcium-activated potassium channels; $SK_{Ca}$ and $IK_{Ca}$, respectively) on the endothelial cell membrane and releases potassium ions. This results in the propagated hyperpolarization and relaxation of neighboring vascular smooth muscle cells by the activation of the inward-rectifier potassium channel and sodium-potassium pump on the vascular smooth muscle cell membrane (37). The second pathway is not associated with endothelial cell hyperpolarization. Instead, increased calcium concentration activates the cytochrome P450 enzyme in vascular endothelial cells which facilitates epoxyeicosatrienoic acid formation, an EDHF, using arachidonic acid as substrate. Epoxyeicosatrienoic acid activates big conductance calcium-activated potassium channels ($BK_{Ca}$), and ATP-sensitive potassium channels ($K_{ATP}$), leading to vascular smooth muscle cell hyperpolarization and relaxation (37).

**Endothelin-1**

Endothelins are vascular endothelium-derived peptides and have four isoforms: endothelin-1, endothelin-2, endothelin-3, and endothelin-4 (38). Among them, endothelin-1 is the most predominant isoform in vascular endothelial cells (39). The formation of the active 21-residue endothelin-1 peptide from the inactive 38-residue big endothelin-1 is facilitated by the endothelin-converting enzyme in vascular endothelial cells (40).

Endothelin-1 combines with endothelin receptor type A ($ET_A$) or B ($ET_B$) on the vascular smooth muscle cell membrane, and causes either vasoconstriction or vasodilation depending on the receptor (38). The activation of the $ET_A$ receptor by endothelin-1 contributes to maintaining vascular tone through vasoconstriction following increased calcium concentration in vascular smooth muscle cells (38). However, the activation of the $ET_B$ receptor by endothelin-1 leads to vascular smooth muscle
relaxation by increasing NO and PGI2 production in the vascular endothelium (38). Data from previous studies demonstrate that plasma endothelin-1 is elevated in chronic heart failure patients, and that ET\textsubscript{A} blockade induces brachial artery vasodilation even in healthy individuals. Taken together, these data suggest that the activation of ET\textsubscript{A} receptors by endothelin-1 has detrimental effects on vascular function (38).

**Prostaglandins**

The precursor of prostaglandins is generated from arachidonic acid by COX-1/2 enzymes and plays a role as a vasoconstrictor (41). Prostaglandins is isomerized to several eicosanoids in the vascular endothelium: PGI2, prostaglandin D\textsubscript{2}, prostaglandin E\textsubscript{2}, prostaglandin F\textsubscript{2}, and thromboxane A\textsubscript{2} (TxA\textsubscript{2}) (41). In a normal physiological environment, the most potent prostaglandin PGI2 is produced by the COX-1/2 enzymes, and acts as a vasodilator in human vasculature. However, in pathophysiological conditions, PGI2 generation is inhibited and COX-1/2 enzymes produce TxA\textsubscript{2}, which following binding with its receptor on the vascular smooth muscle cell membrane leads to vasoconstriction (41).

**Angiotensin II**

Traditionally, the classical view of the Renin-Angiotensin-Aldosterone System (RAAS) involves regulation of blood volume and pressure. Renin which is released from the kidneys, cleaves angiotensinogen, which is secreted into the blood stream by the liver, to form angiotensin I. Angiotensin I is then converted into angiotensin II by the angiotensin converting enzyme. Angiotensin II combines with angiotensin II type I (AT1) receptors and facilitates aldosterone generation from the adrenal glands (42). Aldosterone combines with mineralocorticoid receptors on epithelial cells of the collecting duct in the kidneys where it facilitates the reabsorption of sodium ions and
water molecules into the blood stream, which results in increased blood volume and pressure (42).

In addition to the classical RAAS view, more recently the presence of local tissue RAAS has been recognized. All of the components of RAAS can be synthesized, secreted, and can act in local tissues and cells including the blood vessels (43). Angiotensin II can induce vascular smooth muscle constriction and proliferation by activating AT1 receptors and by increasing superoxide production via activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in the blood vessel wall (44). In addition, angiotensin II increases pro-inflammatory cytokines and leukocyte adhesion molecule activity in blood vessels, which increases the release of angiotensin converting enzyme and produces more angiotensin II in a vicious cycle (45). The elevated production of superoxide and inflammation and leukocyte adhesion molecules, in turn, reduce NO bioavailability in the vascular endothelium, and cause impaired vascular endothelial function and vascular smooth muscle hypertrophy (44).

**Vascular Endothelial Dysfunction in Aging**

The impairment of vascular endothelial function is an early event in the development of atherosclerosis, the main cause of cardiovascular disease events and deaths (46). The vascular endothelium gradually begins to become functionally impaired at approximately age 40 in men and age 50 in women (47). In aging, there is substantial evidence demonstrating reduced NO-mediated endothelium-dependent dilation (9, 48) compared with young adults (49). However, PGI$_2$- and EDHF-mediated endothelium-dependent dilation appear to be less influenced by aging, and may even play a supplementary role in preserving endothelium-dependent dilation when the NO-mediated pathway is impaired (36, 50, 51).
Compared to other parts of the vascular tree, conduit vessels are more vulnerable to atherosclerosis, thus, they are more prone to atherosclerotic plaque formation leading to narrowing of the lumen (52). Ultrasonography-based flow-mediated vasodilation is used in peripheral conduit arteries (most commonly the brachial artery) to evaluate NO-mediated endothelium-dependent dilation, an early marker of atherosclerosis development (12).

Previous studies have demonstrated that flow-mediated vasodilation is gradually decreased even in healthy aging (47, 49). Older sedentary adults have been reported to exhibit ~ 45% lower flow-mediated vasodilation compared with young sedentary adults (7). However, in healthy aging there is no change in endothelium-independent nitroglycerin-induced vascular smooth muscle relaxation (53). Resistance artery peak forearm blood flow in response to acetylcholine, a pharmacological stimulus to induce endothelium-dependent dilation, has also been found to be ~ 25% lower in healthy middle-aged and older adults compared with young adults (49). The major underlying mechanisms of age-associated endothelial dysfunction are thought to be decreased NO bioavailability and oxidative stress, and chronic low grade inflammation (7, 53-57).

**NO Bioavailability and Oxidative Stress**

NO bioavailability decreases with aging and this is either due to decreased production or increased NO degradation by superoxide. Based on experiments using eNOS inhibition with NG-monomethyl-L-arginine (L-NMMA), NO production decreases with human aging (9). Data from freshly biopsied human arterial and venous endothelial cells demonstrate that eNOS protein expression does not change, whereas eNOS serine 1177 phosphorylation, which activates eNOS, increases to compensate for the reduced generation of NO (57).
Intact eNOS cofactor activity is required to maintain NO production in aging. Tetrahydrobiopterin (BH₄), the most critical cofactor for functional eNOS enzyme activity, is oxidized to dihydrobiopterin (BH₂) in aging, resulting in decreased BH₄ activity and NO bioavailability (53, 54). Increased asymmetric dimethylarginine (ADMA) concentration, increased arginase activity, and decreased dimethylarginine dimethylaminohydrolase activity can inhibit the binding of eNOS with L-arginine, leading to impaired NO production (58, 59). However, there is lack of evidence supporting that these mechanisms are responsible for impaired production of NO in human aging.

Aging is associated with an imbalance between free radical production and antioxidant defenses, known as oxidative stress (60, 61). Increased superoxide levels react with NO to generate peroxynitrite (NO₃⁻), thus leading to decreased NO bioavailability (62). In addition, reactive oxygen species can trigger eNOS uncoupling. Without appropriate cofactor activity, eNOS generates more superoxide anions than NO, and reactive NO is likely to combine with elevated superoxide anions, and thus exacerbate oxidative stress in the vascular endothelium and vascular endothelial dysfunction (63).

