MECHANISMS ASSOCIATED WITH VASCULAR STRUCTURE AND FUNCTION

MECHANISMS ASSOCIATED WITH THE REGULATION OF VASCULAR STRUCTURE AND FUNCTION IN HUMANS

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Abstract

A comprehensive understanding of the mechanisms regulating vascular structure and function may assist in designing effective strategies to decrease cardiovascular disease risk. The current studies were designed to investigate a) relationships between collagen markers and arterial stiffness and markers of vasoconstriction and inflammation and endothelial function in humans with a wide range of vascular health, including overweight women, elderly healthy men, individuals with coronary artery disease, individuals with spinal cord injury and young healthy men and b) changes in arterial structure and function and circulating serum markers of type I collagen synthesis and degradation, vasoconstriction and inflammation in overweight pre-menopausal women before and after a 16- week diet and exercise intervention. Resting brachial artery flow mediated dilation (FMD), upper limb and/or central pulse wave velocity (PWV_{c-r} and PWV_{c-f}) and carotid artery distensibility were assessed at baseline in all groups and, in the overweight population, after the 16-week intervention. Pro-collagen type I C-peptide (PIP), C-telopeptide of type I collagen (CTX), markers of collagen synthesis and degradation respectively, endothelin-1 (ET-1) a vasoconstrictor and interleukin-6 (IL-6) an inflammatory marker were measured. In the spectrum of vascular health, a negative relationship exists between collagen markers and central PWV (CTX–PWV_{c-f}: $r = -0.41$, $p = 0.001$ and PIP – PWV_{c-f}: $r = -0.32$, $p = 0.01$) and a positive relationship between markers and carotid distensibility (CTX: $r = 0.59$, $p<0.001$ and PIP: $r = 0.45$, $p<0.001$). ET-1 is negatively associated with absolute and relative FMD (relative: $r = -0.41$, $p<0.001$ and absolute: $r = -0.41$, $p<0.001$). Relative FMD and PWV_{c-r} increased over time in the overweight population (FMD pre: 4.1 ± 0.5 % vs. post: 6.9 ± 0.7 %, $p<0.05$ and PWV_{c-r} pre: 8.1 ± 0.3 m/s vs. post: 8.9 ± 0.3 m/s, $p<0.05$). CTX was increased after the intervention (pre: 0.65 ± 0.01 ng/mL vs. post: 0.80 ± 0.02 ng/mL, $p<0.001$). These studies increase the comprehensive understanding of factors associated with the regulation of vascular structure and function in a spectrum of populations.

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List of Abbreviations and Symbols

CVD – cardiovascular disease
WHO – World Health Organization
PWV – pulse wave velocity
PWVc-f – carotid to femoral pulse wave velocity
PWVc-r – carotid to radial pulse wave velocity
PWVb-a – brachial to ankle pulse wave velocity
CV - cardiovascular
FMD – flow-mediated dilation
ET-1 – endothelin-1
SCI – spinal cord injury
CAD – coronary artery disease
NO – nitric oxide
AIx – augmentation index
LDL – low-density lipoprotein
PIP – pro-collagen type I
ICTP – carboxy terminal peptide of collagen type I
CTX – cross-linked telopeptide of type I collagen
TNF∝ – tumor necrosis factor alpha
IL-6 – interleukin-6
MMPs - metalloproteases
AGEs – advanced glycation end products
eNOS – endothelial nitric oxide synthase
ROS – reactive oxygen species
BH_4 - tetrahydrobiopterin

List of Original Manuscripts

This thesis, presented in sandwich format, is based on the following three original manuscripts.

1. **Cotie LM,** Currie K.D, Totosy de Zepetnek, McGill G, Phillips S.M & MacDonald M.J. **Associations between functional measures and serum markers of vascular structure and function**

2. **Cotie LM,** Josse A.R, Phillips S.M & MacDonald M.J. **Endothelial function increases after a 16-week diet and exercise intervention in overweight and obese young women.**

3. **Cotie LM,** Josse A.R, Phillips S.M & MacDonald M.J. **16-weeks of combined aerobic and resistance training and hypocaloric diet and measures of arterial stiffness in overweight young women.**

Preface: Authors Contributions to Multi-Authored Papers

1. **Cotie LM,** Currie K.D, Totosy de Zepetnek J.O, McGill G, Phillips S.M & MacDonald M.J. **Associations between functional measures and serum markers of vascular structure and function**

   L.M Cotie's role: YOUNG STUDY: Co-author of ethics application, co-investigator responsible for study design and coordination, participant recruitment, data collection and arterial structure and function analysis, blood analysis. **OLD STUDY:** Co-author of ethics application, lead investigator of vascular measures, study design and coordination associated with vascular measures, vascular data collection and analysis, blood collection, handling and analysis. **CAD STUDY:** Blood analysis and interpretation. **SCI STUDY:** Blood analysis and interpretation. **ALL STUDIES COMBINED:** Statistical analysis and interpretation and primary author of the manuscript.

2. **Cotie LM,** Josse A.R, Phillips S.M & MacDonald M.J. **Endothelial Function increases after a 16-week diet and exercise intervention in overweight and obese young women.**

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3. **Cotie LM,** Josse A.R, Phillips S.M & MacDonald M.J. **16-weeks of combined aerobic and resistance training and hypocaloric diet and measures of arterial stiffness in overweight pre-menopausal women.**

   L.M Cotie's role: Author of ethics application associated with vascular measures, study design and coordination associated with vascular measures, assisted in participant exercise training for cohort involved in vascular measures, vascular data collection, analysis and interpretation, blood collection, handling and analysis, primary author of manuscript

1.0 CHAPTER 1:

INTRODUCTION

Cardiovascular disease (CVD) is caused by disorders of the heart and blood vessels, and includes coronary heart disease, cerebrovascular disease, elevated blood pressure (hypertension), peripheral artery disease, rheumatic heart disease, congenital heart disease and heart failure. The World Health Organization (WHO) lists CVD as the leading cause of death in the world [1]. According to the Global status report on non-communicable disease (2010), approximately 17.3 million people died from CVD in 2008 and it is predicted that by 2030, almost 25 million people will die from CVD [2]. In Canada, CVD is the leading cause of death in women and the second leading cause of death in males [3] and therefore represents a major global health concern. As such, it is important to be able to accurately and efficiently predict CVD risk, in order to prevent and effectively treat CVD progression. A more comprehensive understanding of the factors affecting the structure and function of the arterial system as a whole may help in the development of strategies to predict and treat CVD.

Measures of vascular structure and function are valuable markers of CVD risk. Aortic stiffness, as measured most commonly by carotid to femoral pulse wave velocity ($PWV_{c\text{-}f}$), has strong independent predictive power for all cause and CV mortality, CVD, coronary events and stroke in populations with various levels of cardiovascular (CV) risk [4-14]. PWV is a surrogate measure of arterial stiffness, as the speed of travel of the pulse wave over a given distance is

interpreted to indicate arterial stiffness [15]. Conversely, distensibility is a regional index of arterial elasticity, most commonly measured at the common carotid artery. Distensibility is the reciprocal of arterial stiffness, and decreased common carotid and aortic distensibility have been related to all-cause mortality and CV events [16].

Vascular endothelial dysfunction is a hallmark of several different forms of CVD [17-19] and is most often assessed in the brachial artery using the flow mediated dilation (FMD) test. Major CVD risk factors including smoking, hypercholesterolemia, hypertension, hyperglycemia, diabetes and obesity are often associated with endothelial dysfunction [17, 18] and studies have suggested endothelial dysfunction has CVD predictive power associated with some of the inherent risk factors of CVD [17]. Specifically, Thijssen and colleagues have demonstrated decreased FMD is associated with an increased risk of cardiovascular morbidity and mortality [20].

A comprehensive understanding of the mechanisms regulating changes in vascular structure and function may assist in designing effective strategies for decreasing CVD risk. Investigating circulating serum markers of collagen turnover, inflammation and vasoconstriction, which may mechanistically explain some of the key arterial structure and function features leading to CVD, may assist with refinement and development of targeted interventions aimed to decrease the risk of CVD. There is conflict in the literature, with respect to the association between some circulating serum blood markers and functional

measures of vascular structure and function [21-30]. Very little data exists demonstrating the link between several proposed blood markers and established CVD risk factors in a range of populations spanning from clinical patients to healthy individuals.

Interventions designed to target specific regulatory mechanisms may lead to decreased prevalence and attenuated progression of CVD. Lifestyle modification has the ability to reverse some of the detrimental changes to both vascular structure and function observed with CVD. For example, long-term (>1 year) sustained weight loss involving structured diet and exercise interventions have demonstrated improved vascular function [31] and structure [32] and thus potentially reducing CVD risk. Specifically. Miyaki *et al*. (2009) demonstrated that 12-weeks of an aerobic exercise intervention leads to increased carotid artery compliance and decreased plasma endothelin-1 (ET-1) concentration, a potential marker of endothelial function [32].

2.0 CHAPTER 2

REVIEW OF LITERATURE

2.1 Populations at elevated CVD risk

Certain individuals are at an increased risk for CVD and an increasing number of risk factors have been identified and evaluated for their power in predicting CVD risk. Current CVD risk factors include age, sex, obesity, ethnicity, family history, high cholesterol, harmful use of alcohol, tobacco use, dyslipidemia, diabetes, physical inactivity and high blood pressure [33]. The risk factors most directly relevant to the studies conducted in this thesis, including aging, obesity, spinal cord injury (SCI) and coronary artery disease (CAD) will be discussed in more detail below. Other relevant risk factors that might influence the outcomes of the studies included in this thesis such as sex, ethnicity, alcohol intake, smoking, dyslipidemia, diabetes and hypertension, will also be discussed.

The risk of CVD increases progressively with age [34]. Increased arterial stiffness and reduced endothelial function are typical with aging and are present in many disease states common in the aging process [35-37]. A 40-50% difference in large elastic artery stiffness (increasing) and compliance (decreasing) between age 25 and 75 in healthy adults has been observed [38] and age is thought to be the main clinical determinant of large artery stiffness [39]. As individuals age, the large central arteries progressively stiffen, whereas peripheral muscular arteries are thought to change to a much lesser degree [39, 40]. Structurally, aging is associated with medial arterial wall layer breakdown thereby leading to stiffening

of the artery [41]. It is also possible that calcium plays a role in the structural stiffening of arteries with age. Calcium content in the arterial wall increases with age, causing calcium accumulation and thus a potential decrease in arterial distensibility [42]. Endothelial dysfunction is also present in the aging process [43]. Numerous studies have shown that age impairs nitric oxide (NO) - dependent vasodilation in skeletal muscle [44, 45]. Research suggests this age associated decrease in NO dependent vasodilation is triggered by a decrease in antioxidant capacity and an increase in oxidative stress [46, 47].

Obesity is another risk factor associated with CVD and insulin resistance and inflammation are recognized as important links between obesity and CVD [48]. It is thought that obese individuals may have increased risk of developing hypertension, diabetes and atherosclerosis compared to non-obese individuals and there is a relationship between the degree of obesity and the risk of morbidity and mortality from CVD and other conditions [49]. Prospective studies have documented that obesity is an independent predictor of clinical CVD [50]. Recent studies suggest arteries are stiffer in overweight and obese middle-aged individuals than their normal weight peers [51-54]. Specifically, PWV is approximately 0.5 m/s higher in obese compared with non-obese individuals and research suggests this increase in PWV is equivalent to 5-10 years of aging [53]. Obesity has also been linked to decreased endothelial function as measured by FMD [55-57], while several studies involving interventions leading to weight loss have been shown to improve endothelial function [58-62].

Garshick et al. (2005) suggest that CVD has emerged as the leading cause of mortality in individuals with chronic SCI [63]. CAD is of particular concern for individuals with SCI and is thought to occur earlier in individuals with SCI than ambulatory populations [63, 64]. Supporting the concept of accelerated age-associated CVD risk in chronic SCI, traditional risk factors associated with CVD have been shown to be higher in individuals with SCI compared to able-bodied population reference values [65]. The elevation in risk factors, with SCI, is thought to be associated with the increases in sedentary lifestyle and reduced physical function from the loss of motor function accompanying SCI [66]. Specifically, aortic PWV is decreased following spinal cord injury compared to able-bodied individuals [67, 68]. No other measures of arterial stiffness are shown to be different between SCI and able-bodied individuals, including upper [68] and lower limb PWV [67, 68], augmentation index (AIx) [69] and carotid artery compliance [67, 69]. This peripheral arterial maintenance of arterial stiffness in comparison to able bodied peers is especially evident in individuals with SCI who are regularly physically active [69]. FMD in the brachial artery, used to measure NO-mediated endothelial function, remains similar to that of able-bodied individuals following SCI [70]. Surprisingly, FMD is preserved and sometimes increased in the superficial femoral artery of the paralyzed limbs following SCI [70, 71]. This appears to be specific to endothelial dependant dilation, as endothelial independent dilation is not affected by SCI [70].

CAD is the most common form of CVD and accounts for approximately 54% of CVD deaths in Canada and 12% of CVD deaths worldwide (2011) [3]. As such, individuals with CAD are an ideal population to study in terms of CVD risk factors, as most, if not all, of their risk factors would be present in the most advanced diseased state. Individuals with coronary atherosclerosis appear to have impaired FMD in both the coronary [72] and brachial [73, 74] arteries. Additionally, decreased carotid artery distensibility is associated with atherosclerotic plaque formation in the aorta and carotid arteries [75]. Coronary artery distensibility is has been shown to be decreased in coronary arteries with atherosclerosis development [76].

Increased blood pressure, otherwise termed hypertension, is a major risk factor for CVD [77]. It is thought to be the most important risk factor for premature CVD [78]. Increased aortic PWV, indicative of increased aortic stiffness exists in individuals with hypertension [79, 80]. Endothelial dysfunction is the hallmark of essential hypertension [81]. It is characterized by impaired NO bioactivity determined by reactive oxygen species (ROS), which scavenge NO [82].

Sex differences in CVD risk factors exist. Men are at greater risk of heart disease then pre-menopausal women. However, after menopause, a women's risk is similar to that of a man [33]. Recent data suggests CVD is the number 1 killer of women and although CVD has historically primarily affected men, it is now estimated that 1 in 2 women will die of heart disease or stroke, compared with 1

in 25 women who will die of breast cancer [83]. Two of every three women have at least one of the classic risk factors for heart disease, including age (>55 years), family history of premature heart disease, cigarette smoking, high blood pressure, dyslipidemia, obesity, and diabetes [83]. In 2010, reference values for arterial stiffness were published and highlighted that there was negligible differences in aortic PWV between men and women [84]. It appears that FMD decreases more rapidly in women than men during ageing [85], which may help to explain the increased CVD risk in women after menopause.

Higher rates of CVD mortality are observed in some racial and ethnic groups [86]. Genetics may partly explain some of the difference however behavioural, social, cultural and economic factors explain these problems to a greater extent [87]. Increased CVD risk factors exist among Americans with low socioeconomic status, particularly among non-Hispanic Blacks. In individuals with high socioeconomic status, Mexican Americans and non-Hispanic Blacks have a higher risk of CVD than non-Hispanic Whites [88]. Among males, South Asians (39.1%) have a significantly higher prevalence than White Europeans (33.1%) of high CVD risk [89]. This is not the case among women [89].

Diabetes is an independent risk factor for CVD in both men and women [90]. Women with diabetes seem to lose most of their inherent gender associated protection against developing CVD [91] and patients with diabetes that develop clinical CVD have a worse prognosis than those without diabetes [92]. There are several predisposing factors, which simultaneously affect the development of

CVD and diabetes mellitus. These include obesity, physical inactivity, heredity, sex, and advancing age [93]. Previous studies have shown that aortic PWV is greater in individuals with diabetes than in controls [6, 94-96], however results are inconsistent when measuring arterial stiffness with augmentation index [97, 98]. Brachial FMD measured in individuals with Type 2 diabetes mellitus is decreased compared with healthy controls [99-102].