The main source of superoxide generation in the vascular wall is NADPH oxidase. In animal models of aging, NADPH oxidase activity and nitrotyrosine, a marker of protein oxidation, have been found to be elevated (64). In humans, vascular endothelial cell protein expression of both NADPH oxidase p47^{phox} subunit and nitrotyrosine have also been reported to be higher in older compared with young adults. In older adults, plasma oxidized low-density lipoprotein levels, a systemic marker of lipid oxidation, is also elevated (7, 8).
Alterations in endogenous antioxidant enzyme expression and activity also play an important role in age-associated vascular endothelial dysfunction. Systemic total antioxidant status (55) and local vascular endothelial cell protein expression of mitochondrial manganese superoxide dismutase (MnSOD) and extracellular superoxide dismutase (ecSOD) have been found to be lower in older compared with young adults (8). However, there is no evidence to support lower cytosolic copper-zinc superoxide dismutase (CuZnSOD) in aged human blood vessels. Interestingly, in aged compared with young animal vessels, vascular protein expression and activity of CuZnSOD, MnSOD, and ecSOD are not different (64).

Inflammation

Plasma and vascular inflammatory factors increase with age independent of the presence of overt clinical disease. Elevated cardiovascular disease risk and impaired endothelial function are strongly associated with increased systemic and local inflammation in aging (65, 66). Nuclear factor kappa B (NFκB), a transcription factor, is elevated in aging and has recently received increasing attention regarding its role in vascular endothelial dysfunction (8). NFκB translocates from the cytosol into the nuclei of vascular endothelial cells, leading to stimulation of mRNA expression of inflammatory cytokines such as interleukin-6, tumor necrosis factor-alpha (TNF-α), and monocyte chemoattractant protein-1 (67, 68). These inflammatory cytokines, in turn, activate NFκB, which leads to more inflammation. NFκB has also been linked to NADPH oxidase gene expression (69), and nitrotyrosine levels (56), suggesting a causal role in age-related oxidative stress and impaired endothelial function.
Exercise and Age-Related Vascular Endothelial Dysfunction

Enhanced NO bioavailability and reduced oxidative stress are thought to be the major mechanisms related to exercise-induced improvements in vascular endothelium-dependent dilation in human aging (7, 48). In aerobically trained compared with sedentary older adults, eNOS inhibition with L-NMMA results in greater reductions in maximal forearm blood flow in response to acetylcholine infusion (48). Thus, it is reasonable to speculate that, greater brachial artery flow-mediated vasodilation and forearm blood flow response to acetylcholine result from preserved NO-mediated endothelium-dependent dilation in habitually exercising older adults. In addition, oxidative stress decreases and endogenous antioxidant defenses increase, in response to habitual aerobic exercise in older adults (8). Furthermore, regular aerobic exercise prevents the oxidation of BH₄ to BH₂, which contributes to preserved NO bioavailability and vascular endothelium-dependent dilation (70). Moreover, regular aerobic exercise suppresses the age-associated elevation in local NFκB, a major pro-inflammatory cytokine (8).

Data from cross-sectional studies demonstrate that habitually active older adults have preserved flow-mediated vasodilation. Aerobically trained older men have ~ 1.5-fold higher brachial artery flow-mediated vasodilation than age-matched sedentary men, and have similar flow-mediated vasodilation with young men (7). However, no difference has been found between habitually active and sedentary older adults in either peripheral conduit artery or resistance artery smooth muscle responsiveness to a NO donor.

Based on longitudinal data, home-based moderate-intensity aerobic exercise training has been reported to result in ~30% improvement in resistance artery
endothelium-dependent dilation in older adults (49). In addition, moderate intensity aerobic training using cycle ergometers enhanced flow-mediated vasodilation in middle-aged and older sedentary men (71). However, the effect of aerobic exercise training on vascular endothelial function in postmenopausal women is not clearly evident. Eight weeks of aerobic walking exercise improved brachial artery flow-mediated vasodilation in older men, but not in postmenopausal women (72). Another aerobic exercise training intervention reported only slight increases in flow-mediated vasodilation in obese, hypertensive postmenopausal women (73).

To date, a limited number of studies have examined the effect of high intensity interval training on vascular endothelial function in patient populations, but we are not aware of any published studies in older adults free from overt cardiovascular disease. Compared to isovolumic moderate intensity continuous training, high intensity interval training resulted in superior improvements in brachial artery flow-mediated vasodilation in metabolic syndrome patients (19) and heart failure patients (20).

**Arterial Stiffness in Aging**

Large elastic arteries play an important role in buffering sudden increases in pulsatile energy, blood flow and pressure when the heart pumps blood into the systemic vascular beds. Arterial buffering enables stable and continuous blood supply to microvessels throughout the cardiac cycle (74). Central elastic arteries become stiffer with increasing age, but peripheral muscular arteries are not affected (75). In older compared to young adults, pulse waves travel faster via stiffer large elastic arteries to the peripheral vascular beds and when they are reflected at the smaller peripheral blood vessels they return to the heart earlier (75). With advancing age the early reflection of pulse waves reaches the heart during systole, which increases the afterload, thus
contributing to adverse structural alterations in the heart including left ventricular hypertrophy (74, 76). In young adults, however, the delayed reflection of pulse waves reaches the heart during diastole, which enhances myocardial perfusion by increasing blood flow in the coronary circulation (76).

Large elastic artery stiffness can be assessed using a number of methodological approaches. However, the gold standard non-invasive measure of arterial stiffness is aortic pulse wave velocity. Another commonly used measure of arterial stiffness is augmentation index. Augmentation index represents the left ventricular load imposed by increased arterial stiffness and wave reflection. In humans, aortic pulse wave velocity and augmentation index are increased and arterial compliance is decreased with advancing age (77-81). However, the mechanisms responsible for age-related large elastic artery stiffness are not fully understood, but are thought to be associated with age-related alterations in arterial structure and function.

Central elastic arteries in older adults have altered arterial wall composition. In aged human arteries, increased elastase activity facilitates the breakdown of elastin fibers, causing increased elastin fragmentation (82). Furthermore, collagen formation is also facilitated in aged vascular smooth muscle cells (82). Because advanced glycation end-products (AGEs) are elevated in aged human blood vessels, collagen combines with AGEs, resulting in facilitated crosslinking between collagen and AGEs (11, 82). Taken together, decreased intact elastin fibers, increased elastin fragmentation and elevated collagen formation and crosslinking with AGEs can lead to stiffening of large elastic arteries by decreasing arterial wall elasticity and by thickening the arterial wall.
Arterial stiffness in human aging is also due to age-related increases in sympathetic nerve activity and reductions in vascular endothelial function (47, 83). The elevated sympathetic nerve activity augments vascular smooth muscle tone, which leads to increased central artery stiffness with aging. The decreased NO bioavailability and/or increased vascular tone impair vascular endothelium-dependent dilation, which may also contribute to elevated arterial stiffness in human aging (57, 84).

Exercise and Age-Related Arterial Stiffness

Habitual endurance exercise appears to have beneficial effects on age-related arterial stiffness. Recent evidence indicates that more time spent performing light intensity physical activity is related with greater decreases in aortic pulse wave velocity in older adults (77). Data from cross-sectional studies demonstrate that older endurance athletes who regularly participate in local distance running events have lower aortic pulse wave velocity and augmentation index than their age-matched sedentary counterparts (79). Furthermore, older endurance-trained men have 25-30% higher arterial compliance than sedentary and recreationally active men (81). Moreover, endurance exercise-trained postmenopausal women have higher arterial compliance than age-matched sedentary women (80).

Based on longitudinal data, long-term progressive and vigorous exercise training improved arterial compliance in previously sedentary older adults (85). In response to short-term aerobic training (walking and/or jogging), aortic pulse wave velocity and arterial compliance were improved in middle-aged and older sedentary men (81, 86). In postmenopausal women, moderate intensity aerobic exercise (walking, jogging, and cycling) enhanced arterial compliance by about 40% compared to pre-training measures (80, 87). However, central blood pressure and augmentation index did not improve in
response to moderate intensity continuous cycling exercise in previously sedentary postmenopausal women (88). Moderate intensity aerobic exercise training as short as only four weeks appears to have beneficial effects on arterial stiffness. Collier et al. demonstrated that four weeks of moderate intensity treadmill walking improved both central and peripheral PWV in middle-aged, pre- and stage-1 hypertensives (89).

High intensity interval training also demonstrated beneficial effects on central elastic artery stiffness (aortic pulse wave velocity) in hypertensive adults (90). In healthy young adults, low volume high intensity interval training resulted in similar improvements in peripheral artery distensibility compared with high volume moderate intensity continuous training, but there was no beneficial effect on central artery distensibility with either training protocol (91). However, we are not aware of any published studies examining the effect of high intensity interval training on arterial stiffness in older adults.
Figure 2-1. Endothelium-derived vasodilators and vasoconstrictors. ACh, acetylcholine; eNOS, endothelial nitric oxide synthase; NO, nitric oxide; Cyt P450, cytochrome P450; EDHF, endothelium-derived hyperpolarizing factor; COX-1/2, cyclooxygenase-1 and 2; PGI2, prostacyclin; ET-1, endothelin-1; PGH2, prostaglandins; TXA2, thromboxane; ACE, angiotensin converting enzyme; AT-1, angiotensin-1; AT-2, angiotensin-2; GTP, guanosine triphosphate; sGC, soluble guanylyl cyclase; cGMP, cyclic guanosine monophosphate; cAMP, cyclic adenosine monophosphate; AC, adenylyl cyclase; ATP, adenosine triphosphate; R, receptor.
CHAPTER 3
STUDY DESIGN AND METHODS

Subjects

One hundred and fifty older adults (55-79 years of age) volunteered to participate in this study, of which forty-four passed the preliminary phone screening and provided written informed consent to participate in the study. Subjects were non-smokers, without overt cardiovascular and clinical disease (e.g., diabetes, liver and renal disease) as assessed by medical history, physical examination, resting ECG, blood chemistries and hematological evaluation. All subjects demonstrated normal ECG and blood pressure responses to a clinically-supervised diagnostic graded exercise test. None of the subjects were on hormone replacement therapy (e.g., estrogen, progesterone or testosterone) for at least 2 years. All female subjects were postmenopausal, defined as a cessation of menses for at least 2 years (mean: 13±2 years, range: 4-30 years). The study was approved by the Institutional Review Board of the University of Florida. The purpose, nature and risk of study procedures were explained to the subjects and questions were answered prior to obtaining written informed consent. All of the procedures and supervised exercise training were performed at the Integrative Cardiovascular Physiology Laboratory at the University of Florida.

Study Exclusion Criteria

- Age <55 or >79 years
- Premenopausal or perimenopausal women
- Use of tobacco products
- Use of hormone replacement therapy
- >5% weight change in the prior 6 months
• Participation in regular aerobic exercise in the prior 1 year
• Stage 2 hypertension (≥160 mmHg systolic or ≥100 mmHg diastolic)
• History of cardiovascular disease (myocardial infarction, angina pectoris, history of coronary artery bypass surgery or angioplasty, congestive heart failure, or arrhythmia)
• History of renal impairment, gout or hyperuricemia
• History of diabetes, hepatic disease or infection with hepatitis B, C
• History of seizures, or other relevant on-going or recurrent illness
• Recent or recurrent hospitalizations

**Study Design**

Subjects were recruited and screened according to the study inclusion/exclusion criteria. Subjects who were enrolled in the intervention were randomized to one of three groups (Figure 3-1): HIIT, MICT, or CONT. The randomization order was developed by a computer random-number generator and randomization was stratified by initial peak oxygen consumption (L/min). Subjects assigned to CONT were asked to maintain their normal sedentary lifestyle for the duration of the intervention. Subjects assigned to HIIT and MICT were also asked to maintain their normal sedentary lifestyle except for the scheduled supervised exercise sessions. Furthermore, all subjects were asked not to alter their diet or use of medication during their participation in the study.

**Exercise Intervention**

HIIT and MICT were designed to provide an equal volume of training (i.e., isocaloric expenditure) per exercise session and were performed on Airdyne bicycles (AD4, Schwinn) using the arms and legs simultaneously. Supervised HIIT and MICT were performed four days/week (Monday, Tuesday, Thursday, and Friday) for eight weeks.
The intensity of exercise training was based on the subject’s peak exercise heart rate (HRpeak) that was achieved during the maximal exercise test. A heart rate monitoring system (Polar Team 2 Pro, version 1.4.3) was used to monitor and record heart rate throughout each training session. Subjects were instructed to alter the pedaling speed and arm movement to reach and maintain the target heart rate. MICT consisted of 47 min of continuous Airdyne exercise at 70% of HRpeak. HIIT consisted of 40 min of interval Airdyne exercise: 10-min warm-up at 70% of HRpeak, four 4-min intervals at 90% of HRpeak alternated by 3-min intervals of active recovery at 70% of HRpeak, and 5-min cool-down at 70% of HRpeak (Figure 3-2).

Prior to the eight-week exercise intervention, a period of pre-conditioning was performed with the goal of completing 40 minutes of continuous Airdyne exercise at 70% of HRpeak. The pre-conditioning program began with a 15-min session of Airdyne exercise at a moderate pace. Subsequent sessions gradually increased in duration as tolerated until 40 min of continuous Airdyne exercise at 70% of HRpeak could be completed. The preconditioning period took an average of 6 sessions (min: 2 and max: 10).

**Study Procedures**

Pre- and post-intervention measures were obtained at the same time of day after a 12-hour overnight fast (including abstinence from alcohol and caffeine). Subjects rested supine for at least 20 minutes in a semi-darkened temperature-controlled quiet room prior to measurements.

**Brachial Artery Flow-Mediated Dilation**

Brachial artery flow-mediated vasodilation non-invasively assesses conduit artery endothelial function in response to reactive hyperemia, a physiological stimulus (12, 13).
An ultrasound system with a 7.5 MHz linear transducer (Aplio XV, Toshiba) was used to acquire brachial artery images 4 to 8 cm proximally to the antecubital fossa, strictly following well-established guidelines. Brachial artery flow-mediated vasodilation was assessed as previously described in our published studies (92, 93).

Briefly, the arm was abducted at heart level and fixed in position using a commercially available arm support (Versaform, Sammons Preston Rolyan). A rapid inflation/deflation system (E20 and AG 101, D. E. Hokanson) was connected to a pressure cuff placed around the widest and most proximal part of the forearm. Once the brachial artery image was optimized, the ultrasound transducer was fixed in place with a clamp (Flexbar, Flexbar Machine Corporation) to maintain optimal image quality and to prevent arm movement during data collection.

ECG R-gated B-mode 2D ultrasound images and spectral Doppler waveforms with an insonation angle set at $\leq 60^\circ$ were digitally recorded using the Vascular Imager software (Medical Imaging Applications, LLC) for one minute at baseline and for two minutes following reactive hyperemia to measure peak brachial artery diameter. Reactive hyperemia was induced by inflating the forearm cuff to 250 mmHg for five minutes followed by rapid deflation. End-diastolic brachial artery diameters were analyzed using a commercially available, semi-automated edge-detection wall tracking software (Brachial Analyzer, Medical Imaging Applications, LLC). Peak brachial artery diameter was identified after applying a moving average (bin: 3 images) to smooth the data.