Dyslipidemia is characterized by an abnormal amount of lipids in the bloodstream. It is recognized as a prominent risk factor for CVD [103]. Recent data has shown lowering low-density-lipoprotein-cholesterol by 39 mg/dl (1 mmol/l) is associated with approximately one-fifth reduction in the 5-years incidence of major cardiovascular events [104]. Pitsavos et al. (1998) measured ascending aortic stiffness in young (<40years) individuals with familial hypercholesterolemia in comparison to their normolipidemia siblings to determine the effects of dyslipidemia on the elastic properties of the aorta. They suggested that aortic dimensions were similar in the two groups however aortic stiffness, as measured by aortic distensibility and aortic stiffness index, was significantly increased in the hypercholesterolemia group [105]. Wilkinson et al. (2002) used pulse wave analysis to better understand the stiffness profile of individuals with hypercholesterolemia. Aortic augmentation and estimated aortic PWV were higher in individuals with hypercholesterolemia as compared to age-matched controls [106]. Endothelial dysfunction and reduced nitric oxide (NO)

bioavailability are associated with hypercholesterolemia and may contribute to the increase in arterial stiffness and AIx observed in these individuals [106].

Smoking has long been implicated as one of the leading causes of CVD [107]. Specifically, smoking has been associated with a 2.5 times increased risk of coronary heart disease [108]. It is one of the most modifiable and preventable risk factors. Studies have shown that 12 months of smoking cessation is associated with improved arterial stiffness as measured by brachial-ankle pulse wave velocity (PWV_{b-a}) in otherwise healthy individuals [109, 110]. Johnson et al. demonstrated that smoking cessation leads to prolonged improvements in endothelial function, however, may lead to weight gain and thus small changes in CVD risk [111].

Studies have demonstrated that the effects of alcohol on the CV system suggest a higher risk of CVD in both non-drinkers and heavy drinkers and a protective effect of moderate alcohol intake; as such a U-shaped association exists between drinking frequency and CVD risk [112]. Alcohol abuse leads to impairment of endothelial-dependent vasodilation as measured by FMD [113]. This appears to be a long-term issue, as three months of abstinence does not seem to improve FMD values [113]. In comparison, a J-shaped association appears to exist between alcohol consumption and PWV [114]. As such, moderate consumption has been shown to lead to a decreased risk of CVD, however heavy consumption largely increasing CVD risk [114].

2.2 CVD risk reduction and exercise

Physical activity is one of the most important factors necessary for maintaining health and eliminating risk factors for CVD. Studies as early as 1953 showed the beneficial relationship between increased physical activity and decreased incidence of CVD [115]. A systematic review in 2008 reported that physical activity was associated with 35% risk reduction for CVD mortality and 33% risk reduction for all-cause mortality [116]. Current guidelines recommend regular physical activity for primary CVD prevention [117, 118] and treatment [119].

Many studies have suggested that arterial stiffness is lower in individuals who performed aerobic exercise regularly compared with their sedentary peers [120-122]. Central, but not peripheral arterial stiffness increases with age in sedentary healthy females however, this is not observed in highly physically active women [121]. Conversely, central artery compliance decreases with age in men even in healthy physically active men [122]. However, regular aerobic exercise does attenuate these reductions in central arterial compliance observed with age in this population [122]. Additionally, as little as 13-14 weeks of regular aerobic exercise can restore some of the loss of central arterial compliance in previously sedentary middle-aged and older men [122]. Despite the well-documented benefit of aerobic exercise on both decreasing and/or preventing arterial stiffening, the mechanisms behind this are not completely understood.

It is suggested that 60% of risk reduction from physical activity is associated with traditional risk factors and the remaining 40% might be attributed to vascular adaptations [123]. Exercise leads to increases in shear stress on the endothelium, which may elicit favourable adaptations to the vessel wall. Research has shown that regular aerobic exercise prevents age-related declines in endothelium-dependent vasodilation in healthy individuals [124] and improves endothelial function in patients with CVD [125-127].

2.3 Vascular Structure

2.3.1 Arterial wall – layers

In young healthy arteries the intima is a biologically functional membrane located between the media and the blood. The intima is primarily a single layer of endothelial cells lining the arterial wall and resting on a thin basal membrane. In the intima there is a high content of collagen, with 67% type I and 33% type III collagen [128].

The medial layer of the arterial wall consists of a complex three-dimensional network of bundles of collagen fibrils, elastin and smooth muscle cells [129]. In the media of elastic arteries, collagen, elastin and smooth muscle cells are organized in a varying number of medial lamellar units [130]. Similar to the intima, there is a large amount of both type I and III collagen in the media with 56%% type I and 44% type III [128].

The adventitia is surrounded continuously by loose perivascular tissue and consists mainly of fibroblasts and fibrocytes, histological ground matrix and

collagen fibers organized in thick bundles. The collagen fibers, primarily type I (62%), are arranged within the ground matrix and form a typically fibrous tissue [128]. In contrast to the media, in the adventitial layer the orientation of the collagen fibers is dispersed.

2.3.2 Collagen

Collagen is the most abundant protein in the human body and is most commonly found in tendons, ligaments, skin, the cornea, cartilage, bone, blood vessels, the gut, and intervertebral discs [131]. Fibroblasts are the main cell source of collagen in the body and collagen is the main load-bearing element in the artery wall [132]. Collagen is relatively inextensible and acts as a stiff structural element within the wall. The function and integrity of arteries are maintained by the tension in collagen fibers [132]. Collagen in the arterial wall exists in different subtypes. These subtypes differ in both structure and function. Their proportional ratios found in human arteries are type-1 (70-75%), type-III (20-25%) and type V (1-2%) [128].

Type III is thought to be associated with extensibility of the vessel wall, whereas changes in type I collagen may be associated with arterial stiffening [133]. Peripheral blood vessels are remodeled in hypertension and during aging and fibrous tissue accumulation in the medial layer of the vessel wall is an important feature of this process [24]. Fibrous tissue accumulation in the arterial wall may be a result of increased total collagen formation, decreased collagen degradation or a combination of both. However, changes in the ratio of collagen

subtypes may also affect the mechanical properties of the vessel wall [133]. Unfortunately, there is little known about the change in ratio of collagen subtypes in the arterial stiffening process.

Evidence exists suggesting that changes in the composition of the collagen subtypes occur in hypertensive rats with stiffer arteries [134, 135]. Alterations in collagen type-I have been shown to independently determine not only large artery stiffness, but also play an important role in the control of the smaller vessels further downstream, in both normo- and hypertensives [24]. McNulty and colleagues (2006) showed that alterations in serum collagen type-I were related to large artery stiffness. Specifically McNulty et al. (2006) demonstrated an association between blood markers of collagen synthesis and degradation, measuring carboxy terminal peptide of pro-collagen type-I (PIP) (collagen synthesis) and carboxy terminal telopeptide of collagen type-I (ICTP) (collagen degradation), and measures of PWV in normotensive and hypertensive individuals.

There is, however, limited other research investigating the relationships between circulating serum markers of type I collagen synthesis and degradation and measures of arterial stiffness [21, 23-25]. The data from the existing studies has resulted in conflicting outcomes, with regards to the nature and direction of the relationship between collagen turnover and arterial stiffness. Some studies that have reported associations between increased collagen synthesis [23] or decreased degradation [21] with increasing arterial stiffness and others that have reported the opposite relationship of increased collagen degradation with increasing arterial

stiffness [23, 24]. However, all of these studies suggest that an altered collagen environment exists during stiffening. All of the previous research was conducted in middle-aged to elderly (45- 90 years) clinical populations including stable chronic heart failure [21], chronic kidney disease [22] and medicated [23, 25] and non-medicated hypertensives [24]. A better understanding of these relationships in a wider range of populations is necessary to comprehensively determine the role of type I collagen turnover in arterial stiffening and to evaluate if serum markers of type 1 collagen turnover are useful markers of central and peripheral arterial stiffness.

2.3.3 Arterial Stiffness

In recent years, an emphasis has been placed on examining the role of arterial stiffness in the development of CVD [136-138]. Arterial stiffness assessment is currently being used more commonly in the clinical assessment of patients [139] and stiffening of the aorta and other large central arteries has been established as a potential risk factor for increased cardiovascular morbidity and mortality [39].

The term arterial stiffness refers to changes in the mechanical properties within an artery or a segment of the arterial tree. Mechanical behaviours of large arteries are extremely complex and vascular stiffening develops from a complicated interaction between stable and dynamic changes involving structural and cellular elements of the vessel wall [35]. Collagen and elastin are chemical components of the arterial wall [140] and changes in the amounts and

arrangements of these components may be important factors in the development of arterial stiffness. However, there is much conflict in the literature with regard to the role of collagen and elastin in the process of arterial stiffening [21-25]. The stability, resilience and compliance of the vascular wall are dependent on the relative contribution of the two prominent proteins; collagen and elastin [35]. The relative content of these molecules is normally held stable by a slow, but dynamic, process of production and degradation and an interruption of this balance may contribute to the development and progression of vascular stiffness [35].

Arterial stiffening has been used as a marker for increased CVD risk [141-144], however, arterial stiffness is a descriptive term that cannot be directly measured or quantified non-invasively in humans. As such, surrogate measures have been developed to represent the stiffness of arteries. Some surrogate measures commonly used to estimate arterial stiffness include arterial PWV, AIx, compliance and distensibility [139].

2.3.4 Pathophysiology of Arterial Stiffness

Arterial stiffening develops from changes within the structural and cellular components in the vessel wall, and is influenced by hemodynamic forces, physical and mechanical factors, changes in sympathetic nervous system outflow and deposition of lipids [145]. A better understanding of the mechanisms regulating these processes is necessary to fully understand the process of artery wall stiffening and its causes. The elastic properties of conduit arteries vary along the arterial tree; with more elastic arteries located proximal and stiffer arteries distal.

This difference throughout the arterial tree is caused by the molecular, cellular, histological structure of the arterial tree [145].

Aortic stiffening is thought to be associated with a variety of processes, including breaks in elastin fibers, increases and accumulation of collagen, fibrosis, inflammation, medial smooth muscle necrosis, calcifications, abnormal and unorganized endothelial cells, and diffusion of macromolecules within the arterial wall [145, 146].

Collagen and elastin are strongly regulated by matrix metalloproteases (MMPs) that degrade the extracellular matrix of the vessel wall by creating uncoiled, less effective collagen and broken elastin [35]. Disruption of the cross-linking in collagen and elastin molecules leads to arterial stiffness. Collagen molecules are responsible for the tensile strength of a vessel wall and are enzymatically cross-linked making them insoluble to hydrolytic enzymes. When this cross-linking breaks, the collagen molecule unravels. Collagen has a high chance of non-enzymatic glycation cross-linking thereby leading to increased collagen content, usually in a more unorganized and dysfunctional fiber distribution [147, 148]. Collagen cross-linking also stabilizes elastin molecules and disruption of this cross-linking contributes to weakening of the elastin and thus a predisposition to mineralization by calcium and phosphorous, and increasing arterial stiffness [147, 148]. Advanced glycation end products (AGEs) also lead to arterial stiffening. AGEs result from non-enzymatic protein glycation forming irreversible cross-links in collagen and elastin molecules [149, 150].

AGE-linked collagen is stiffer and AGE-linked elastin reduces the elastin of the vessel wall [150]. AGEs may also affect endothelial cell function by using up NO and increasing the generation of oxidant species [151].

The most accepted model of the arterial tree is the propagative model. Specifically, the propagative model assumes the arterial tree is a series of viscoelastic tubes with distributed elastic properties. This model permits generation of a forward pressure wave through contraction of the heart, which travels along the tube [139]. The numerous branch points and large resistance within the tube's end create reflective retrograde waves [139]. The theory behind this model is that the higher the arterial stiffness, the higher the speed of travel of both the forward and retrograde waves. An increase in sympathetic nervous system outflow may lead to arterial and arteriolar constriction and also result in reflection points closer to the heart [152, 153]. When the forward moving pulse waveform combines with the reflected waves created at these reflection points the amplitude of the waveform is increased. In large elastic arteries, the PWV is often lower, thus the reflected wave arrives back at the aortic root during diastole [139]. However, stiffer arteries cause the reflected waveform to move backward more quickly and arrive during systole creating a larger waveform. A waveform with increased amplitude leads to larger systolic blood pressure values and decreased diastolic values, and therefore increased pulse pressure. Increases in systolic blood pressure produces a greater mechanical load (afterload) on the left

ventricle, therefore increasing myocardial oxygen demand. Increase systolic blood pressure is also associated with left ventricular hypertension [154, 155].

2.3.5 Measurement of Arterial Stiffness with Pulse Wave Velocity (PWV)

The measurement of PWV is generally accepted as a simple, non-invasive, robust, and reproducible method to determine arterial stiffness [139]. The fundamental mechanical principle is that pulse waves travel faster in stiffer arteries; therefore PWV measurement is considered the best surrogate to evaluate arterial stiffness. PWV is now considered a strong independent predictor of CV risk [136].

Each heartbeat initiates the flow of blood into the aorta, creating a pressure wave, which will propagate through the vasculature. PWV is defined as the speed of travel of this pressure wave along an arterial segment of known distance [139]. Higher PWV values reflect stiffer arteries and therefore higher CVD risk as PWV has been shown to be a strong predictor of CVD [39, 156]. PWV is calculated by dividing the distance between two arterial sites by the travel time of the pressure wave between these sites (Equation 1).

Equation 1 $$PWV = \frac{\Delta d}{\Delta t}$$

There are a number of tools available to non-invasively measure the pulse pressure waveform of the arterial tree including applanation tonometry, infrared plethysmography and pulse wave ultrasound. Specifically, applanation tonometry allows assessment of arterial stiffness by measuring the pressure changes detected by the pressure-sensitive tip on the tonometer at different sites on the skin surface.

Aortic PWV is assessed by measuring the speed of travel of the pressure wave from the common carotid artery to the common femoral artery (PWV_{c-f}). Aortic PWV has been shown to be a strong independent predictor of cardiovascular and all-cause mortality in healthy and clinical populations [4, 9, 13]. Because it is measured along the aortic pathway, PWV_{c-f} is most clinically relevant, since the aorta and its first branches are what the left ventricle encounters and are therefore responsible for most of the pathophysiological effects of arterial stiffness [145].

PWV_{c-f} has been used in pivotal studies demonstrating the predictive value of aortic stiffness for CV events [136, 157, 158]. Conversely, PWV measured outside of the aortic track, at the upper (carotid to radial, PWV_{c-r}) or lower limb (femoral to dorsalis pedis), has no known predictive value [15]. Despite the lack of demonstrated predictive value, measures of peripheral PWV may provide valuable information with regards to the mechanisms involved with arterial stiffening.

PWV_{c-f} is considered as the 'gold-standard' measurement of arterial stiffness [139], however, there are some limitations to the required pulse measurement at the femoral site in certain populations. There may be physical barriers, such as abdominal obesity, to obtaining a high quality pressure wave signal at the femoral site in individuals with metabolic syndrome, obesity and diabetes [159]. Additionally, large bust size in women, and large abdominal girth can make distance measurements inaccurate. Di Iorio et al. (2010) state that

$PWV_{c\text{-}r}$ is appropriate for PWV measurement in dialysis patients, because it is more tolerated for patients and simpler for data acquisition than carotid-femoral measurements [160]. McLeod et al. suggest that $PWV_{c\text{-}r}$ might be a useful non-invasive surrogate marker for assessing the extent of coronary atherosclerosis in thirty-five patients undergoing elective diagnostic coronary angiography.[161]. Conversely, Tillin et al. in a study of 159 men with and without known coronary artery disease, concluded that site matters for PWV; they found that $PWV_{c\text{-}f}$ is a better indicator of atherosclerosis than either $PWV_{c\text{-}r}$ or femoral-dorsalis-pedis PWV, and therefore should be used for studies investigating risk of CVD [162].

2.3.6 Measurement of Arterial Stiffness with Arterial Distensibility

Arterial stiffness is the reciprocal of distensibility and the elastic properties of the artery can be assessed through measurements of arterial distensibility. Arterial distensibility is determined from the variation in pressure from systolic to diastolic compared to the change in lumen diameter. Distensibility is the relative arterial diameter change for a given pressure [163]. Equation 2 outlines this calculation.