Absolute change in flow-mediated vasodilation was expressed in mm and was calculated as the difference between maximum and baseline diameter. The relative
change in flow-mediated vasodilation was expressed as (%) and was calculated as
\[(\text{maximum diameter} – \text{baseline diameter})/\text{baseline diameter}\] x100. To quantify the hyperemic response, the first 15 post-occlusion spectral Doppler envelopes and 10 baseline spectral Doppler envelopes were analyzed using the Brachial Analyzer software to obtain blood velocity. Blood flow (mL/min) was calculated as mean blood velocity (m/s) x [(baseline diameter)²/4] x π x 60 (93). Shear stress (dyne/cm²) was calculated as 8 x μ x (mean blood velocity/baseline diameter) x 10³; μ is blood viscosity and used as a constant, 0.035 dyne-sec/cm² (93). Ultrasound images and spectral Doppler envelopes were analyzed by a researcher who was blind to the subject identity and group assignment.

**Aortic Pulse Wave Velocity**

Aortic pulse wave velocity assesses human arterial stiffness (94). The SphygmoCor MM3 system was employed to measure aortic pulse wave velocity in this study as previously described (43). Briefly, aortic pulse wave velocity was determined by recording the pulse waves at the carotid and femoral arteries using a high-fidelity micromanometer (Millar Instruments, Houston, TX) and calculating the ratio of the distance between the recording sites to the time delay between the carotid and femoral pulse waves. The distance was measured with a non-stretchable tape from the suprasternal notch to the carotid site and from the suprasternal notch to the femoral site. The former distance was subtracted from the latter and used in the calculation of aortic pulse wave velocity. The average of three high-quality recordings was used for aortic pulse wave velocity.
Augmentation Index

Augmentation index is a validated measure to assess the load on the left ventricle imposed by a stiffer vascular system (94). Augmentation index was determined using the SphygmoCor MM3 system (AtCor Medical, Sydney, Australia) as previously described (94-96). Briefly, to measure augmentation index, radial artery pressure waveforms were recorded using a high-fidelity micromanometer (Millar Instruments, Houston, TX) and calibrated using brachial artery systolic and diastolic blood pressure. A validated mathematical transfer function was used to generate the central aortic pressure waveform. Aortic augmentation index was estimated as the ratio of wave reflection amplitude to the central aortic pulse pressure (97).

Resting Blood Pressure

Resting blood pressures were measured over the brachial artery using an automated oscillometric device (Dinamap, GE). The average of three measures varying <5 mmHg were used for analysis.

Height, Weight and Body Composition

Height was measured in mm using a stadiometer and body mass was assessed to the nearest 0.1 kg using an electronic scale (Tanita, Arlington Heights, IL, USA). Body mass index was determined as weight divided by height squared (kg/m²). Fat and fat-free mass, android/gynoid fat mass, and % body fat were estimated from a whole body scan using dual-energy x-ray absorptiometry (Lunar Prodigy Advance, GE, enCore version 8.70.005) as previously described (93, 98). Waist and hip circumferences were measured to the nearest mm using a non-stretchable tape. Measurements were performed at the level of the iliac crest for waist circumference and at the level of the maximum girth of the buttocks for hip circumference. The average of
three measures was used for analysis.

**Diagnostic Graded Exercise Test and Maximal Oxygen Consumption**

Oxygen consumption was measured using computer-assisted open-circuit spirometry to evaluate expired gases during a diagnostic graded exercise test on the treadmill (93, 98). A 6 minute warm up at a walking speed corresponding to 70~80% of the age-predicted maximal heart rate was followed by grade increases of 2.5% every 2 minutes until volitional exhaustion. For each subject, post-intervention testing was performed by following precisely their pre-intervention protocol. Peak oxygen consumption was considered to represent maximum oxygen uptake (VO$_{2\text{max}}$) when at least 3 of the following 4 criteria were met: a) plateau in oxygen consumption (<100 ml) with increasing exercise intensity; b) a maximal respiratory exchange ratio of at least 1.15; c) achievement (±10bpm) of age-predicted max heart rate (220-age); and d) a rating of perceived exertion of at least 18 on the Borg Rate of Perceived Exertion scale. Because not all subjects achieved 3 of the 4 criteria, VO$_{2\text{max}}$ is presented as peak oxygen consumption and maximal heart rate is presented as peak heart rate.

**Blood Lipids, Glucose, Insulin and Insulin Resistance**

Fasting blood lipids, glucose and insulin were assessed by a clinical laboratory using standard assays. Insulin resistance was evaluated using the homeostasis model of insulin resistance [HOMA-IR; HOMA-IR = (fasting insulin µU/ml x fasting glucose mg/dl)/405] (99)

**Habitual Physical Activity Level and Dietary Analysis**

The Modifiable Activity Questionnaire was used to establish the baseline habitual physical activity level and to confirm that subjects did not engage in regular exercise training. Physical activity monitors (ActiGraph GT3X, software version 5.10.0) were
used for 4 days (3 weekdays and 1 weekend day) pre- and post-intervention to confirm that habitual physical activity was stable during the intervention. The ActiGraph devices were worn 24 hours/day over the hip at the midaxillary line at the level of the iliac crest. A food diary was completed during the same days as above and was used to analyze dietary intake to confirm that diet was stable during the intervention. The ESHA Food Processor SQL (version 10.7) was used for the analysis.

**Data Analysis**

Statistical analyses were performed using IBM SPSS Statistics (Essentials, Version 22). Statistical significance was set at $P< 0.05$. Data were plotted and examined for accuracy. Descriptive statistics and normality tests were performed to evaluate data distribution. To examine the effect of the intervention on the outcomes of interest, analysis of variance with repeated measures was used including time as the within-subject factor (time; pre- vs. post-intervention measures), group as the between-subject factor (HIIT vs. MICT vs. CONT) and a time x group interaction. To examine group differences, one way analysis of variance was used with Tukey’s post hoc tests. Pre- and post-intervention measures within each group were examined using paired t-tests. The Bonferroni correction was used for multiple comparisons.
Figure 3-1. Flow chart of study design. HIIT: high intensity interval training. MICT: moderate intensity continuous training. CONT: normal sedentary lifestyle non-exercise control group.
Figure 3-2. High intensity interval training (HIIT) protocol. HRpeak refers to peak heart rate.
CHAPTER 4
RESULTS

Forty-four older adults were recruited and screened, of which twenty-three met the inclusion criteria and were randomly assigned to one of three groups (Figure 3-1): high intensity interval training (HIIT, n=7), moderate intensity continuous training (MICT, n=9), and sedentary lifestyle (non-exercise control; CONT, n=7). Two subjects who were assigned to the MICT group withdrew due to lack of motivation. Thus, seven subjects per group completed the intervention period and post-intervention measures.

The volume of exercise training per session was similar between HIIT and MICT (226±25 vs. 241±25 kcal, P=0.7). Exercise intensity was 99.5% and 95% of the time within the prescribed target heart rate zone for HIIT and MICT, respectively (P=0.9). Subjects completed 87±4% of the scheduled exercise sessions for HIIT and 88±4% for MICT (P=0.9). However, a subject assigned to the MICT group completed only 50% of the scheduled exercise sessions and therefore, was excluded from all the analyses.

Habitual physical activity level (excluding activity during scheduled exercise training sessions) did not change pre- compared with post-intervention (493,353±29,272 vs. 529,517±41,098 counts/day, P=0.2 and 5871±394 vs. 6127±466 steps/day, P=0.5). In addition, use of medication and dietary intake remained stable during the intervention (2173±189 vs. 1919±264 kcal/day, P=0.3, pre- vs. post-intervention, respectively), except for one CONT subject who restricted his caloric intake and lost 10.5 kg of body weight during the intervention. This subject was excluded from all the analyses.