Equation 2
$$Distensibility = \frac{\left[\Pi\left(\frac{d_{\max}}{2}\right)^2 - \Pi\left(\frac{d_{\min}}{2}\right)^2\right]}{\Pi\left(\frac{d_{\min}}{2}\right)^2 \times PP}$$

When an artery has lower distensibility it suggests that there is regional arterial stiffness. Studies have shown a relationship between carotid distensibility and all-cause mortality and cardiovascular events [16, 164, 165].

Arterial compliance of an arterial segment represents the increase in cross-sectional volume for a given increase in pressure, but does not account for the diastolic dimension before distension [163].

Equation 3 $$Conpliance = \frac{\left[\Pi\left(\frac{d_{\max}}{2}\right)^2 - \Pi\left(\frac{d_{\min}}{2}\right)^2\right]}{PP}$$

Distensibility is compliance normalized by arterial diameter. Similar to PWV measures, there are various tools commonly used to measure arterial distensibility. Simultaneous collection of carotid artery pulse pressure waveforms using applanation tonometry and B-mode ultrasound imaging of the carotid artery are necessary for the acquisition of carotid artery distensibility and compliance.

Distensibility is pressure dependent, relies on arterial structural mechanics and on the function of the vascular endothelium [163]. An increased pulse pressure reflects decreased arterial distensibility and therefore increased stiffness. As such, elevated systolic blood pressure and pulse pressure are considered to be reliable indicators of cardiovascular risk [163]. Common carotid artery and aortic distensibility and compliance have been studied and related to all-cause mortality and CV events [13]. Carotid distensibility is associated with risk of CVD [75, 164, 166] and ischemic stroke [167]. In particular, Barenbrock et al. (2001) described carotid artery distensibility as an independent predictor of cardiovascular disease in 68 renal transplant recipients [164]. Similarly, van Popele et al. described a strong association between atherosclerosis and carotid artery distensibility in over 3000 elderly subjects aged 60 to 101 years [75].

2.3.7 Measurement of Arterial stiffness with Augmentation Index

The arterial pressure waveform is a combination of the forward pressure wave created by ventricular contraction and a reflected wave. Specifically, waves are reflected from the periphery, mainly at branch points or sites of impedance mismatch [139]. Waves that propagate along an elastic artery with few branches are progressively decreased in magnitude, whereas a pressure wave, which propagates along an elastic artery with numerous branches, is progressively amplified in magnitude, from central to distal conduit arteries, because of the numerous reflections. Wave reflections in peripheral arteries can amplify the incident pressure wave because reflection sites are closer to peripheral sites than to central arteries reflecting a stiffer artery. Therefore, the amplitude of the pressure wave is higher in peripheral arteries than in central arteries [139]. Theoretically, in elastic vessels, where PWV is assumed to be lower, the reflected wave arrives back at the aortic root during diastole; whereas in stiff arteries with higher PWV, the reflected wave arrives back at the central arteries earlier adding to the forward wave and increasing the systolic pressure [139]. This difference in pressure in different segments of the arterial tree can be quantified through what is called the augmentation index (AIx). AIx is defined as the difference between the second and first systolic peaks expressed as a percentage of the pulse pressure.

Equation 4

$$AIx = \left(\frac{P2 - P1}{PP} \right) \times 100\%$$

Pressure waveforms can be measured using applanation tonometry at various pulse sites on the skin surface. There are many variables, which could affect AIx values. High PWV, changes in reflection sites, diastolic blood pressure, height, age and aortic PWV are the main determinants affecting AIx [168]. Arterial waveforms should be analyzed centrally because the ascending aorta represents the true load imposed on the left ventricle and the central large artery walls. Aortic pressure waveforms can be estimated from waveforms acquired from either the common carotid or radial artery waveform, with a transfer function applied. Contrary to what one might expect, the more ideal method is using the radial waveform, as this artery is supported by bone, allowing for a clearer and more optimal waveform [169, 170]. Carotid tonometry requires a higher degree of technical expertise, however a transfer function is not required here because of the proximity of the arterial sites and similarity of the carotid and aortic waveforms.

Studies have demonstrated a relationship between increased AIx and CVD mortality and future events in various healthy and clinical populations [171-173]. Specifically, London *et al.* showed increased carotid artery AIx in dialysis patients was independently predictive of all-cause and cardiovascular mortality [173]. This was later confirmed in other clinical populations with work from Nurnberger et al (2002), showing a significant increase in carotid artery AIx with increasing cardiovascular risk scores and a correlation between carotid AIx and cardiovascular risk in 216 individuals with and without cardiovascular disease

[172]. In contrast, the results of the Australian National Blood Pressure Study 2 showed that AIx did not independently predict cardiovascular disease in 484 elderly hypertensive women [174].

2.3.8 Local vs. Regional Assessments of Arterial Stiffness

The aorta is a major vessel of interest when determining regional arterial stiffness [139] because the thoracic and abdominal aorta makes the largest contribution to the arterial buffering function [175-178] and aortic PWV is an independent predictor of cardiovascular outcomes in a variety of populations [4, 5, 13, 14]. Local arterial stiffness of superficial arteries can be determined from measurements of arterial diameter and pressure in specific arterial segments and carotid artery stiffness may be of particular interest, since atherosclerosis is common in the carotid artery [179]. A major advantage to measurements of local arterial stiffness over systemic or regional assessments is that local arterial stiffness is directly determined, from the change in local pressure causing the change in local volume; i.e. distensibility. Although $PWV_{c\text{-}f}$ and carotid stiffness provide similar information in some populations, this is not necessarily true in all populations [180]. Central PWV and carotid artery distensibility are thought to reflect similar information about large artery stiffness in healthy subjects however; this relationship may not exist in clinical populations, including individuals with high blood pressure, end-stage renal disease or diabetes as it has been demonstrated that the greater the number of cardiovascular risk factors present, the weaker the association between aortic stiffness and carotid stiffness

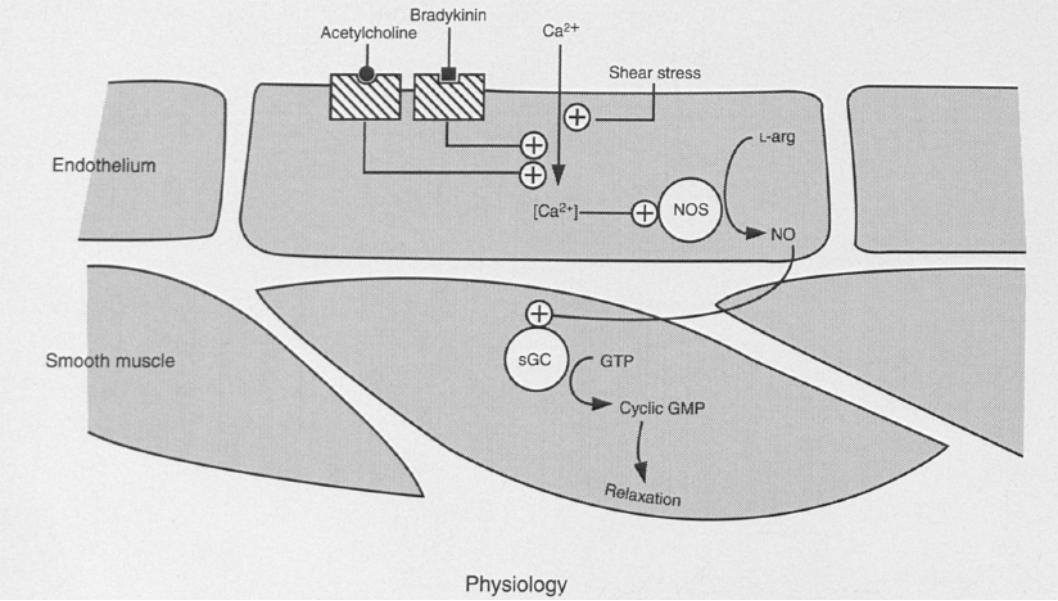
[180]. Therefore, it may be inappropriate to use aortic stiffness and carotid stiffness interchangeably as predictors in some high-risk individuals [139]

2.4 Vascular Function

2.4.1 Endothelial Function

The endothelium is the single layer of cells lining the interior surface of the entire cardiovascular system. Endothelial cells contribute to the control of blood pressure through regulation of vascular smooth muscle tone [181]. Mechanical stresses, such as shear stress, stimulate the release of NO from the endothelial cells, however the mechanisms responsible are not entirely understood [182]. Endothelial cell release of NO involves the opening of calcium-activated potassium-channels followed by membrane hyperpolarization and calcium-mediated activation of endothelial nitric oxide synthase (eNOS). eNOS stimulates the release of NO from the endothelium into the surrounding smooth muscle leading to vasodilation [182] (Figure 1). Optimal function of the endothelial cells is an important component in the local regulation of vasomotor tone.

Figure 1. Vascular relaxation mediated by Nitric Oxide



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The reduced ability of endothelial cells to respond to physical and/or pharmacological stimuli is referred to as endothelial dysfunction. Functional alterations to the typical phenotype of arteries exist, often leading to the development and clinical expression of atherosclerosis and other vascular disorders [17, 18, 183] and impairments in the NO-pathway are commonly implicated in endothelial dysfunction. Endothelial dysfunction is associated with increased cardiovascular disease risk and can be altered with acute and chronic exercise [85]. Endothelial dysfunction may represent an early subclinical event in the development and progression of atherogenesis and is a known independent indicator of CVD [18, 19, 184]. Experimental studies of atherogenesis suggest endothelial dysfunction precedes the formation of plaques [185, 186]. Endothelial

dysfunction is present in children [186] and adults with risk factors for atherosclerosis before any evidence of plaque formation in the arteries [187]. Endothelial dysfunction is thus a critical feature of many cardiovascular disorders including atherosclerosis [188], hypertension [189], coronary artery disease [190] and peripheral artery disease [191]. Endothelial dysfunction can even be used to predict future cardiovascular events in these populations [17].

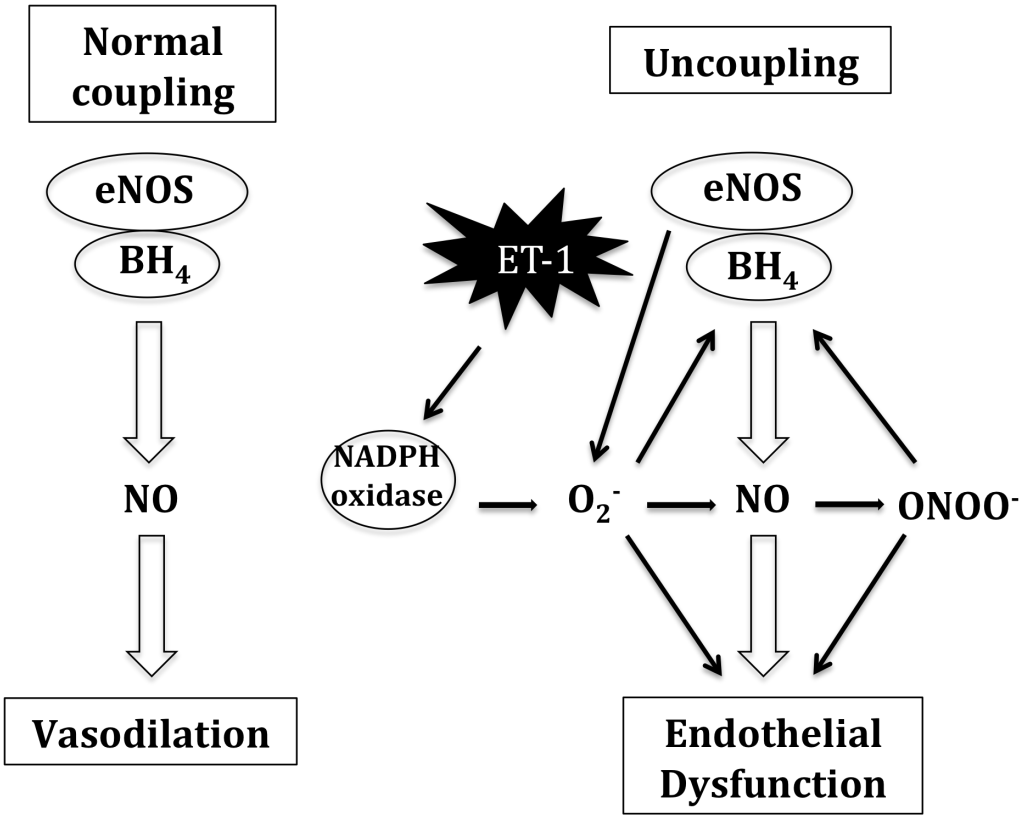
2.4.2 Pathophysiology of endothelial dysfunction

The pathophysiology of endothelial dysfunction is complex and involves multiple mechanisms. Endothelial function is chronically disrupted in many pathological conditions [192]. Endothelial dysfunction is thought to result from an imbalance between vasodilator and vasoconstrictor substances produced by or acting on endothelial cells, elevation in sympathetic muscle tone and/or alterations in the mechanical properties of the arterial wall [182].

As described above, one of the most important vasodilating substances released by the endothelium is NO and a reduction in NO is observed in the presence of impaired endothelial function [193]. This reduction in NO release may be due to a decreased bioavailability of NO and/or reduced eNOS activity. Studies have suggested some potential mechanisms for the observed reduction in NO bioavailability with endothelial dysfunction [82, 193-197]. Reactive oxygen species are known to suppress NO with the formation of peroxynitrite, which leads to the degradation of tetrahydrobiopterin (BH_4), an eNOS cofactor [194]. Specifically, one hypothesis speculates that endothelin-1 (ET-1), a potent

vasoconstrictor, leads to an increase in ROS production. BH_4 is a target for these ROS and its oxidation leads to an impairment of eNOS function [198]. Consequently, soluable NOS becomes a source of ROS, which then leads to endothelial dysfunction.

Figure 2. Mechanisms of ET-1-induced eNOS uncoupling in endothelial cells



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Elevation in asymmetric dimethylarginine, a competitive inhibitor of eNOS, has also been linked to endothelial dysfunction [193, 195]. Finally, it has been suggested that oxidative excess leads to endothelial dysfunction. Studies

have shown improved endothelial-dependent relaxation after the use of anti-oxidants [196]. Oxidative excess in hypertensive patients leads to diminished NO [82] and correlates with the degree of impairment of endothelial-dependent vasodilation and with cardiovascular events [197].

The circulating levels of various biomarkers expressed and or released by the endothelium may provide an indication of the progression of atherosclerosis and the efficacy of therapeutic modalities. The "response-to-injury" hypothesis is the most commonly accepted theory describing the initiation of the atherosclerotic process, the atherogenesis cascade and how endothelial dysfunction increases the susceptibility of the endothelium to injury [199]. Initiation of this cascade begins with an injury to the endothelial cells [199]. This leads to endothelial dysfunction, abnormal cellular interactions and eventually initiation and progression of atherogenesis [199]. Therefore, it is not surprising that the presence of atherosclerosis is common in many individuals with endothelial dysfunction [200]. When there are cardiovascular risk factors present the endothelium becomes dysfunctional resulting in enhanced production of cytokines and greater expression of cellular adhesion molecules by the endothelium [182]. Adhesion molecules play a crucial role in the interaction of the endothelial surface with circulating leukocytes and mediate the recruitment of leukocytes and their accumulation in the intima of the vessel wall [182]. Compared to individuals with smooth coronary arteries, individuals with evidence of coronary atherosclerosis demonstrated impaired flow mediated responses in the coronary [72] and brachial

[73, 74] arteries. Additionally there is evidence of impaired NO production and decreased eNOS protein expression in atherosclerotic human arteries [201].

2.4.3 Measurement of endothelial function with flow-mediated dilation

The functional test used to assess endothelial function is the flow-mediated dilation assessment. This is the most commonly used technique to measure endothelial function non-invasively [186]. This technique is based on the principle that an increase in blood flow through an artery and thus shear stress on the endothelial cells leads to the release of NO and dilation of the artery. FMD assessments involve the measurement of the change in diameter of a conduit artery (i.e. brachial, radial or femoral) in response to an increased flow stimulus.

The increased flow is commonly induced by a period of limb ischemia created by cuff occlusion followed by instantaneous release of the occlusion [186] but can also be induced with heating or mechanical compression of the artery [202, 203]. The FMD technique was first described by Celermejer et al. (1992) and has developed over the years with clear and distinct measurement guidelines being released and updated [20, 204-206]. FMD responses have been shown to be predominantly NO mediated in the radial, brachial and femoral arteries and are reduced in individuals with atherosclerosis and CVD risk factors [74, 186, 207, 208]. The magnitude of FMD response stimulated correlates well with coronary vascular endothelial vasodilator function, as has been examined in studies comparing responses to acetylcholine infusion and FMD techniques [209, 210].

2.4.4 Potential regulatory mechanisms for endothelial function

At a molecular level, endothelial function may be regulated by several factors. Changes in endothelial function may be a mechanistic link in the observed association between CVD and inflammation, as chronic inflammation has also been linked to endothelial dysfunction [211]. Inflammation may impair FMD by increasing vasoconstriction or by reducing the availability of endothelium-derived vasodilators. There may be an association between the pro-inflammatory state, often observed in hypertension, and endothelial dysfunction. Endothelial dysfunction may play a role in the elevation of blood pressure and thus possible hypertension-related vascular damage [182].

The up-regulation of the expression of adhesion molecules in the vascular endothelium allows leukocyte and monocyte adhesion to the endothelial cell surface [182] and leads to further up-regulation of endothelial adhesions. The up-regulation of endothelial adhesions induces leukocyte and monocyte penetration into the sub-endothelial environment, where tumor-necrosis factor (TNFα), interleukin 6 (IL-6) and other cytokines are released [182] and result in the recruitment of additional circulating inflammatory cascade cells [182].

A key determinant of this inflammatory pathway is the increased generation of reactive oxygen species (ROS). ROS reduce nitric oxide synthase activity and increase NO breakdown [182] and since NO and ET-1, a potent vasoconstrictor, are reciprocally regulated, an impaired NO availability could lead to increased ET-1 production, thereby affecting various endothelial cell functions,

and inducing endothelial dysfunction [28]. Endothelial regulatory substances such as ET-1, TNFα, and IL-6 could therefore be markers of endothelial function. Specifically, pro-inflammatory cytokines may induce vasoconstriction by causing increased synthesis of ET-1 [211] as elevations in IL-6 are thought to stimulate the synthesis of ET-1 in the vasculature [211].

2.4.4.1 Vasoconstrictor: Endothelin-1

ET-1 is a 21-amino acid peptide that was discovered in 1988 by Yanagisawa and colleagues [212]. ET-1 is a potent vasoconstrictor with a molecular weight of 2492, free amino and carboxyl termini and two intramolecular disulfide bonds [213]. ET-1 is present in many mammalian species [213]. In humans, vascular endothelial cells are the major source of ET-1, however, many other cell types are also sources, suggesting that the peptides may participate in complex regulatory mechanisms in other organs [214, 215].

Although endothelial dysfunction is thought to be associated with a reduction in NO, it has not been determined if increased ET-1 is a major contributor to this process. Increased ET-1 may contribute to the reduction of NO bioavailability, which is often observed with endothelial dysfunction [27, 216, 217].

2.4.4.2 Inflammatory marker: Interleukin-6

Investigators have hypothesized that inflammation is an important cause of endothelial dysfunction, but prior human studies relating inflammation to endothelium-dependent dilation have been limited to relatively small and selected

samples and yielded conflicting results [218-220]. Risk factors may be the primary cause of vascular dysfunction in conduit arteries, however studies support the possibility that systemic inflammation may represent a mechanistic link between risk factors and vascular dysfunction. In particular, chronic inflammation may impair flow-mediated dilation by reducing the bioavailability of endothelium-derived vasodilators.

IL-6 is an acute inflammatory cytokine of molecular mass 26kDa that has been linked to various pathological states [221-223] and is secreted from a variety of different cells, including vascular endothelial cells [224]. IL-6 is a mediator of the acute inflammatory response and contributes to chronic inflammation in obesity [225, 226]. IL-1 and tumour necrosis factor alpha (TNF∝) largely cause IL-6 expression and secretion [227]. IL-6 has been linked to some traditional risk factors. Specifically, IL-6 increases with age and is associated with increased levels of adiposity, high blood pressure, smoking and insulin sensitivity [228-231].

Inflammatory markers have been shown to decrease the expression of eNOS [232-234]. Approximately 33% of total IL-6 originates from adipose tissue [231] and that it plays a key role in the relationship between adiposity, inflammation and CVD. Importantly, an inverse correlation between circulating IL-6, TNF∝ and CRP concentrations and endothelial function has been observed [26, 235]. However, other studies have seen no relationships between circulating pro-inflammatory markers and measures of endothelial dysfunction [236]. Further research is warranted to better understand the relationship between

circulating markers of vasoconstriction and inflammation and measures of vascular endothelial function. Although these circulating markers may not be responsible for the changes in endothelial function, they may be representative of what is happening at the level of the endothelium. Local and systemic mechanisms may simply be occurring simultaneously due to the shear or biochemical changes at a local level. Local factors, including shear pattern and biochemical changes, might be responsible for the FMD changes we see in arteries with exercise training [237], but circulating markers from venous blood samples might still reflect the changes in the local area as these markers may dominate the venous sample pool.

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3.0 CHAPTER 3:

PURPOSES and HYPOTHESES

The primary purpose of the present thesis was to investigate potential mechanisms associated with the regulation of vascular structure and function in a spectrum of human populations.

The specific aims of the individual studies were:

1. To determine the associations between serum markers and functional measurements of the regulation of vascular structure and function in a spectrum of human populations
2. To investigate the effects of a 16-week combined aerobic and resistance training program and hypocaloric diet on endothelial function, ET-1 and IL-6 in otherwise healthy overweight and obese young pre-menopausal women.
3. To determine the effects of a 16-week diet and combined aerobic and resistance exercise intervention on markers of type I collagen turnover and both central and peripheral measures of arterial stiffness, as assessed by carotid artery distensibility and carotid-radial PWV in overweight young women.

The corresponding hypotheses associated with each of the above purposes are stated below.

1. We hypothesize that there would be an inverse relationships between circulating serum markers of inflammation and FMD and circulating serum markers of vasoconstriction and flow-mediated dilation and a positive

relationship between arterial stiffness and collagen turnover in a wide spectrum of individuals with varying degrees of vascular health.

2. We hypothesize that brachial FMD would increase with an associated decrease in ET-1 and IL-6 after a 16-week diet and exercise intervention in young overweight and women.
3. We hypothesize that both central and peripheral arterial stiffness would decrease and that there would be an associated increase in markers of collagen turnover following the 16-week weight loss intervention in young overweight women.

4.0 CHAPTER 4:

Associations between functional measures and serum markers of vascular structure and function

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Lisa M. Cotie – design of study, prepared manuscript, collected, analyzed and interpreted all blood data and vascular data in OLD & YOUNG, analyzed and interpreted blood data for CAD and SCI populations
Katharine D. Currie – vascular structure and function data collection and analysis in individuals with CAD
Julia O. Totosy de Zepetnek –vascular structure and function data collection and analysis in individuals with SCI
Greg McGill - vascular structure and function data collection on YOUNG
Stuart M. Phillips – lead investigator on the OLD study, edited manuscript
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Short Title: Mechanisms associated with the regulation of vascular structure and function

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4.1 Abstract

A comprehensive understanding of the factors regulating changes in vascular structure and function may assist in designing effective strategies for decreasing cardiovascular disease (CVD) risk. Simultaneous examinations of blood markers and functional measures of arterial structure and function have not been thoroughly studied. Vascular and blood measures were collected on 21 young healthy university students (3 women), 26 individuals with coronary artery disease (CAD), 14 individuals with spinal cord injury (SCI) and 17 healthy older men for a total of 78 individuals across a large spectrum of expected vascular health. Blood markers measured included pro-collagen type I C-peptide (PIP), a marker of collagen synthesis, C-telopeptide of type I collagen (CTX), a marker of collagen degradation, endothelin-1 (ET-1) a vasoconstrictor and interleukin-6 (IL-6), to measure inflammation. Moderate negative relationships existed between central PWV and type I collagen turnover (CTX; $r = -0.41$, $p = 0.001$ and PIP: $r = -0.32$, $p = 0.013$). There were significant positive relationships observed between common carotid distensibility and type I collagen turnover (CTX: $r = 0.59$, $p < 0.001$ and PIP: $r = 0.45$, $p < 0.001$). Significant negative correlations were observed between ET-1 and FMD (relative FMD: $r = -0.41$, $p = 0.001$ and absolute FMD: $r = -0.41$, $p = 0.001$). No relationships were observed between the inflammatory marker (IL-6) and FMD. This study increases the comprehensive understanding of factors associated with the regulation of vascular structure and function in a spectrum of populations spanning a large range of vascular health.

The information provided by this study may assist with focusing on direct targets, such as type I collagen turnover and vasoconstrictors to assist with lifestyle interventions to decrease CVD risk.

4.2 Introduction

A comprehensive understanding of the factors and mechanisms regulating changes in vascular structure and function may assist in designing effective strategies for decreasing cardiovascular disease (CVD) risk. Commonly used functional tests for arterial structure and function include pulse wave velocity (PWV) and carotid artery distensibility for assessing arterial structure and brachial artery flow-mediated dilation (FMD) for assessing arterial function.

PWV is a measure of the speed of travel of the pulse between two sites of a known distance on the body [1]. Specifically, central pulse wave velocity, measured from the carotid artery to the femoral artery, is a strong predictor of CVD risk in healthy and higher risk individuals [2-12]. Carotid artery distensibility, a regional measure of arterial stiffness, used to represent central aortic stiffness, is also a known strong predictor of CVD risk in clinical populations [13, 14].

The endothelium is a single layer of cells lining the entire vascular system and regulates vascular smooth muscle tone. Endothelial dysfunction may represent an early subclinical event in the development and progression of atherogenesis and is a known independent indicator of CVD [15-17]. The dilatory capacity of the endothelium is commonly measured in the brachial artery using the FMD test. Brachial artery FMD is a strong predictor of cardiovascular events in asymptomatic individuals [18] and in patients with established CVD [19].

FMD is thought to provide independent prognostic information, which may supplement the information available from traditional risk factors [18].

To better understand the mechanisms associated with changes in vascular structure and function and translate this information to clinical practice, it is likely informative to examine the potential relationships between functional assessments of arterial structure and function and more easily evaluated blood markers. Examining circulating serum markers of type I collagen synthesis and degradation along with circulating serum markers of vasoconstriction and inflammation may contribute to the comprehensive understanding of potential mechanisms responsible for structural and functional changes in the vascular system following lifestyle interventions and aid in the design of more effective strategies targeted at specific mechanisms to help decrease CVD.

The artery wall contains a large portion of collagen within its different layers. Type I collagen accounts for 60% of the vascular collagen and is present in the intima, media and adventitia of the vessel wall [20] and thus warrants a closer investigation for its involvement in arterial wall stiffening. Previous research has shown relationships do exist between various serum and plasma markers of type I collagen synthesis and degradation with indices of both central [21-23] and peripheral artery stiffness [22, 24], however the literature is conflicted in terms of the direction, strength and significance of this relationship in populations with differing levels of vascular structure and function.

Recent research has suggested that endothelial function is largely governed by local endothelial cell derived factors including nitric oxide (NO), prostacyclin (PGI_2) and endothelial-derived hyperpolarizing factor (EDHF) [25], however past research has also suggested a relationship exists between circulating markers of inflammation and endothelial function [26]. Previous investigations of circulating markers have largely concentrated on the role of C-reactive protein (CRP), as it emerged as a marker of inflammation linked with endothelial function status [26-29]. However, there is no clear reproducibility of these findings in the literature. Specifically, some studies have found an inverse relationship [26, 28] between CRP and endothelial function whereas other have found no relationship [27]. Thus further research is warranted to examine other circulating markers of inflammation, for relationships to endothelial function.

The role of endothelin-1 (ET-1) in regulating endothelial function is not fully understood. Pro-inflammatory markers may induce vasoconstriction by causing an increase in the synthesis of ET-1 [30]. ET-1 can decrease NO bioavailability by decreasing its production or increasing its degradation [31]. Although past research has suggested that endothelial dysfunction is associated with a reduction in NO, it has not been clarified whether an increased concentration of ET-1 is a major contributor in this process [30].

The purpose of this study was two fold. The first purpose was to determine the relationship between functional tests of vascular stiffness and circulating serum markers of type I collagen synthesis and degradation in human populations

demonstrating a wide spectrum of arterial stiffness. The second purpose was to determine the relationship between endothelial function and circulating markers of inflammation (IL-6) and vasoconstriction (ET-1) over a wide range of endothelial function. We hypothesized that arterial stiffness would be associated with altered collagen turnover and that there would be an inverse relationship between endothelial function and markers of both inflammation and vasoconstriction.

4.3 Methods

Vascular and blood measures were collected in 21 young healthy university students (3 women), 26 individuals with coronary artery disease (CAD) (3 women), 14 individuals with spinal cord injury (SCI) and 17 healthy older men for a total of 78 individuals across a large spectrum of expected vascular health.

Cardiovascular Measurements

Heart Rate and Blood Pressure. Testing sessions began with 10 minutes of supine rest to ensure representative resting measurements prior to the commencement of the vascular assessment. Continuous measurements of heart rate via single lead electrocardiograph (ECG) (ML123, ADInstruments Inc. Colorado Springs, CO, USA.) and blood pressure (BP) measurements via an automated applanation tonometer with and without oscillometric cuff calibration (Nexfin monitor, Model 1, BMEYE B.V., Amsterdam, The Netherlands or Finometer Midi, Model 2, Finapres Medical Systems, Amsterdam, The Netherlands) were made.

Arterial Stiffness Measures

Carotid artery distensibility was measured in all population groups. However, PWV was only measured in three of the four populations. PWV was not measured in the CAD group, as this was part of a much larger study and due to time restrictions, this measurement was omitted.

Pulse wave velocity. Central artery stiffness was estimated by carotid-femoral PWV (PWV_{c-f}), respectively, using high fidelity, simultaneous carotid and femoral artery pressure measurements. PWV_{c-f} was defined as the time delay in arrival of the foot of the pulse wave between the carotid and femoral arteries. Arterial waveforms at the common carotid and femoral arteries were collected using a hand-held tonometer (model SPT-301, Millar Instruments Inc., Houston, TX) positioned on the right common carotid and common femoral arteries at the point of greatest pulsation to produce continuous arterial pressure waveforms. Pulse transit time was determined using the subtraction method (Weber T, 2009). In short, central pulse transit time was calculated by subtracting the transit time between the sternal notch and common carotid from the transit time between the sternal notch and femoral artery. Transit time was calculated by determining the time delay between ventricular depolarization (R-wave peak), and the "foot" of the artery waveforms. The pressure waveforms obtained were band-pass filtered (5-30Hz) with the lower (≤ 5Hz) and higher frequencies (≥ 30Hz) removed in order to assist in the detection of the foot of each waveform. The foot was identified as the minimum value of the digitally filtered signal and corresponded

to the end of diastole, when the steep rise in the wave begins and appears as a sharp inflection of the original signal [32].

Similarly the path length between the pulse measurement sites was calculated by subtracting the surface distance between the sternal notch and the carotid tonometer placement from that of the sternal notch and the femoral tonometer placement. An anthropometric measuring tape was used to measure the straight-line distance between skin sites along the surface of the body. Resting PWV data was recorded during 20 continuous heart cycles using the equation:

Equation 1: $$PWV = \frac{\Delta d}{\Delta t}$$

where Δd is the distance between measurement sites, and Δt is the pulse transit time.