Pre-intervention values for subject characteristics, body composition, blood profiles, cardiorespiratory fitness, blood pressure, flow-mediated dilation and arterial
stiffness are presented in Tables 4.1 to 4.7. There was no significant difference in these factors between HIIT, MICT and CONT prior to the intervention \( (P \geq 0.1) \).

**Body Composition Response**

Body weight decreased by 1.2 kg in HIIT \( (P=0.1) \) and 1.2 kg in MICT \( (P=0.004) \) and increased by 1.6 kg in CONT \( (P=0.04, \) not significant after Bonferroni correction; Figure 4-1 and Table 4-2). There was a significant time by group interaction for body weight \( (P=0.01) \). The change in body weight was significantly different in HIIT vs. CONT \( (P=0.02) \) and in MICT vs. CONT \( (P=0.02) \), but was not different between HIIT and MICT \( (P=0.9; \) Figure 4-1). Fat mass decreased by 1.2 kg in HIIT \( (P=0.007) \) and 0.9 kg in MICT \( (P=0.07) \) and increased by 1.1 kg in CONT \( (P=0.2; \) Figure 4-2 and Table 4-2). There was a significant time by group interaction for fat mass and group \( (P=0.03) \). The change in fat mass was different in HIIT vs. CONT \( (P=0.03) \), but the difference in MICT vs. CONT did not reach statistical significance \( (P=0.08) \). Fat mass changed similarly in HIIT vs. MICT \( (P=0.9; \) Figure 4-2). Fat-free mass did not change in response to the intervention \( (P=0.8; \) Table 4-2).

Body mass index and % body fat were measured to evaluate total adiposity. Body mass index increased by 0.7 kg/m² in CONT \( (P=0.045, \) not significant after Bonferroni correction) and decreased by 0.5 kg/m² in MICT \( (P=0.003) \) and 0.4 kg/m² in HIIT \( (P=0.1; \) Figure 4-3 and Table 4-2). There was a significant time by group interaction for body mass index \( (P=0.01) \). The change in body mass index was significantly different in HIIT vs. CONT \( (P=0.02) \) and in MICT vs. CONT \( (P=0.03) \), but was not different between HIIT and MICT \( (P=0.9; \) Figure 4-3). Body fat % did not change in response to the intervention \( (P=0.6; \) Table 4-2).
To evaluate body fat distribution, waist/hip circumference and android/gynoid fat mass were examined, however, neither one of these measures changed in response to the intervention (P=0.5 and P=0.1, respectively; Table 4-2). Waist circumference, a surrogate measure of abdominal adiposity, was also not affected by the intervention (P=0.3; Table 4-2).

**Blood Lipids, Glucose and Insulin Response**

Total, LDL and total/HDL cholesterol did not change in response to the intervention (P≥0.2; Table 4-3). There was a significant main effect for HDL cholesterol (P=0.04), but there was no time by group (P=0.2) or significant individual group changes (P≥0.03, not significant after Bonferroni correction; Table 4-3). Triglycerides were not affected by the intervention (P=0.6; Table 4-3). There was also a significant main effect for fasting glucose (P=0.048), but there was no time by group interaction (P=0.9) or significant individual group changes (P≥0.05; Table 4-3). A significant time by group interaction was found for insulin (P=0.03; Table 4-3). The change in insulin was significantly different in HIIT vs. CONT (P=0.03), but was not different in MICT vs. CONT (P=0.2) and in HIIT vs. MICT (P=0.5; Figure 4-4 and Table 4-3). Insulin decreased by 1.6 µIU/mL in HIIT (P=0.06) and 0.5 µIU/mL in MICT (P=0.2) and increased by 1.3 µIU/mL in CONT (P=0.04, not significant after Bonferroni correction; Table 4-3). Insulin resistance (HOMA-IR) decreased by 0.3 in HIIT (P=0.08) and 0.1 in MICT (P=0.3), and increased by 1.3 in CONT (P=0.06; Figure 4-5 and Table 4-3). There was a time by group interaction for insulin resistance (P=0.046). The change in insulin resistance was different in HIIT vs. CONT (P=0.04), but was not different in MICT vs. CONT (P=0.3) and in HIIT vs. MICT (P=0.6; Figure 4-5 and Table 4-3).
Cardiorespiratory Fitness Response

There was a significant main effect for peak oxygen consumption expressed in ml/kg/min (P=0.03), but there was no time by group interaction (P=0.2) or significant individual group changes (P≥0.02, not significant after Bonferroni correction; Table 4-4). Peak oxygen consumption expressed in L/min did not significantly change in response to the intervention (P=0.053; Table 4-4). Maximal exercise test duration improved by 2.3 min in HIIT (P=0.001), 0.9 min in MICT (P=0.06) and 0.1 min in CONT (P=0.5; Figure 4-6 and Table 4-4). The time by group interaction for test duration approached significance (P=0.05). The change in test duration was different in HIIT vs. CONT (P=0.045), but was not different in MICT vs. CONT (P=0.6) and in HIIT vs. MICT (P=0.2; Figure 4-6 and Table 4-4). Peak heart rate was not affected by the intervention (P=0.3; Table 4-4).

Resting Heart Rate and Blood Pressure Response

Resting heart rate did not change in response to the intervention (P=0.4; Table 4-5). Similarly, brachial systolic and diastolic blood pressure, aortic systolic blood pressure and augmentation pressure were also not influenced by the intervention (P≥0.3; Table 4-5).

Brachial Artery Flow-Mediated Dilation Response

Baseline brachial artery diameter, blood velocity and shear stress did not change in response to the intervention (P≥0.5; Table 4-6). Hyperemic blood velocity decreased by 0.16 m/s in HIIT (P=0.002), 0.07 m/s in MICT (P=0.1), and 0.08 m/s in CONT (P=0.1; Table 4-6). However, there was no time by group interaction for hyperemic blood velocity (P=0.4; Table 4-6). Hyperemic shear stress decreased by 14.7 dyne/cm² in HIIT (P=0.006), 5.9 dyne/cm² in MICT (P=0.2), and 12.9 dyne/cm² in CONT (P=0.1;
Table 4-6). However, there was no time by group interaction for hyperemic shear stress (P=0.6; Table 4-6). Flow-mediated dilation increased by 2 % in MICT (P=0.09) and decreased by 1 % in HIIT (P=0.3) and 1% in CONT (P=0.1; Figure 4-7 and Table 4-6). The time by group interaction for flow-mediated dilation, however, did not reach statistical significance (P=0.2; Table 4-6). The results for flow-mediated dilation normalized for hyperemic shear stress and flow-mediated dilation expressed as absolute change in mm were similar.

**Arterial Stiffness Response**

Aortic pulse wave velocity decreased by 0.63 m/s in MICT (P=0.002), but increased by 0.16 m/s in HIIT (P=0.4) and 0.55 m/s in CONT (P=0.2; Figure 4-8 and Table 4-7). However, the time by group interaction for aortic pulse wave velocity did not reach statistical significance (P=0.1; Table 4-7). Augmentation index normalized at heart rate of 75 beats/min decreased by 3.2% in HIIT (P=0.2), 0.2% in MICT (P=0.5) and increased by 0.6% in CONT (P=0.3; Figure 4-9 and Table 4-7), but these changes were not significant. There was also no time by group interaction for augmentation index normalized at heart rate of 75 beats/min (P=0.6).
Table 4-1. Subject characteristics at pre-intervention