Carotid artery distensibility. Central artery stiffness was estimated with direct measurements of carotid artery distensibility using a combination of high-resolution, two-dimensional, brightness mode ultrasound images (Vivid Q; GE Medical Systems, Horten, Norway) and applanation tonometry (model SPT-301; Millar Instruments, Houston, TX, USA). Common carotid artery images were collected using a 12MHz ultrasound probe and were collected at 23.2 frames·sec^{-1}. A hand-held tonometer was positioned over the point of greatest pulsation of the right common carotid artery and held in a fixed position for ten consecutive heart cycles while ultrasound images of the left common carotid artery were collected simultaneously. Absolute carotid artery systolic blood pressures were calculated by calibrating the relative values acquired using applanation tonometry to the

calibrated brachial artery blood pressure acquired simultaneously using the Nexfin or Finometer [33, 34]. Ultrasound images were stored offline in Digital Image and Communications in Medicine (DICOM) format for later analysis using semi-automated edge tracking system [AMS, (Artery Measurement System) Image and Data Analysis: Tomas Gustavsson, gustav@alumni.chalmers.se]. In each frame, carotid (minimum and maximum) lumen diameters were calculated from roughly 100 measurement markers along the vessel wall within a chosen region of interest, for a total of 110,000 measures in the 10 heart cycles. Distensibility was calculated using the following equation [35]:

Equation 2: $$Distensibility = \frac{\left[\Pi\left(\frac{d_{max}}{2}\right)^2 - \Pi\left(\frac{d_{min}}{2}\right)^2\right]}{\Pi\left(\frac{d_{min}}{2}\right)^2 \times PP}$$

where d_{max} is the maximum diameter, d_{min} is the minimum diameter, and PP is carotid pulse pressure, the difference between DBP and SBP.

Flow-mediated dilation assessment. An FMD test was conducted to assess brachial artery endothelium-dependent function. With the participant in the supine position, the right arm was supinated and abducted 80-90° so that an optimal image of the brachial artery could be obtained in a comfortable position. An inflatable cuff was placed on the forearm, approximately 5 cm below the medial epicondyle [36] and remained deflated while baseline data were collected. B-mode ultrasound images of the common brachial artery were collected through two-dimensional grayscale ultrasound imaging using a 12 MHz linear array probe

(System FiVe; GE Medical Systems, Horten, Norway) and at a frame rate of 7.7 frames/second. A baseline longitudinal image of the brachial artery (30 seconds) was acquired. Simultaneous image and pulse wave Doppler was collected at all time points. The forward and reverse audio signals from the pulse wave Doppler spectrum were processed by an external spectral analysis system (Neurovision 500M, Multigon Ind; Yonkers NY) and an intensity-weighted calculated mean signal was acquired (Powerlab model ML795).

To create the flow stimulus, a forearm cuff was instantaneously inflated to a standardized, supra-systolic pressure of 200mmHg to ensure arterial inflow occlusion and ischemia of downstream vessels and tissue [37]. The cuff was instantaneously deflated (Hokkanson) after 5 min. of occlusion. Similar to baseline measures, simultaneous B-mode imaging and mean blood velocity signals were obtained for 3-minutes following cuff release. A semi-automated edge detection software program (Artery Measurement System, Image and Data Analysis, Tomas Gustavsson, gustav@alumni.chalmers.se) was used to detect the vessel diameters within a specific region of interest at the end diastolic frames of each heart cycle collected as described above. Five-diameter rolling averages were used to calculate the peak dilation of the vessel in the 180 seconds post cuff release. From this data, the absolute FMD (mm) and relative FMD (%FMD) were calculated as follows [37]:

Equation 3: $AbsoluteFMD = PeakDiameter(mm) - BaselineDiameter(mm)$

Equation 4: $$\text{Re}\,lativeFMD = \left(\frac{AbsoluteFMD}{BaselineDiameter}\right)x100\%$$

The following equation was used to calculate shear rate (SR) for each participant [38]:

Equation 5: $$ShearRate = 8x\left(\frac{Velocity}{Diameter}\right)$$

where velocity represents the mean of the velocity profile until peak diameter and the baseline brachial diameter (mm) as the artery diameter value. The area under the curve of the shear rate was calculated from the mean of the first point, using the trapezoid rule to obtain the area under the entire curve (GraphPad Prism version 4.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com). Relative FMD (%FMD) was normalized to the area under the entire SR curve and reported as

Equation 6: $$NormalizedFMD = \left(\frac{\%FMD}{SR_{AUC}}\right)$$

All analogue signals (including those described below) were converted to digital by fast Fourier transform and sampled simultaneously at a sampling rate of 200Hz using a commercially available data acquisition system (Power lab Model ML 795, ADInstruments, Colorado Springs, USA) and software program (LabChart 7.0; ADInstruments Inc. Colorado Springs, CO, USA).

Blood Analysis

Overnight fasted venous blood samples were collected at baseline from all participants for further analysis. Serum was stored at -20°C until analysis was completed. Serum samples were analyzed using ELISAs for concentrations of pro-collagen type I (PIP) as a marker of type 1 collagen synthesis (Takara, Mountain View, CA), cross-linked telopeptide of type I collagen (CTX) as a marker of degradation (Immunodiagnostic Systems, Scottsdale, AZ), interleukin-6 (R&D Systems Quantikine, Minneapolis, MN) and endothelin-1 (Enzo, Life Sciences Assay Designs, Farmingdale, NY) using the corresponding immunoassay kits.

Statistics

Results are presented as mean ± SE and relationships were considered significant at $p<0.05$. Data was analyzed using SPSS (Version 11.5 for Windows). Pearsons correlations were used to assess relationships between PWV_{c-f} and both of the collagen markers (PIP and CTX), carotid artery distensibility and both of the collagen markers (PIP and CTX), FMD and the inflammatory marker (IL-6) and FMD and the vasoconstrictor (ET-1).

4.4 Results

Vascular Structure. There were moderate negative relationships observed between PWV_{c-f} and markers of type I collagen turnover (CTX; $r = -0.41$, $p = 0.001$ and PIP: $r = -0.32$, $p = 0.01$, Figure 1A&1B). When these relationships were assessed in the specific populations, the only one, that achieved statistical

significance, was in the young healthy population (CTX: $r = - 0.46$, $p = 0.04$). There were significant positive relationships observed between common carotid distensibility and markers of type I collagen turnover (CTX: $r = 0.59$, $p < 0.001$ and PIP: $r = 0.45$, $p < 0.001$, Figure 2). When analyzed separately the only populations with statistically significant relationships were the young healthy (CTX: $r = 0.63$, $p = 0.003$) and SCI (CTX: $r = 0.75$, $p = 0.002$ and PIP: $r = 0.72$, $p = 0.003$).

Figure 1. Associations between PWV_{c-f} and type I collagen markers

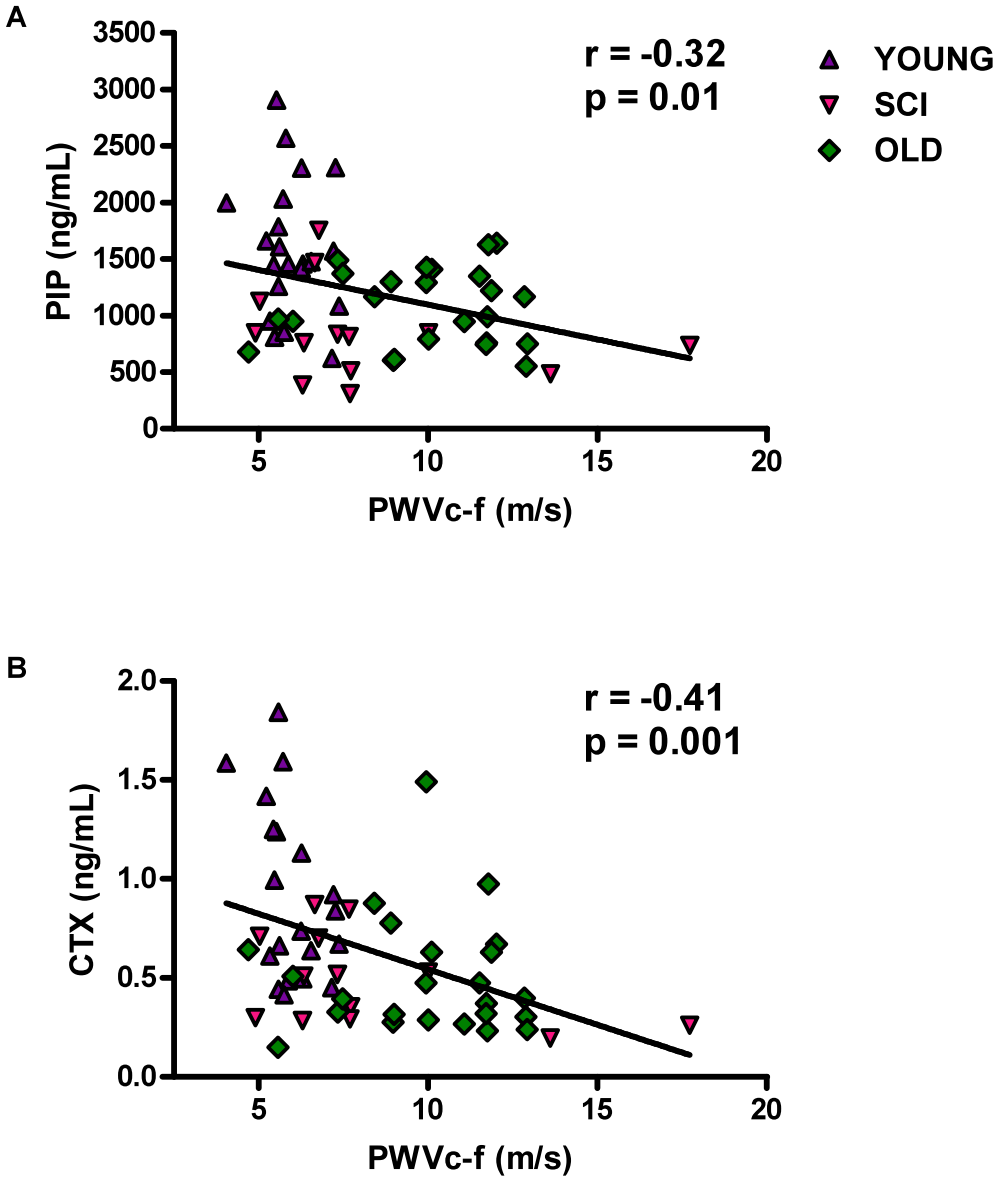
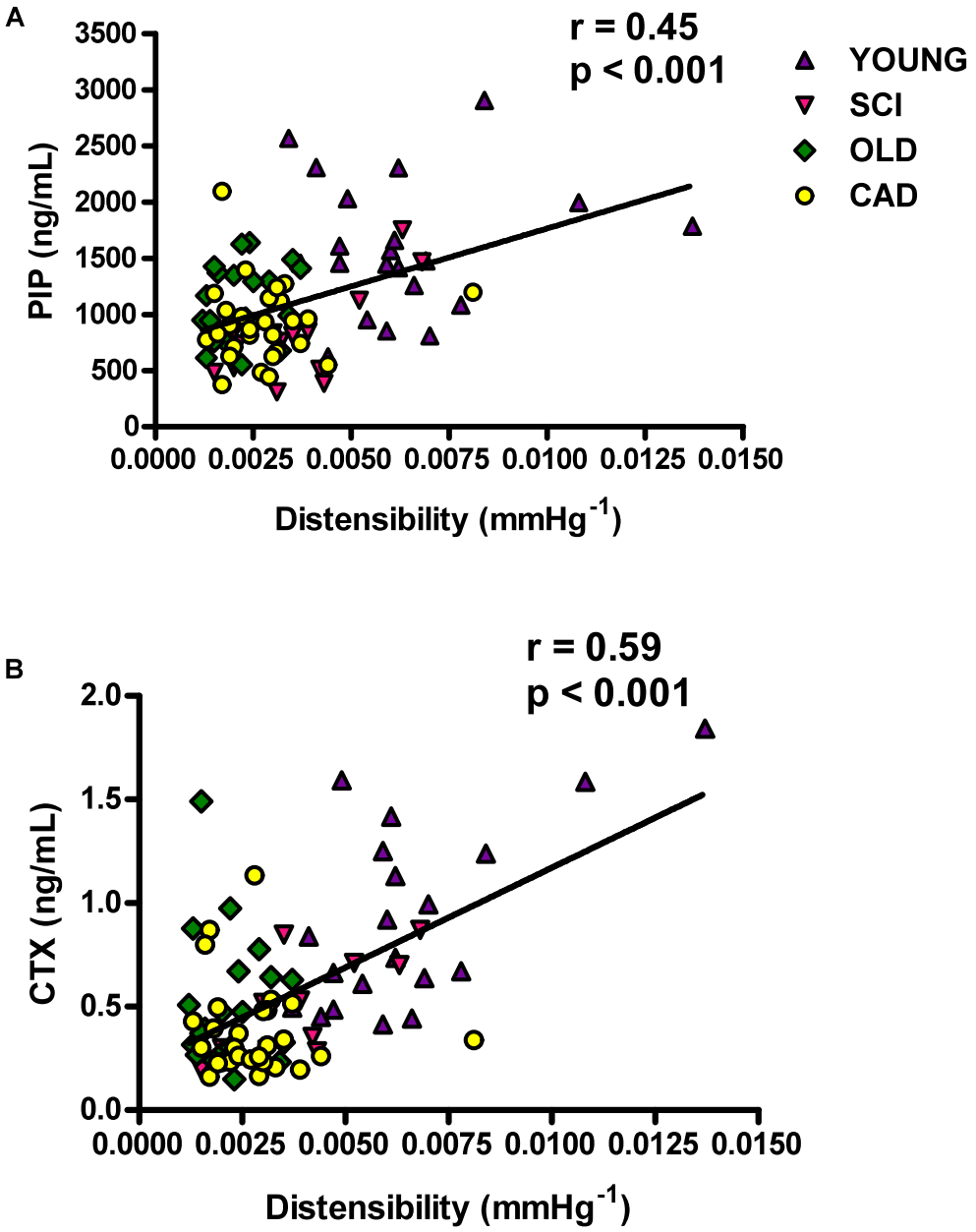


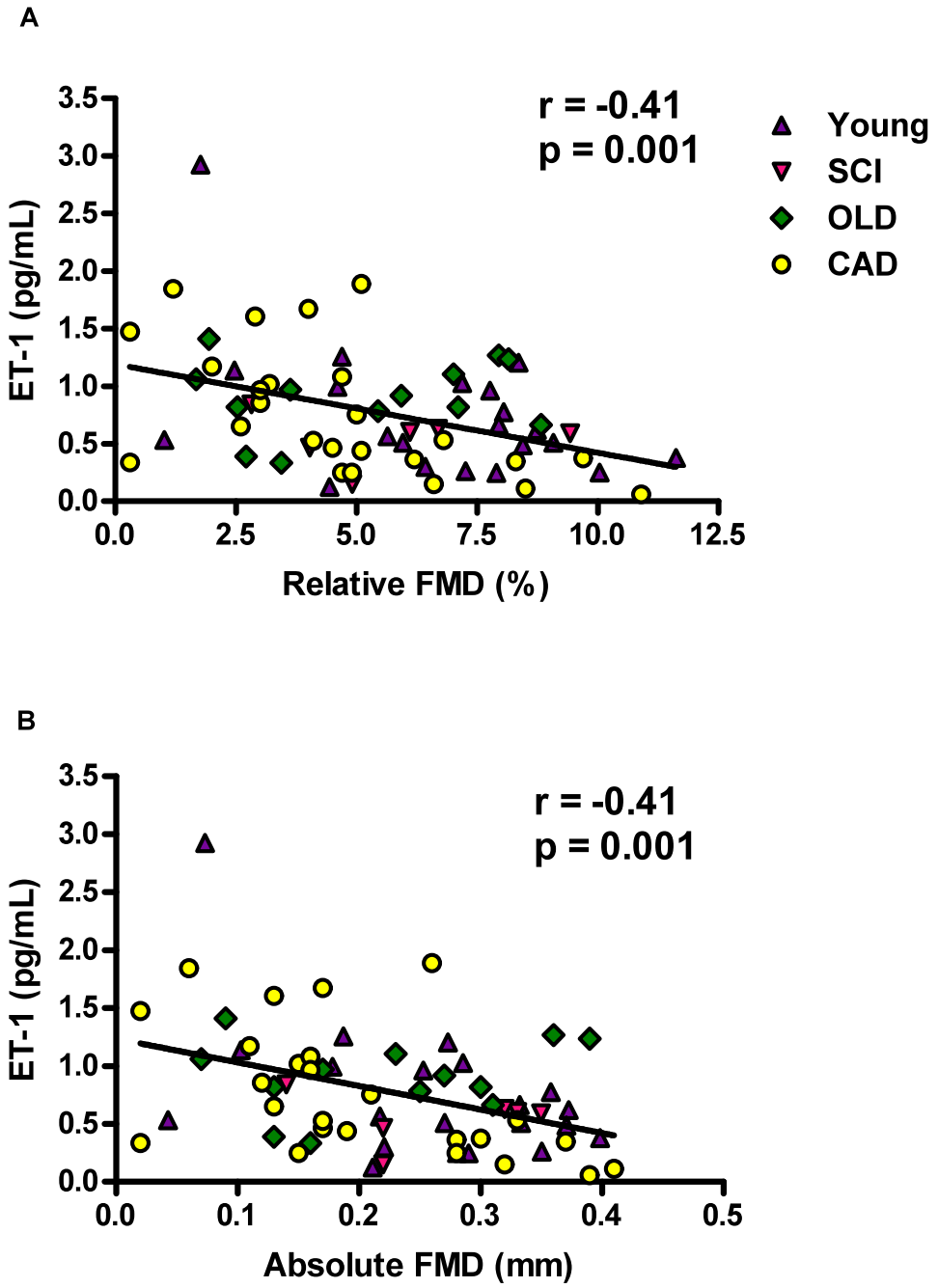
Figure 2. Associations between distensibility and type I collagen markers



Carotid artery distensibility was inversely correlated to $PWV_{c\text{-}f}$ ($r = -0.44$, $p = 0.001$).