<table>
<thead>
<tr>
<th></th>
<th>HIIT</th>
<th>MICT</th>
<th>CONT</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, m/f</td>
<td>1/6</td>
<td>2/4</td>
<td>1/5</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>65.7±2.0</td>
<td>64.3±2.7</td>
<td>62.2±2.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Height, cm</td>
<td>159.5±2.8</td>
<td>160.3±3.3</td>
<td>163.8±3.8</td>
<td>0.6</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>75.4±7.0</td>
<td>74.7±5.3</td>
<td>72.4±10.0</td>
<td>0.9</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>29.4±2.3</td>
<td>29.1±2.5</td>
<td>26.6±2.5</td>
<td>0.7</td>
</tr>
<tr>
<td>Physical activity, counts/day</td>
<td>492733±32861</td>
<td>538613±72235</td>
<td>493225±49853</td>
<td>0.8</td>
</tr>
<tr>
<td>Steps, counts/day</td>
<td>5461±269</td>
<td>6461±1043</td>
<td>5980±616</td>
<td>0.6</td>
</tr>
<tr>
<td>Medications, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-blockers</td>
<td>0 (0%)</td>
<td>1 (17%)</td>
<td>1 (17%)</td>
<td></td>
</tr>
<tr>
<td>Diuretics</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (17%)</td>
<td></td>
</tr>
<tr>
<td>Statins</td>
<td>1 (14%)</td>
<td>2 (33%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Acetylsalicylic acid</td>
<td>3 (43%)</td>
<td>0 (0%)</td>
<td>2 (33%)</td>
<td></td>
</tr>
</tbody>
</table>

Data are mean±SE.
<table>
<thead>
<tr>
<th></th>
<th>HIIT</th>
<th></th>
<th>MICT</th>
<th></th>
<th>CONT</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>75.4±7.0</td>
<td>74.2±6.5</td>
<td>74.7±5.3</td>
<td>73.4±5.0**</td>
<td>72.4±10.0</td>
<td>74.0±10.1</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>31.3±2.9</td>
<td>30.1±2.9**</td>
<td>28.5±4.2</td>
<td>27.6±3.9</td>
<td>27.1±5.6</td>
<td>28.2±5.4</td>
</tr>
<tr>
<td>Fat-free mass, kg</td>
<td>44.1±4.6</td>
<td>44.1±4.1</td>
<td>46.1±2.5</td>
<td>45.9±2.7</td>
<td>45.4±4.8</td>
<td>45.8±5.0</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>29.4±2.2</td>
<td>29.0±1.9</td>
<td>29.1±2.0</td>
<td>28.6±2.0**</td>
<td>26.6±2.9</td>
<td>27.3±3.0</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>41.6±1.7</td>
<td>40.4±1.9</td>
<td>37.4±3.5</td>
<td>36.9±3.4</td>
<td>35.9±2.9</td>
<td>36.9±2.5</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>101.1±5.9</td>
<td>98.8±5.2</td>
<td>98.0±6.2</td>
<td>95.4±5.9</td>
<td>88.6±10.1</td>
<td>89.3±9.5</td>
</tr>
<tr>
<td>Waist/hip circumference</td>
<td>0.93±0.03</td>
<td>0.92±0.03</td>
<td>0.90±0.03</td>
<td>0.90±0.04</td>
<td>0.85±0.05</td>
<td>0.84±0.05</td>
</tr>
<tr>
<td>Android/gynoid fat mass</td>
<td>0.59±0.07</td>
<td>0.58±0.07</td>
<td>0.50±0.05</td>
<td>0.49±0.05</td>
<td>0.46±0.09</td>
<td>0.44±0.08</td>
</tr>
</tbody>
</table>

Data are mean±SE; **P<0.01, pre- vs. post-intervention.
Table 4-3. Blood lipids, fasting glucose, and insulin at pre- and post-intervention

<table>
<thead>
<tr>
<th></th>
<th>HIIT</th>
<th>MICT</th>
<th>CONT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>Total Cholesterol, mg/dL</td>
<td>205±20</td>
<td>190±17</td>
<td>189±12</td>
</tr>
<tr>
<td>HDL, mg/dL</td>
<td>57±6</td>
<td>58±8</td>
<td>56±3</td>
</tr>
<tr>
<td>LDL, mg/dL</td>
<td>123±12</td>
<td>110±11</td>
<td>115±10</td>
</tr>
<tr>
<td>Total/HDL cholesterol</td>
<td>3.8±0.4</td>
<td>3.5±0.3</td>
<td>3.4±0.2</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>126±40</td>
<td>109±21</td>
<td>92±8</td>
</tr>
<tr>
<td>Fasting glucose, mg/dL</td>
<td>91±2</td>
<td>93±3</td>
<td>90±4</td>
</tr>
<tr>
<td>Insulin, µIU/mL</td>
<td>5.7±1.1</td>
<td>4.1±0.6</td>
<td>3.3±0.6</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.28±0.24</td>
<td>0.96±0.15</td>
<td>0.74±0.12</td>
</tr>
</tbody>
</table>

Data are mean±SE; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HOMA-IR, homeostatic model assessment-insulin resistance.
<table>
<thead>
<tr>
<th></th>
<th>HIIT</th>
<th></th>
<th>MICT</th>
<th></th>
<th>CONT</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td><strong>VO₂peak, L/min</strong></td>
<td>1.73±0.17</td>
<td>1.91±0.19</td>
<td>1.67±0.15</td>
<td>1.82±0.15</td>
<td>1.56±0.08</td>
<td>1.59±0.09</td>
</tr>
<tr>
<td><strong>VO₂peak, mL/kg/min</strong></td>
<td>21.8±0.7</td>
<td>24.2±0.6</td>
<td>21.9±3.0</td>
<td>25.1±2.5</td>
<td>23.2±2.7</td>
<td>23.1±2.6</td>
</tr>
<tr>
<td><strong>Test duration, min</strong></td>
<td>9.08±0.68</td>
<td>11.40±0.95**</td>
<td>10.97±0.88</td>
<td>11.87±0.97</td>
<td>10.85±1.22</td>
<td>10.97±1.00</td>
</tr>
<tr>
<td><strong>Peak heart rate, beats/min</strong></td>
<td>160±3</td>
<td>159±3</td>
<td>160±6</td>
<td>156±6</td>
<td>160±11</td>
<td>156±10</td>
</tr>
</tbody>
</table>

Data are mean±SE; VO₂peak, peak oxygen consumption; **P<0.01, pre- vs. post-intervention.
<table>
<thead>
<tr>
<th></th>
<th>HIIT</th>
<th>MICT</th>
<th>CONT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>65±2</td>
<td>64±2</td>
<td>59±1</td>
</tr>
<tr>
<td>Brachial SBP, mmHg</td>
<td>117±3</td>
<td>115±5</td>
<td>113±4</td>
</tr>
<tr>
<td>Brachial DBP, mmHg</td>
<td>68±2</td>
<td>68±2</td>
<td>68±2</td>
</tr>
<tr>
<td>Aortic SBP, mmHg</td>
<td>112±3</td>
<td>109±5</td>
<td>108±3</td>
</tr>
<tr>
<td>Augmentation pressure, mmHg</td>
<td>16±2</td>
<td>14±3</td>
<td>13±2</td>
</tr>
</tbody>
</table>

Data are mean±SE; SBP, systolic blood pressure; DBP, diastolic blood pressure.
Table 4-6. Brachial artery flow-mediated dilation at pre- and post-intervention