Vascular Function. There were significant negative correlations observed between ET-1 and both relative and absolute FMD (relative FMD: $r = -0.41$, $p = 0.001$ and absolute FMD: $r = -0.41$, $p = 0.001$, Figure 3A & 3B). No relationships were observed with normalized FMD and ET-1. No relationships were observed between the inflammatory marker (IL-6) and any measure of FMD (relative, absolute or normalized). When separated by population both the young healthy population and individuals with CAD had significant associations between FMD (absolute and relative) and ET-1 (Young: relative FMD vs. ET-1 – $r = -0.47$, $p < 0.001$ and absolute FMD vs. ET-1 – $r = -0.50$, $p = 0.02$ and CAD: relative FMD vs. ET-1 – $r = -0.57$, $p = 0.003$ and absolute FMD vs. ET-1 – $r = -0.54$, p 0.005).

Figure 3. Associations between ET-1 and FMD



4.5 Discussion

The most important findings in this study are that over a broad range of vascular health, relationships exist between markers of type I collagen turnover and functional measures of arterial stiffness and between a marker of vasoconstriction and endothelial function. Specifically, negative relationships exist between both central artery stiffness (PWV_{c-f}) and collagen synthesis (PIP) and breakdown (CTX) markers. Coinciding to these findings is that a positive relationship exists between carotid artery distensibility (the inverse of stiffness) and both CTX and PIP. These findings suggest there may be a link between type I collagen turnover and aortic stiffness, however previous reports of similar relationships have been inconsistent in the literature. We know that changes in the composition of collagen subtypes occur in hypertensive rats with stiff arteries [39, 40], however it is less clear whether this relationship exists and in what direction it exist in humans.

Some previous studies have reported increased collagen synthesis [22] or decreased degradation [24] with increasing stiffness, whereas others have reported increased collagen degradation with increasing stiffness [21, 22]. Interestingly, our results demonstrate that markers of both collagen synthesis and degradation are diminished with increased arterial stiffness suggesting that increased type I collagen turnover is associated with healthier, less stiff arteries.

A challenge exists when comparing the current results to previous literature due to the extensive use of different methodologies. All previous

studies were conducted in relatively narrow sample populations including middle-aged to elderly (45-90 years) clinical populations, such as stable chronic heart failure [24], chronic kidney disease [41] and medicated [23] and non-medicated hypertensives [21, 22]. The current results are the only study to include participants with a wide range of vascular stiffness. Our findings show that relationships existed when all of the data was pooled together, representing a large range of vascular health. However, when the populations were separated, population specific relationships emerged in the SCI, young healthy and CAD populations only. This may mean that the relationships were specific to certain populations, however it is possible that the populations in this study had larger ranges in vascular health within themselves in comparison to the previous research and that the existing relationships observed may not be sensitive enough to be seen in samples representing a smaller range of vascular stiffness.

Central PWV and carotid artery distensibility are both accepted in the literature as representatives of aortic vascular structure. Central PWV estimates are expected to be dominated by the stiffness of the descending aorta, whereas carotid artery distensibility measures the inverse of stiffness in the carotid artery, which is also considered to be a central elastic artery. It is important to highlight that our measures of aortic stiffness were statistically related to one another and as expected there was an inverse association between central PWV and carotid artery distensibility. In addition, both of these measures were also similarly correlated with blood markers of type I collagen synthesis and degradation. These findings

strengthen the robust nature of our finding that type I collagen turnover is implicated in the regulation of central arterial stiffness.

Endothelial function may be regulated by both local [25] and systemic factors [26]. We found a moderate negative association between circulating serum levels of the potent vasoconstrictor ET-1 and both absolute and relative FMD, suggesting that circulating markers of vasoconstriction may be indicative of the functional status of the brachial artery in our spectrum of endothelial function. Studies have shown that the functional status of the brachial artery is indicative of coronary functional status and thus overall vascular health [42]. The literature suggests that increased ET-1 may contribute to the reduction in NO bioavailability often associated with endothelial dysfunction [31, 43, 44] however, there is no clear evidence to date, as to whether or not ET-1 plays a role in this process. The current findings suggest that ET-1 may in fact moderate brachial artery FMD responses.

There were no relationships observed between a marker of systemic inflammatory status (IL-6) and any measure of FMD (relative, absolute or normalized) suggesting that IL-6 may not be linked to endothelial function or that circulating IL-6 may not be sensitive enough to use as a surrogate marker of endothelial health. Vita et al. (2004), found a weak but significant relationship between circulating IL-6 and CRP and FMD [26], suggesting that there may be some promise in further investigating the involvement of systemic inflammatory status and endothelial function. The authors suggested inflammation might

represent a mechanistic link between risk factors and vascular dysfunction [26]. We were unable to reproduce these results in the current study. Recent research has suggested that endothelial function is largely governed by local endothelial cell derived factors including nitric oxide (NO), prostacyclin (PGI_2) and endothelial-derived hyperpolarizing factor (EDHF) [25]. Stoner et al. suggest that the relative contribution of each of these other vasodilators may vary with different clinical populations [25]. We did not measure any local blood markers, as our samples were taken as baseline circulating measures, however this hypothesis of primarily local factor regulation of endothelial function warrants further research.

The current study increases the comprehensive understanding of factors associated with the regulation of vascular structure and function in a spectrum of populations spanning a large range of vascular health. The information provided by this study may assist with focusing on direct targets, such as type I collagen turnover and vasoconstrictors to assist with lifestyle interventions set to decrease CVD risk.

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5.0 CHAPTER 5:

Endothelial Function increases after a 16-week diet and exercise intervention in overweight and obese young women

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Short title: Endothelial function increases with diet and exercise

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5.1 Abstract

Weight loss and exercise reduce the risk of cardiovascular morbidity in overweight populations. Aerobic exercise training leads to increased brachial artery endothelial function in many populations; however, endothelial function remains unchanged or is decreased with resistance exercise training. Weight loss improves endothelial function in overweight individuals. The combined effects of aerobic and resistance training and weight loss through caloric restriction on in vivo measures and blood markers thought to regulate endothelial function have not been comprehensively examined. Therefore, in this study we investigated brachial artery endothelial function and potential regulatory blood markers in twenty overweight pre-menopausal women who participated in 16-weeks of aerobic exercise, 5 d/wk and resistance training, 2 d/wk while consuming a hypocaloric (-500 kcal/d versus requirement) diet. Resting brachial artery flow mediated dilation (FMD) was assessed at baseline and following the intervention. Endothelin-1 (ET-1) and interleukin-6 (IL-6) were assessed as circulating systemic markers of vasoconstriction and inflammation, respectively. Relative FMD and absolute FMD increased (pre: 4.1 ± 0.5 % vs. post: 6.9 ± 0.7 %, $p<0.05$ and pre: 0.14 ± 0.02 mm vs. post: 0.23 ± 0.02 mm, $p<0.05$, respectively), while body mass decreased (pre: 86.8 ± 2.4 kg vs. post: 80.7 ± 2.4 kg $p<0.05$) following the 16-week diet and exercise intervention. There were no changes in either of the blood markers (IL-6 - pre: 1.7 ± 0.2 ng/mL vs. post: 1.6 ± 0.1 ng/mL, $p>0.05$ and ET-1 – pre: 0.6 ± 0.1 pg/mL vs. post: 0.6 ± 0.1 pg/mL, $p>0.05$). 16-

weeks of combined aerobic-resistance training and diet-induced weight loss improved endothelial function in overweight and obese young women, but this increase was not associated with changes in blood markers of vasoconstriction or inflammation.

Keywords: flow-mediated dilation, Endothelin-1, Interleukin-6, overweight women, combined aerobic and resistance training

5.2 Introduction

The vascular endothelium plays many roles, including contributing to the regulation of vascular smooth muscle tone. Endothelial dilatory capacity is commonly investigated using the flow-mediated dilation (FMD) test. Endothelial dysfunction may represent an early subclinical event in the development and progression of atherogenesis and is a known independent indicator of cardiovascular disease (CVD) [1, 2]. Aerobic exercise training improves endothelial function [3] and studies have reported a positive relationship between diet-induced weight loss and endothelial function in overweight and obese women with increased cardiovascular risk [4-6]. A positive relationship between exercise-induced weight loss and endothelial function has been reported in overweight and obese men and women with established coronary heart disease [7]. No data exists on the effects of combined diet and exercise – induced weight loss on FMD in otherwise healthy overweight and obese women. FMD is measured in the brachial artery and has been documented to correlate with pharmacological evaluations of coronary artery endothelial function [8]. At the molecular level, endothelial regulatory substances such as endothelin-1, a potent vasoconstrictor and interleukin-6 (IL-6), a pro-inflammatory cytokine, have been measured in a number of exercise training studies [9-11]. Changes in endothelial function could be a key mechanistic link in the previously observed association between CVD and inflammation, as chronic inflammation has also been linked to endothelial dysfunction [9]. Specifically, pro-inflammatory cytokines may induce

vasoconstriction by causing increased synthesis of endothelin-1 (ET-1) [9]. Although endothelial dysfunction is thought to be associated with a reduction in nitric oxide (NO), it has not been determined if increased ET-1 is a major contributor to this process. Increased ET-1 may contribute to the reduction of NO bioavailability, which is often observed with endothelial dysfunction [10].

IL-6 has been linked to various pathological states [12-14] and is secreted from a variety of different cells, including vascular endothelial cells [15]. IL-6 is a mediator of the acute inflammatory response and contributes to chronic inflammation in obesity [16, 17]. Elevations in IL-6 are thought to stimulate the synthesis of ET-1 in the vasculature [9]. Approximately 33% of total IL-6 originates from adipose tissue [18] and that it plays a key role in the relationship between adiposity, inflammation and CVD. Importantly, an inverse correlation between circulating systemic inflammatory markers and endothelial function has been observed in healthy [19] and clinical populations [11, 20] .

Aerobic exercise training leads to improved arterial health in many populations [21] and is involved in cardiovascular risk reduction. Research examining the effects of resistance training on arterial structure and function has yielded conflicting results [22, 23] and the effects of combined aerobic and resistance training on arterial structure and function has not been comprehensively examined [24, 25].

The purpose of this study was to investigate the effects of a 16-week combined aerobic and resistance training program and hypocaloric diet on

endothelial function, ET-1 and IL-6 in otherwise healthy overweight and obese young women. We hypothesized that brachial FMD would increase and ET-1 and IL-6 would decrease after the 16-week diet and exercise intervention.

5.3 Methods

Participants. This study was part of a larger lifestyle intervention study: Improving Diet, Exercise and Lifestyle (I.D.E.A.L) for Women Study, which involved 90 participants [26]. A small subset of participants (n = 20) from the larger lifestyle intervention study volunteered to participate in the cardiovascular measurements for the current study, based on participant availability and consent. The Research Ethics Board of Hamilton Health Sciences approved the study. Twenty young, overweight female subjects with an average age of 30 ± 2 years (mean ± SE) participated in this study. Participants were all otherwise healthy, premenopausal and overweight or obese women (BMI: 32.4 ± 0.8 kg/ m^2). Other general inclusion criteria were: sedentary lifestyle, regular menstrual cycle, and no vitamin or mineral supplementation. Participants were deemed healthy and thus eligible to participate based on their responses to a short medical screening questionnaire and measurement of serum lipids, glucose, and insulin concentrations all of which were normal (data not shown). All participants provided written informed consent before participating in the study.

Intervention

Diet. Participants were divided into three different dietary groups, based on dairy intake, throughout the 16-weeks. The dietary specifications of the three groups

have been previously described [26]. Briefly, the groups were 1. Adequate protein, low dairy (APLD), 2. Adequate protein, medium dairy (APMD) and 3. High protein, high dairy (HPHD). Of the twenty participants in this study, five were in APLD, eight were in APMD and seven were in HPHD. The targeted total daily energy reduction throughout the study was -750 kcal/d (500 kcal/d by diet and 250 kcal/d through exercise). All participants received individualized diet counseling by study dieticians and research nutritionists on a biweekly basis. Every 2 weeks, participants provided a 3-d food record to track compliance with the intervention [26].

Exercise Training. Participants completed 16 weeks of combined aerobic and resistance training as part of a targeted body composition-changing protocol. Participants exercised at the main fitness center at McMaster University. They engaged in various modes of aerobic exercise (stationary cycling, jogging on a treadmill, walking on an indoor track) 5 d/wk and resistance exercise 2 d/wk with supervision. Each exercise session was designed to result in the expenditure of 250 kcal. During the week (Monday – Friday), subjects reported to the study office and were given a SenseWear Pro (BodyMedia, Pittsburgh, PA, US) energy expenditure arm band device to track energy expenditure [27]. Participants were requested to wear the SenseWear Pro device at home on several occasions randomly throughout the study in order to assess compliance with weekend workouts. The aerobic and resistance training programs have been described previously [26]. Briefly, participants engaged in a 2 d/wk resistance training

protocol (upper body, lower body split). Weight progressions were made once the participants were able to successfully complete 3 sets of 10 repetitions at a given weight.

Cardiovascular Measurements

Heart Rate and Blood Pressure. Testing sessions began with 10 minutes of supine rest to ensure representative resting measurements prior to the commencement of the vascular assessment. Continuous measurements of heart rate *via* single lead electrocardiograph (ECG) (model ML123, ADInstruments Inc. Colorado Springs, Colo.) and brachial blood pressure (BP) measurements via an automated applanation tonometer with oscillometric cuff calibration (model CBM-7000; Colin Medical Instruments, San Antonio, TX.) were made. An FMD test was conducted to assess brachial artery endothelium-dependent function on the basis of previously established guidelines [28, 29]. All analogue signals (including those described below) were converted to digital by fast Fourier transform and sampled simultaneously at a sampling rate of 200Hz using a commercially available data acquisition system (Power lab Model ML 795, ADInstruments, Colorado Springs, USA) and software program (LabChart 7.0; ADInstruments Inc. Colorado Springs, CO, USA).

Flow-mediated dilation assessment. FMD was assessed as previously described [30]. Briefly, with the participant in the supine position, the right arm was positioned and stabilized so that an optimal image of the brachial artery could be obtained in a comfortable position. An inflatable cuff was placed on the forearm,

below the medial epicondyle [31] and remained deflated while baseline data were collected. B-mode ultrasound images of the left brachial artery were collected through two-dimensional grayscale ultrasound imaging using a 10 MHz linear array probe (System FiVe; GE Medical Systems, Horten, Norway) and at a frame rate of 10 frames/second. A baseline longitudinal image of the brachial artery (17 seconds of consecutive heart cycles) was acquired by a single ultrasonographer. Following acquisition of the B-mode image a continuous blood velocity in the brachial artery was obtained using pulsed wave mode Doppler at a frequency of 4MHz with the sample volume width set to insonate the entire artery. The forward and reverse audio signals from the pulse wave mode Doppler spectrum were processed by an external spectral analysis system (Neurovision 500M, Multigon Ind; Yonkers NY) and an intensity-weighted calculated mean signal was acquired (Powerlab model ML795).

To create the flow stimulus, a forearm cuff was instantaneously inflated to a standardized, supra-systolic pressure of 200 mmHg to ensure arterial inflow occlusion and ischemia of downstream vessels and tissue [28]. The cuff was instantaneously deflated after 5 min. of occlusion and during the first 90 seconds after cuff release reactive hyperemic intensity weighted mean blood velocity signals were obtained as described above. Subsequently B-mode ultrasound images of the brachial artery were obtained at 10 frames/sec from 90 to 107 seconds following cuff release; to encompass the time period of the estimated maximum shear rate induced dilation. A semi-automated edge detection software

program (Artery Measurement System, Image and Data Analysis, Tomas Gustavsson, gustav@alumni.chalmers.se) was used to detect the vessel diameters within a specific region of interest within the end diastolic frames of each heart cycle collected within the 17 second recording. The peak dilation of the vessel was established as the single largest end-diastolic diameter (mm) in the 17 second recording between 90 and 107 seconds post cuff release. From this data, the absolute FMD (mm) and relative FMD (%FMD) were calculated as follows [28]:

Equation 3: $$AbsoluteFMD = PeakDiameter(mm) - BaselineDiameter(mm)$$

Equation 4: $$\mathrm{Re}\,lativeFMD = \left(\frac{AbsoluteFMD}{BaselineDiameter}\right)x100\%$$

The following equation was used to calculate shear rate (SR) for each participant [32]:

Equation 5: $$ShearRate = 8x\left(\frac{Velocity}{Diameter}\right)$$

where velocity represents the mean of the velocity profile for the first 30 second post cuff release and the baseline brachial diameter (mm) is used for the artery diameter value. The area under the curve of the shear rate was calculated from the mean of the first point, using the trapezoid rule to obtain the area under the entire curve (GraphPad Prism version 4.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com). Relative FMD (%FMD) was normalized to the area under the entire SR curve and reported as

Equation 6: $$NormalizedFMD = \left(\frac{\%FMD}{SR_{AUC}}\right)$$

This method of analysis provides values of absolute maximum dilation (mm), time to reach peak dilation (sec.) and a raw calculation of the SR stimulus (SR_{AUC}).

Blood Analysis

Overnight fasted venous blood samples were collected prior to and after 16 wk of the intervention for further analysis. Serum was stored at -20°C for further analysis. Serum samples were analyzed using ELISA for concentrations of interleukin-6 (R&D Systems Quantikine, Minneapolis, MN) and endothelin-1 (Enzo, Life Sciences Assay Designs, Farmingdale, NY) using the corresponding immunoassay kits.

Statistics

Results are presented as mean± SE and differences were considered significant at $p<0.05$. Data was analyzed using SPSS (Version 11.5 for Windows). The three dietary groups were compared for differences in all measures. No dietary group differences were observed and thus the data was pooled for all further analysis of the intervention. The effect of the intervention (Pre vs. Post) was examined using one-way repeated measures ANOVAs. Correlations were used to assess relationships between flow-mediated dilation measures and blood markers.

5.5 Results

There were no differences between the dietary groups APLD, APMD or HPHD in any of the vascular or blood marker measures; therefore all groups were pooled for subsequent analysis.

Participants. A total of twenty (n = 20) young healthy women (mean [± SE] age = 30 ± 2 years; height = 163 ± 1 cm) completed the laboratory vascular testing. Following the 16-week intervention weight and body mass index (BMI) decreased **(Table 1).**

Table 1. Body composition measures before and following the 16-week intervention.

	Pre (mean±SE)	**Post** (mean±SE)	**p value**
Body Mass (kg)	86.8 ± 2.4	80.6 ± 2.4	<0.001
BMI (kg/m^2)	32.4 ± 0.8	30.1 ± 0.7	<0.001

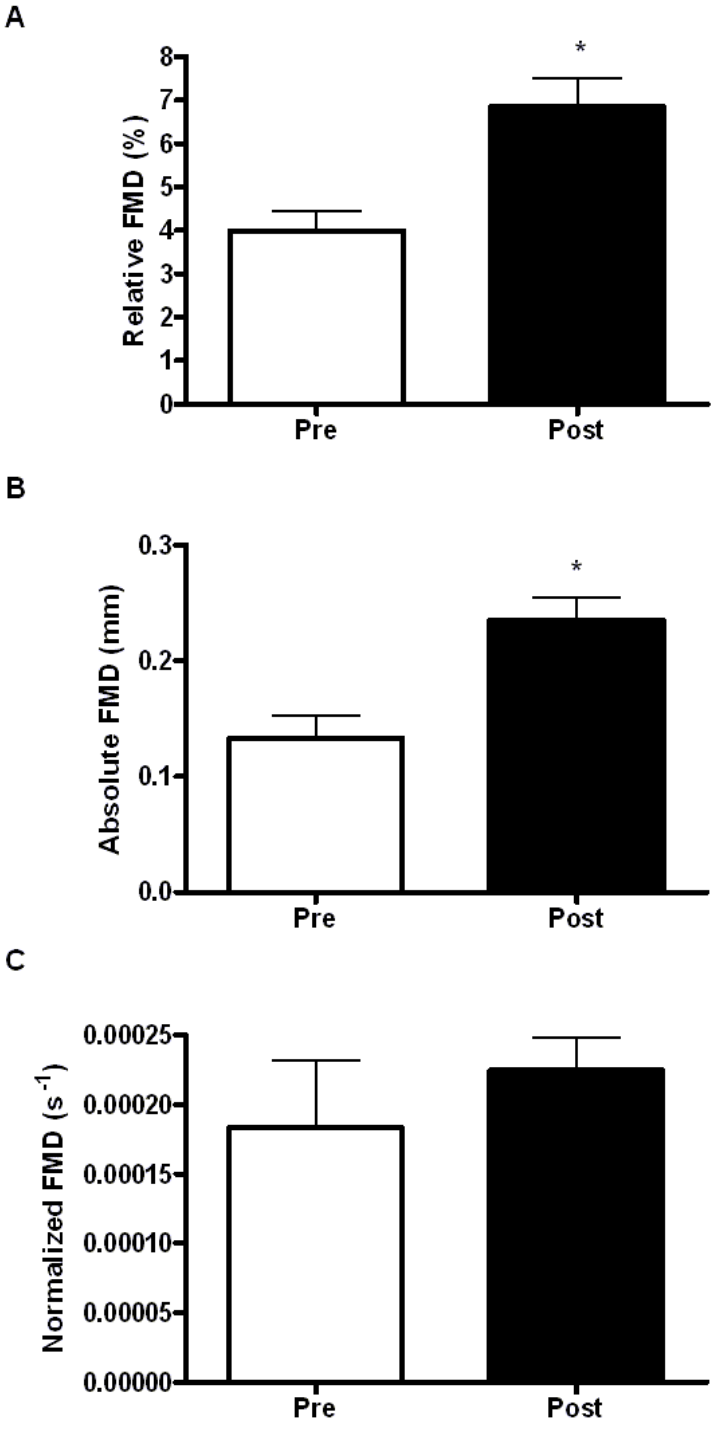
There was no change in brachial artery diameter, heart rate, mean arterial pressure (MAP), systolic blood pressure (SBP) or diastolic blood pressure (DBP) at rest following the intervention. **(Table 2)**

Table 2. Resting vascular measures before and following the 16-week intervention.

Resting Variable	**Pre**	**Post**
Brachial Diameter (mm)	3.39 ± 0.08	3.48 ± 0.09
HR (bpm)	65 ± 1	62 ± 1
MAP (mmHg)	81 ± 2	81 ± 3
SBP (mmHg)	116 ± 2	114 ± 3
DBP (mmHg)	63 ± 2	63 ± 3

Arterial Function. Relative (Figure 1A) and absolute FMD (Figure 1B) increased after the 16-week diet and exercise intervention (relative: pre: 4.1 ± 0.5 % vs. post: 6.9 ± 0.7 %, p = 0.001, absolute: pre - 0.14 ± 0.02 mm vs. post - 0.23 ± 0.02 mm, p = 0.001). However, when FMD was normalized to shear rate, it was unchanged after 16-weeks (normalized: pre: $1.9x10^4 \pm 5.0x10^5$ vs. post: $2.2x10^4 \pm 2.4x10^5$, p = 0.45; Figure 1C).

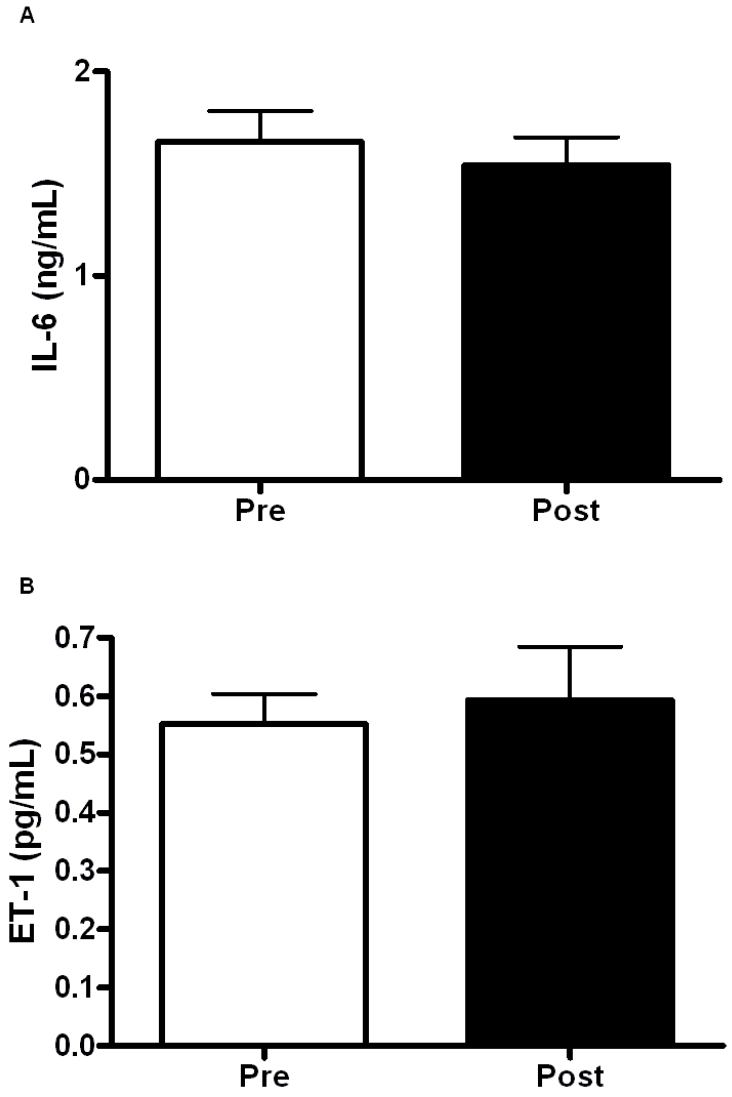
Figure 1. Changes in FMD from Week 0 to Week 16.



There were no relationships observed between weight loss and increased FMD (relative, absolute or normalized) following the intervention (weight loss vs. change in absolute FMD: $r = -0.12$, $p = 0.62$, weight loss vs. change in relative FMD: $r = -0.07$, $p = 0.77$, weight loss vs. change in normalized FMD: $r = -0.17$, $p = 0.47$). There were no relationships between weight loss and changes in IL-6 or ET-1 (weight loss vs. change in IL-6: $r = 0.28$, $p = 0.17$, weight loss vs. change in ET-1: $r = 0.11$, $p = 0.65$).

Serum IL-6 did not change (pre = 1.7 ± 0.2 ng/mL to post = 1.6 ± 0.1 ng/mL, $p = 0.79$, Figure 2A) after the 16-week intervention**.** Serum ET-1 also did not change (pre = 0.55 ± 0.01 pg/mL to post = 0.60 ± 0.05 pg/mL, $p = 0.45$, $N = 19$, Figure 2B) after the 16-week intervention**.** There were no relationships observed between any measures of FMD and IL-6 before or after the intervention. There were no relationships observed between any measures of FMD and ET-1 ($N = 19$) before or after the intervention. One participant was removed from this analysis, as her ET-1 concentrations were undetectable.

Figure 2: IL-6 and ET-1 – Week 0 and Week 16



5.6 Discussion

The main findings of this study were that brachial artery endothelial function, assessed by relative and absolute FMD improved after the 16-week diet and exercise intervention, however, IL-6 and ET-1 were unchanged, in obese and

overweight women. Given the importance of endothelial function as an independent risk factor for CVD [1, 2] we view our findings as a relevant demonstration of what a multi-faceted exercise- and diet-based weight loss intervention can do to alleviate CVD risk. The women in this study, while overweight or obese by BMI, were healthy by a number of other criteria (blood lipids, glycemia); however, their sedentary lifestyle may have contributed to a reduction in endothelial function. Our combined aerobic and strength training program, which improved both fitness and strength [26], was likely responsible in part for the improved endothelial function we observed. While all of our subjects lost weight, the weight loss was not associated with changes in FMD or with reductions in markers of inflammation and so it is difficult to ascribe the changes in FMD to changes in body weight or indirectly through the influence this might have had on inflammation.

Weight loss with diet [5, 6] and/or aerobic exercise [7] interventions has previously been linked with increased endothelial function as measured by brachial FMD in overweight and obese women. We did not observe this relationship between weight loss and improved endothelial function via brachial FMD measures. The degree of weight loss was relatively small; therefore it may not have been enough to elicit changes in FMD. However the vascular stimulus provided by the exercise intervention (increased shear) may be responsible for the observed changes. Thus we do not attribute the changes in FMD to the weight

loss of the individuals but rather possibly to the vascular stimulus provided by the exercise intervention.

Aerobic exercise training has been shown to improve endothelial function in a variety of populations [33] including overweight post-menopausal women [23, 34]. We found a significant increase in FMD in overweight pre-menopausal women who performed combined aerobic and resistance training for 16 weeks. Our findings agree with those of Kwon and colleagues [23] who reported increases in relative FMD after an aerobic training program in overweight post-menopausal women ($p=0.03$). Other studies have also shown increases in endothelial function as assessed by FMD using combined aerobic and resistance training interventions in other populations including young healthy men and men and women with T2DM [24, 25]. To our knowledge there are no studies to date that have investigated the effects of a combined weight loss plus aerobic and resistance program on FMD in young overweight pre-menopausal women.

Mechanistically, circulating systemic concentrations of ET-1 and IL-6 are potential regulators of endothelial function. Inflammation has the ability to impair FMD as cytokines may lead to increased vasoconstriction [9]. IL-6 contributes to chronic inflammation in conditions such as obesity and studies have identified elevated IL-6 levels in obese individuals [16, 17]. ET-1 can decrease NO bioavailability either by decreasing its production or by increasing its degradation [10]. Recently, it has been reported that endothelial function is largely regulated by local endothelial-derived factors including PGI_2, EDHF and NO, [35] however

studies have reported a weak but significant inverse correlation ($r = -0.12$, $p<0.0001$) between circulating serum IL-6 concentration and endothelial function [11]. Chronic inflammation has been linked to endothelial dysfunction [9], and thus a decrease in endothelial dysfunction may be related to changes in inflammatory markers. However, in the current study, ET-1 and IL-6 were unchanged following the 16-week intervention and there was no relationships observed between IL-6 or ET-1 and any of the FMD measurements suggesting that in our subject population IL-6 and ET-1 are not sensitive markers for changes in FMD during this type of lifestyle intervention.