<table>
<thead>
<tr>
<th></th>
<th>HIIT</th>
<th>MICT</th>
<th>CONT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>Baseline diameter, mm</td>
<td>3.54±0.29</td>
<td>3.57±0.23</td>
<td>3.48±0.23</td>
</tr>
<tr>
<td>Baseline velocity, m/sec</td>
<td>0.19±0.03</td>
<td>0.17±0.03</td>
<td>0.15±0.02</td>
</tr>
<tr>
<td>Baseline SS, dyne/cm²</td>
<td>14.11±2.69</td>
<td>14.02±2.69</td>
<td>11.19±1.40</td>
</tr>
<tr>
<td>Hyperemic velocity, m/sec</td>
<td>1.06±0.05</td>
<td>0.90±0.06**</td>
<td>1.01±0.10</td>
</tr>
<tr>
<td>Hyperemic SS, dyne/cm²</td>
<td>86.19±6.82</td>
<td>71.53±5.52**</td>
<td>84.92±13.23</td>
</tr>
<tr>
<td>FMD, mm</td>
<td>0.20±0.04</td>
<td>0.18±0.05</td>
<td>0.10±0.02</td>
</tr>
<tr>
<td>FMD, %</td>
<td>5.85±1.27</td>
<td>4.89±1.17</td>
<td>2.97±0.85</td>
</tr>
<tr>
<td>FMD/Hyperemic SS</td>
<td>0.067±0.014</td>
<td>0.069±0.017</td>
<td>0.034±0.007</td>
</tr>
</tbody>
</table>

Data are mean±SE; SS, shear stress; FMD, flow-mediated dilation; **P<0.01, pre- vs. post-intervention.
Table 4-7. Arterial stiffness at pre- and post-intervention

<table>
<thead>
<tr>
<th></th>
<th>HIIT</th>
<th>MICT</th>
<th>CONT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>Aortic pulse wave velocity, m/sec</td>
<td>9.59±0.39</td>
<td>9.75±0.60</td>
<td>9.44±0.59</td>
</tr>
<tr>
<td>Augmentation index @HR75, %</td>
<td>31.1±3.4</td>
<td>27.9±3.4</td>
<td>25.6±3.0</td>
</tr>
</tbody>
</table>

Data are mean±SE; Augmentation index @ HR75, augmentation index normalized at heart rate of 75 beats/min; **P<0.01, pre- vs. post-intervention.
Figure 4-1. Change in body weight in response to the intervention. HIIT, high intensity interval training; MICT, moderate intensity continuous training; CONT, sedentary lifestyle (non-exercise control). *P=0.02 vs. CONT; †P=0.02 vs. CONT.
Figure 4-2. Change in fat mass in response to the intervention. HIIT, high intensity interval training; MICT, moderate intensity continuous training; CONT, sedentary lifestyle (non-exercise control). *P=0.03 vs. CONT.
Figure 4-3. Change in body mass index in response to the intervention. HIIT, high intensity interval training; MICT, moderate intensity continuous training; CONT, sedentary lifestyle (non-exercise control). *P=0.02 vs. CONT; †P=0.03 vs. CONT.
Figure 4-4. Change in insulin in response to the intervention. HIIT, high intensity interval training; MICT, moderate intensity continuous training; CONT, sedentary lifestyle (non-exercise control). *P=0.03 vs. CONT.
Figure 4-5. Change in insulin resistance (HOMA-IR) in response to the intervention. HIIT, high intensity interval training; MICT, moderate intensity continuous training; CONT, sedentary lifestyle (non-exercise control). *P=0.04 vs. CONT.
Figure 4-6. Change in maximal exercise test duration in response to the intervention. HIIT, high intensity interval training; MICT, moderate intensity continuous training; CONT, sedentary lifestyle (non-exercise control). *P=0.045 vs. CONT.
Figure 4-7. Change in brachial artery flow-mediated dilation in response to the intervention. HIIT, high intensity interval training; MICT, moderate intensity continuous training; CONT, sedentary lifestyle (non-exercise control).
Figure 4-8. Change in aortic pulse wave velocity in response to the intervention. HIIT, high intensity interval training; MICT, moderate intensity continuous training; CONT, sedentary lifestyle (non-exercise control).
Figure 4-9. Change in augmentation index normalized to heart rate of 75 beats/min (@ HR75) in response to the intervention. HIIT, high intensity interval training; MICT, moderate intensity continuous training; CONT, sedentary lifestyle (non-exercise control).
CHAPTER 5
DISCUSSION

The present randomized controlled trial investigated for the first time the effect of HIIT compared with MICT on brachial artery flow-mediated dilation and arterial stiffness in healthy older adults without overt cardiovascular disease. The major findings are: 1) Eight weeks of HIIT decreased fat mass and increased maximal exercise test duration. However, it did not improve brachial artery flow-mediated dilation and arterial stiffness. 2) MICT decreased body weight and body mass index and improved aortic pulse wave velocity. But, it did not influence augmentation index or flow-mediated dilation in older adults. 3) Contrary to our hypothesis, HIIT was not superior to MICT in improving flow-mediated dilation and arterial stiffness.

**Body Composition, Blood Profile and Cardiorespiratory Fitness Response**

Body weight and body mass index decreased following MICT, but did not change following HIIT in our study. In addition, fat mass decreased in response to HIIT, but did not change in response to MICT. Kohrt et al. also reported significant decreases in body weight and fat mass in older adults in response to long-term (9 to 12 months) moderate intensity walking/jogging (100). Blood lipids, fasting glucose, insulin and insulin resistance were not improved in response to HIIT and MICT in our study. Our findings agree with the reported lack of effect of HIIT and MICT on these factors in older heart failure patients (20). We are not aware of published data comparing the effect of MICT to HIIT on body composition and blood profile in older adults free from cardiovascular disease.

Maximal exercise test duration improved ~2 min in response to HIIT, but did not change in response to MICT. Test duration is closely associated with cardiopulmonary
fitness, an important predictor of cardiovascular disease events and mortality. For every minute increase in test duration there is an associated 7.9% decrease in mortality risk (101). Pierce et al demonstrated in older adults that eight weeks of moderate intensity walking exercise improved test duration by 1.8 minutes (72). Peak oxygen consumption was not improved in that study, which is similar to the lack of effect in response to HIIT and MICT in the present study. We are not aware of published data comparing the effect of MICT to HIIT on cardiorespiratory fitness in older adults free from cardiovascular disease.

**Brachial Artery Flow-Mediated Dilation Response**

Brachial artery flow-mediated dilation progressively declines with advancing age. In the present study, HIIT and MICT did not significantly influence flow-mediated dilation, but in MICT flow-mediated dilation increased by 74% (from 3.0% to 5.2%; pre- vs. post-intervention). Although this is not statistically significant, this magnitude of change might be physiologically important. For every 1% improvement in flow-mediated dilation there is an associated ~13% reduction in cardiovascular disease risk (102). We are not aware of other published data examining the effect of HIIT on flow-mediated dilation in older adults free from cardiovascular disease. However, in older heart failure patients, both HIIT and MICT improved flow-mediated dilation and HIIT was associated with a greater improvement in flow-mediated dilation compared with MICT (20). In that study, ~80% of the subjects were men, whereas in our study ~73% were postmenopausal women.

Habitually active older men have preserved flow-mediated dilation compared with age-matched sedentary men. In addition, moderate intensity aerobic exercise training has been reported to improve flow-mediated dilation in older sedentary men (71, 103).
However, the effect of aerobic exercise training on vascular endothelial function in postmenopausal women is not clear. Pierce et al. demonstrated that eight weeks of aerobic walking exercise improved brachial artery flow-mediated dilation in older men, but not in postmenopausal women (72). They found that eight weeks of moderate intensity continuous exercise did not improve flow-mediated dilation (5% vs. 6%, pre vs. post-intervention) in the whole group of 26 older adults (11 older men & 15 older postmenopausal women). However, flow-mediated dilation increased in ten out of the eleven older men in response to exercise training (4.5% vs. 7%, pre vs. post-intervention). On the contrary, flow-mediated dilation decreased in eleven out of the fifteen older women (5% vs. 5.3%, pre vs. post-intervention).

In our study, the number of men in HIIT and MICT was small which prevented examining the effect of sex in each protocol separately. But, when combining the HIIT and MICT groups, there were a total of 13 older adults of which 10 were postmenopausal women (77%). In the whole group, flow-mediated dilation did not change in response to HIIT and MICT combined (4.52 vs. 5.02%, P=0.7, n=13, pre vs. post-intervention). However, the change in flow-mediated dilation in response to exercise training (HIIT and MICT combined) was different in men compared with women (4.77% vs. -0.8%, P=0.03, respectively). Taken together, these data suggest that our findings might have been influenced by the larger number of female subjects.

The increase in shear stress following reactive hyperemia is an important stimulus for NO production and brachial artery flow-mediated endothelium-dependent dilation. In the current study, hyperemic shear stress significantly decreased in response to HIIT, which might have also influenced our flow-mediated dilation results.
However, the results were similar when flow-mediated dilation was normalized for hyperemic shear stress, indicating that this might not have been a serious confounding factor. It is unclear why hyperemic shear stress decreased in response to exercise training in our study. The decrease in hyperemic shear stress was associated with a decrease in hyperemic blood velocity. Our data are in agreement with Eskurza et al. who reported decreased hyperemic blood velocity in aerobically trained compared with sedentary older men (53).

The HIIT and MICT protocol in the present study were based on a published protocol by Wisloff et al. (19, 20) demonstrating significant improvements in flow-mediated dilation in heart failure and metabolic syndrome patients. However, some differences exist between the Wisloff protocol and the protocol used in the current study. First, their exercise training protocol involved “uphill” treadmill walking, whereas our protocol involved Airdyne bicycle exercise. Walking “uphill” involves larger muscle mass compared with cycling. However, the simultaneous arm and leg movement during Airdyne bicycle exercise in our study might have led to similar amount of muscle mass activation. Second, their exercise intervention was 12-weeks long and involved no pre-conditioning, whereas our study was 8-weeks long and included an additional period of time for pre-conditioning (mean: 6 pre-conditioning sessions and range: 2 to 10). However, the training frequency in our protocol was 4 times per week, whereas, theirs was 3 times per week. Therefore, the total volume of training was comparable (38 vs. 36 sessions; current study protocol vs. Wisloff protocol) (19, 20). But despite the similar total training volume we cannot rule out that the length of training might be a more influential factor on vascular physiology than training volume.
Arterial Stiffness Response

In the current study, MICT significantly decreased aortic pulse wave velocity by 0.6 m/sec in sedentary older adults, but HIIT did not affect arterial stiffness. A change of 1 m/s in aortic pulse wave velocity is associated with ~7% change in cardiovascular disease risk (104). We are not aware of published data comparing the effect of MICT to HIIT on aortic pulse wave velocity in older adults free from cardiovascular disease. Some previous studies have compared the effect of HIIT vs. MICT on arterial stiffness in hypertensive patients and young normotensive women with high familial risk for hypertension. They demonstrated that sixteen weeks of HIIT improved aortic pulse wave velocity but MICT had no effect (90, 105).

Regarding the significant improvement in aortic pulse wave velocity in response to MICT in our data, a previous study in middle-aged adults also demonstrated that 16 weeks of moderate intensity walking or jogging improved aortic pulse wave velocity by 0.7 m/s (86). HIIT and MICT did not improve brachial blood pressures, aortic systolic blood pressure and augmentation index. In agreement with our findings, Sugawara et al. demonstrated that eight weeks of moderate intensity cycle exercise does not improve aortic systolic blood pressure and augmentation index in sedentary postmenopausal women (88).

Conclusions

Brachial artery flow-mediated dilation and aortic pulse wave velocity are important therapeutic targets for prevention of cardiovascular disease in older adults. Aerobic exercise training is often prescribed for reducing the risk for cardiovascular disease, but the current exercise prescription guidelines for older adults remain generic. HIIT has recently been reported to be superior to MICT in improving cardiovascular...
disease risk factors in heart failure patients, but the effect of HIIT in older adults free of cardiovascular disease has not been previously reported.

The major findings of the current study are as follows: 1) Eight weeks of HIIT decreased fat mass and increased maximal exercise test duration. However, it did not improve brachial artery flow-mediated dilation and arterial stiffness in older adults. 2) MICT decreased body weight and body mass index, and improved aortic pulse wave velocity. But, it did not influence augmentation index or flow-mediated dilation in older adults. 3) Contrary to our hypothesis, HIIT was not superior to MICT in improving flow-mediated dilation and arterial stiffness.

**Study Limitations and Future Directions**

This study is limited by the small sample size. It is possible that increasing the number of subjects per group might lead to improved statistical power and significant findings for those outcomes that approached but did not reach statistical significance.

The predominance of female subjects in this study limits the generalizability of the findings. The inclusion of both male and female subjects might be confounding the response to exercise training because some studies show effects in older men but not in older postmenopausal women. Future studies should investigate how sex affects the vascular responses to HIIT and MICT in older adults. Currently, there is no definitive information if exercise training improves flow-mediated dilation in postmenopausal women.

The duration of the current intervention is eight weeks. It is possible that a more long-term exercise intervention (e.g., 1 year) might lead to different findings. However, the cost and feasibility of such studies are prohibitive. In addition, previous short-term interventions, as short as two weeks have resulted in significant effects on flow-
mediated dilation (91) and arterial stiffness (106). Thus, eight weeks should be sufficient time to detect improvements in flow-mediated dilation and arterial stiffness.

Although peripheral artery flow-mediated dilation is intended to test endothelium-dependent dilation, the results of this test can be influenced by vascular smooth muscle function. In this study we did not assess endothelium-independent dilation by measuring brachial artery dilation to sublingual nitroglycerin, a NO donor. However, vascular smooth muscle responsiveness to a NO donor is not impaired with advancing age and is not influenced by aerobic exercise (49). Thus, one can postulate that any change in flow-mediated dilation in response to exercise training would not reflect changes in vascular smooth muscle function.

The current study did not investigate whether HIIT and MICT differentially affect cellular/molecular factors associated with vascular endothelial function and arterial stiffness. Future studies using endothelial cell biopsies to collect and study protein levels associated with vascular endothelia function in freshly isolated human vascular endothelial cells will be valuable in providing mechanistic insight into the underlying mechanisms of exercise-induced blood vessel adaptations in human aging.

Finally, exercise training studies in the future should focus on subpopulations of older adults with increased risk factors for cardiovascular disease including hypertension and hypercholesterolemia. Moreover, more interventions are needed to establish the optimal type and dose of exercise for preventing and treating arterial aging.
LIST OF REFERENCES


BIOGRAPHICAL SKETCH

Moon Hyon Hwang was born in the Republic of Korea in 1976. He grew up in Seoul with his younger sister. He graduated from Chungdam High School in Seoul, Korea in 1995. He earned his Bachelor’s degree in Physical Education from Korea University in 1999, and his Master’s degree in Exercise Physiology also from Korea University in 2001. He was then recruited from the Korea Air Force Academy to teach cadets Physical Education classes. As officer and full-time instructor, he successfully completed the required military duty for 40 months. To receive advanced training in the field of Exercise Physiology, he decided to pursue a doctoral degree in the United States. In August 2006, he began graduate work at Texas A&M University. He joined the Integrative Cardiovascular Physiology Laboratory at the Department of Health and Kinesiology, where, under Dr. Demetra Christou’s mentorship, he was actively involved in NIH- and AHA-funded research studies. In August 2010, his PhD advisor and the Integrative Cardiovascular Physiology Laboratory relocated to the University of Florida. Thus, he transferred to the Department of Applied Physiology and Kinesiology, at the University of Florida to be able to continue to work with his advisor. His research focused on examining the effects of aerobic exercise training on cardiovascular health in human aging and type II diabetes. He earned his Doctor of Philosophy degree in Health and Human Performance with a concentration in Exercise Physiology at the University of Florida in August 2014. He is married to Jaeun Gu, and they have a pretty little girl, La-Eum Elizabeth Hwang, who was born in Gainesville, Florida in 2012.