Another potential explanation for our observation of no change in IL-6 in conjunction with the observed increase in FMD is the inclusion of resistance training in the exercise regime for our participants. There are conflicting results with respect to the effects of resistance training on circulating markers of inflammation. A recent study by Patterson et al. demonstrated that IL-6 levels were elevated following resistance training in older men [36], while Phillips *et.al.* observed no change in IL-6, after resistance training in post-menopausal women [37]. It is also possible that the women in this study did not have chronic inflammation and thus we are observing a 'floor' effect where even an intervention involving weight loss and exercise would not decrease their inflammatory markers further.

It appears ET-1 generally decreases following aerobic exercise training [38-40]. A recent study showed that ET-1 is reduced after just three weeks of

aerobic training in middle-aged obese type 2 diabetic men and women [38]. Kasimay and colleagues (2010) observed a decrease in ET-1 when aerobic exercise training was accompanied with a low-calorie diet [39]. Maeda and colleagues (2003) observed a reduction in plasma ET-1 concentrations in older healthy women after 3 months of aerobic training [40]. The populations involved in these studies compared to our study were slightly different, however, all of the above participants were overweight, similar to our study and many were overweight middle-aged women. We observed no differences in ET-1 concentration following 16-weeks of exercise training. Similar to our suggestion of the potential confounder of added resistance exercise on our IL-6 it is possible the resistance-training component in our intervention attenuated any aerobic exercise training stimulated decreases in ET-1. Very little information exists regarding the effects of resistance training on ET-1, however Maeda et al. (2004) observed a decrease in plasma ET-1 in young healthy men after 8 weeks of resistance training [41]. In this study we found no change in ET-1 after resistance training. More studies are necessary to better understand the role of ET-1 after resistance training, as there are no other studies to our knowledge investigating the effects of this type of training on ET-1 in women.

In conclusion, we observed an increase in endothelial function as measured by absolute and relative FMD following 16-weeks of a diet and exercise intervention that resulted in weight loss in overweight and obese pre-menopausal women. No changes were observed in blood markers, IL-6 and ET-1, which are

often proposed as being mechanistically relevant in inflammation and vasoconstriction. Our study demonstrates that endothelial function is improved with weight loss and combined aerobic and resistance exercise and may be helpful for designing lifestyle interventions for overweight individuals at elevated CVD risk. Further research is warranted to better understand the mechanisms responsible for the observed changes.

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6.0 CHAPTER 6

16-weeks of combined aerobic and resistance training and hypocaloric diet on measures of arterial stiffness in overweight pre-menopausal women

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Andrea R. Josse – lead student investigator on the larger IDEAL study, edited manuscript
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6.1 Abstract

Overweight and obesity is a major risk factor for heart disease and hypertension. While diet and exercise reduce the risk of cardiovascular morbidity in overweight populations the effects of combined aerobic and resistance training on arterial health has not been comprehensively examined. In this study we investigated changes in carotid artery distensibility and carotid to radial pulse wave velocity (PWV) in twenty-five overweight, pre-menopausal women who participated in 16-weeks of 5-7 d/wk aerobic exercise, 2 d/wk resistance training, and a hypocaloric diet intervention. Pro-collagen type I C-peptide (PIP) was used as a marker of type I collagen synthesis and C-telopeptide of type I collagen (CTX), as a marker of type I collagen degradation. All twenty-five of the participants lost body mass after the 16-weeks (mean ± SE- Pre: 86.8 ± 2.4 kg vs. Post: 80.6 ± 2.4 kg, $p<0.05$). Carotid artery distensibility was not altered (Pre: $5.1 \times 10^{-3} \pm 3.9 \times 10^{-4}$ vs. Post: $5.5 \times 10^{-3} \pm 3.5 \times 10^{-4}$, $p = 0.26$); however, carotid - radial PWV increased significantly after the 16-week intervention (Pre: 8.1 ± 0.3 m/s vs. Post: 8.9 ± 0.3 m/s, $p<0.05$). There were no changes in the concentration of PIP (Pre: 1188 ± 91 ng/mL vs. Post: 1222 ± 94 ng/mL, $p = 0.69$). However, the ratio of PIP:CTX as an indicator of turnover was decreased (Pre: 1851.6 ± 140.8 vs. Post: 1514.7 ± 111.6, $p = 0.008$) and the concentration of CTX increased with the intervention (0.65 ± 0.01 ng/mL vs. Post: 0.80 ± 0.02 ng/mL, $p<0.001$). We observed no relationships between markers of collagen turnover and arterial function measures. We conclude that 16-weeks of combined aerobic-resistance

training and diet induced weight loss did not alter carotid artery distensibility or circulating markers of type I collagen synthesis but was associated with increased carotid-radial PWV and a marker of type I collagen degradation. It appears that 16-weeks of diet and combined aerobic and resistance training may lead to increased peripheral artery stiffness, as measured by upper limb pulse wave velocity, however, cardiovascular risk assessed by carotid artery distensibility remained unchanged.

6.2 Introduction

Obesity has been identified as an independent risk factor for cardiovascular disease (CVD) [1, 2]. Increases in central artery stiffness, assessed by carotid artery compliance [3], stiffness index [3] and carotid-femoral PWV [3, 4], is a strong predictor of cardiovascular disease risk in healthy and individuals at elevated risk. Aerobic exercise has been shown to increase central arterial compliance and decrease carotid-femoral PWV and augmentation index and thus the risk of CVD [5, 6] in a variety of populations including young healthy men [7], hypertensive men and women [8] and active and inactive pre- and postmenopausal women [9]. It is unclear whether arterial health benefits are limited to aerobic training or if other exercise modalities such as resistance training or combined aerobic and resistance training can decrease arterial stiffness and therefore cardiovascular risk in overweight and obese individuals. Rakobowchuk and colleagues found no change in carotid artery compliance after 6 and 12 weeks of whole body resistance training in young healthy men [10]. In contrast, other studies have observed an increase in central (carotid-femoral) [8, 11] and peripheral (femoral-dorsalis pedis) PWV [8] after 4 weeks of 3-times/week resistance training in moderately active middle-aged men [8, 11] and women with pre- and stage 1 hypertension [8]. There are minimal data, however, on the effects of combined aerobic and resistance training on arterial stiffness. Yang reported a decrease in brachial-ankle PWV in obese women (30-60 years) after three months of combined aerobic and resistance training [12]. The

variations in methods and measurement sites in different studies likely contributes to the variability in outcomes with respect to the effects of different exercise training programs on arterial stiffness.

Information on circulating markers of collagen turnover have been used as proxies for arterial wall remodeling, for example using markers of type I collagen synthesis and degradation, may contribute to a better understanding of potential mechanisms responsible for changes in arterial stiffness observed with exercise/weight loss interventions [13-16]. Few studies have investigated the relationship between arterial stiffness and markers of type I collagen synthesis and degradation and the results in the existing studies are mixed [13-16]. Some studies have reported positive associations between collagen synthesis and arterial stiffness [14] or inverse relationships between collagen degradation and arterial stiffness [13] while others have reported the opposite and found positive relationships between collagen degradation and arterial stiffness [14, 15]. Clearly, more data are needed to comprehensively understand the role of type I collagen turnover in arterial stiffening and if markers of type 1 collagen turnover are useful markers of arterial stiffness.

Studies have shown that 10 weeks of either low or high intensity aerobic training does not change serum markers of type I collagen degradation (ICTP) or synthesis (PINP) [17] in post-menopausal women. Despite this there are no studies examining changes in arterial stiffness and collagen type 1 turnover with a combined exercise and diet intervention in pre-menopausal women who are at

elevated risk for CVD. Thus, the purpose of this study was to determine the effects of a 16-week diet and combined aerobic and resistance exercise intervention on markers of type I collagen turnover and both central and peripheral measures of arterial stiffness, as assessed by carotid artery distensibility and carotid-radial PWV (PWV_{c-r}) in obese and overweight pre-menopausal women.

6.2 Methods

Participants. This study was part of a larger lifestyle intervention study: Improving Diet, Exercise and Lifestyle (I.D.E.A.L) for Women Study, which involved 90 participants [18]. A small subset of participants (n = 25) from the larger lifestyle intervention study volunteered to participate in the cardiovascular measurements for the current study, based on participant availability and consent. The Research Ethics Board of Hamilton Health Sciences approved the study. Twenty-five young, (30 ± 2 years; mean ± SE) obese (BMI: 32.4 ± 0.7 kg/m^2) female subjects participated in this study. Other general inclusion criteria were: sedentary lifestyle, regular menstrual cycle, and no vitamin or mineral supplementation. Participants were deemed healthy and thus eligible to participate based on their responses to a short medical screening questionnaire and measurement of serum lipids, glucose, and insulin concentrations all of which were normal (data not shown). All participants provided written informed consent before participating in the study.

Intervention

As previously described, participants took part in both diet and exercise interventions over a 16 week period [18].

Diet. Participants were asked to consume a hypocaloric diet that was -750 kcal/d less than their estimated requirements (500 kcal/d by diet and 250 kcal/d through exercise). All participants received individualized diet counseling by study dieticians and research nutritionists on a biweekly basis. Every 2 weeks, participants provided a 3-d food record to track compliance. Participants were divided into three dietary groups, based on dairy intake, throughout the 16-weeks. The dietary specifications of the three groups have been previously described [18]. Briefly, the groups included a) Adequate protein, low dairy (APLD) b) Adequate protein, medium dairy (APMD) and c) High protein, high dairy (HPHD). Of the twenty-five participants in this study, eight were in APLD, eight in APMD and nine in HPHD.

Exercise Training. Participants completed 16 weeks of combined aerobic and resistance training as part of a targeted body composition-changing protocol. Participants exercised at the main fitness centre at McMaster University. They engaged in a number of modes of aerobic exercise (stationary cycling, jogging on a treadmill, walking on an indoor track) 5 d/wk and resistance exercise 2 d/wk with supervision. Each exercise session was designed to result in the expenditure of 250 kcal. During the week (Monday – Friday), participants reported to the study office and were given a SenseWear Pro (BodyMedia, Pittsburgh, PA, US)

energy expenditure arm band device to track energy expenditure [19]. Participants were requested to wear the SenseWear Pro device at home on random occasions in order to assess compliance with weekend workouts. The aerobic and resistance training programs have been described previously [18].

Cardiovascular Measurements

Heart Rate and Blood Pressure. Testing sessions began with 10 minutes of supine rest to ensure representative resting measurements prior to the commencement of the vascular assessment. Continuous measurements of heart rate *via* single lead electrocardiograph (ECG) (model ML123, ADInstruments Inc. Colorado Springs, Colo.) and brachial blood pressure (BP) measurements via an automated applanation tonometer with oscillometric cuff calibration (model CBM-7000; Colin Medical Instruments, San Antonio, TX.) were made. All analogue signals were converted to digital using a fast Fourier transform and were sampled simultaneously at 200 Hz using a commercially available data acquisition system (Power lab Model ML 795, ADInstruments, Colorado Springs, USA) and software program (LabChart 7.0; ADInstruments Inc. Colorado Springs, CO, USA).

Arterial Stiffness Measures

Peripheral Artery Stiffness. Peripheral artery stiffness was estimated using $PWV_{c\text{-}r}$ using high fidelity, simultaneous carotid and radial artery pressure measurements. $PWV_{c\text{-}r}$ was defined as the time delay in arrival of the foot of the pulse wave between the carotid and radial arteries. Arterial waveforms at the

common carotid and radial arteries were collected using a hand-held tonometer (model SPT-301, Millar Instruments Inc., Houston, TX) positioned on the right common carotid and radial artery at the point of greatest pulsation to produce continuous arterial pressure waveforms. Peripheral pulse transit time was determined using the subtraction method (Weber T, 2009). This was achieved by determining the time delay between ventricular depolarization (R-wave peak), and the "foot" of the radial and carotid artery waveforms. The pressure waveforms obtain were band-pass filtered (5-30Hz) with the lower (≤ 5Hz) and higher frequencies (≥ 30Hz) removed in order to assist in the detection of the foot of each waveform. The foot of each waveform was identified as the minimum value of the digitally filtered signal and corresponded to the end of diastole, when the steep rise in the wave begins and appears as a sharp inflection of the original signal [20].

Similarly the path length between the pulse measurement sites was calculated by subtracting the surface distance between the sternal notch and the carotid tonometer placement from that of the sternal notch and the radial tonometer placement. An anthropometric measuring tape was used to measure the straight-line distance between skin sites along the surface of the body. Resting PWV data was recorded during 20 continuous heart cycles using the equation:

Equation 1: $$PWV_{c-r} = \frac{D}{\Delta T}$$

where D is the distance between measurement sites, and Δt is the pulse transit time.

Central Artery Stiffness. Central artery stiffness was estimated with direct measurements of carotid artery distensibility, as previously described [21], using a combination of high-resolution, two-dimensional, brightness mode ultrasound images (SystemFiVe; GE Medical Systems, Horten, Norway) and applanation tonometry (model SPT-301; Millar Instruments, Houston, TX, USA). Common carotid artery images were collected using a 10MHz ultrasound probe and were collected at 10 frames·sec^{-1}. A hand-held tonometer was positioned over the point of greatest pulsation of the right common carotid artery and held in a fixed position for ten consecutive heart cycles while ultrasound images of the left common carotid artery were collected simultaneously. Absolute carotid artery systolic blood pressures were calculated by calibrating the relative values acquired using applanation tonometry to the calibrated brachial artery blood pressure acquired simultaneously [22, 23]. Ultrasound images were stored offline in Digital Image and Communications in Medicine (DICOM) format for later analysis using semi-automated edge tracking system [AMS, (Artery Measurement System) Image and Data Analysis: Tomas Gustavsson, gustav@alumni.chalmers.se]. In each frame, carotid (minimum, mean and maximum) lumen diameters were calculated from roughly 100 measurement markers along the vessel wall within a chosen region of interest, for a total of 110,000 measures in the 10 heart cycles. Distensibility was calculated using the following equations [24]:

Equation 2: $$Distensibility = \frac{\left[\Pi\left(\frac{d_{max}}{2}\right)^2 - \Pi\left(\frac{d_{min}}{2}\right)^2\right]}{\Pi\left(\frac{d_{min}}{2}\right)^2 \times PP}$$

where d_{max} is the maximum diameter, d_{min} is the minimum diameter, and PP is carotid pulse pressure, the change in pressure DBP and SBP. The mean carotid diameter was calculated using the average of all diameters acquired throughout the ten heart cycles.

Blood Analysis

Overnight fasted venous blood samples were collected prior to and after 16 weeks of the intervention for further analysis. Serum was stored at -20°C for further analysis. Serum samples were analyzed for levels of pro-collagen type I (PIP) as a marker of type 1 collagen synthesis (Takara, Mountain View, CA) and cross-linked telopeptide of type I collagen (CTX) as a marker of degradation (Immunodiagnostic Systems, Scottsdale, AZ) using the corresponding immunoassay kits.

Statistics

Results are presented as mean ± SE and differences were considered significant at $p<0.05$. Data were analyzed using SPSS (Version 11.5 for Windows). The three dietary groups were compared for difference in all measures. No differences were observed and thus the data was pooled for all further analysis. The effect of the intervention (pre vs. post) was examined using

paired t-tests. Possible relationships between the blood markers and functional markers of arterial stiffness were analyzed using Pearson correlation coefficient.

6.4 Results

There were no differences between the dietary groups APLD, APMD or HPHD in any of the vascular or blood marker measures; therefore all dietary groups were pooled for subsequent analysis.

Participants. A total of twenty-five (n = 25) young healthy women (mean [± SE] age (years) = 30 ± 2; height (cm) = 163 ± 1) completed the vascular assessments before and after the lifestyle intervention. Following the 16-week intervention body mass and BMI decreased significantly (Table 1).

Table 1. Body composition measures before and following the 16-week intervention.

	Pre	Post	P value
Body Mass (kg)	86.8 ± 2.4	80.6 ± 2.4	<0.001
BMI (kg/m^2)	32.4 ± 0.8	30.1 ± 0.7	<0.001

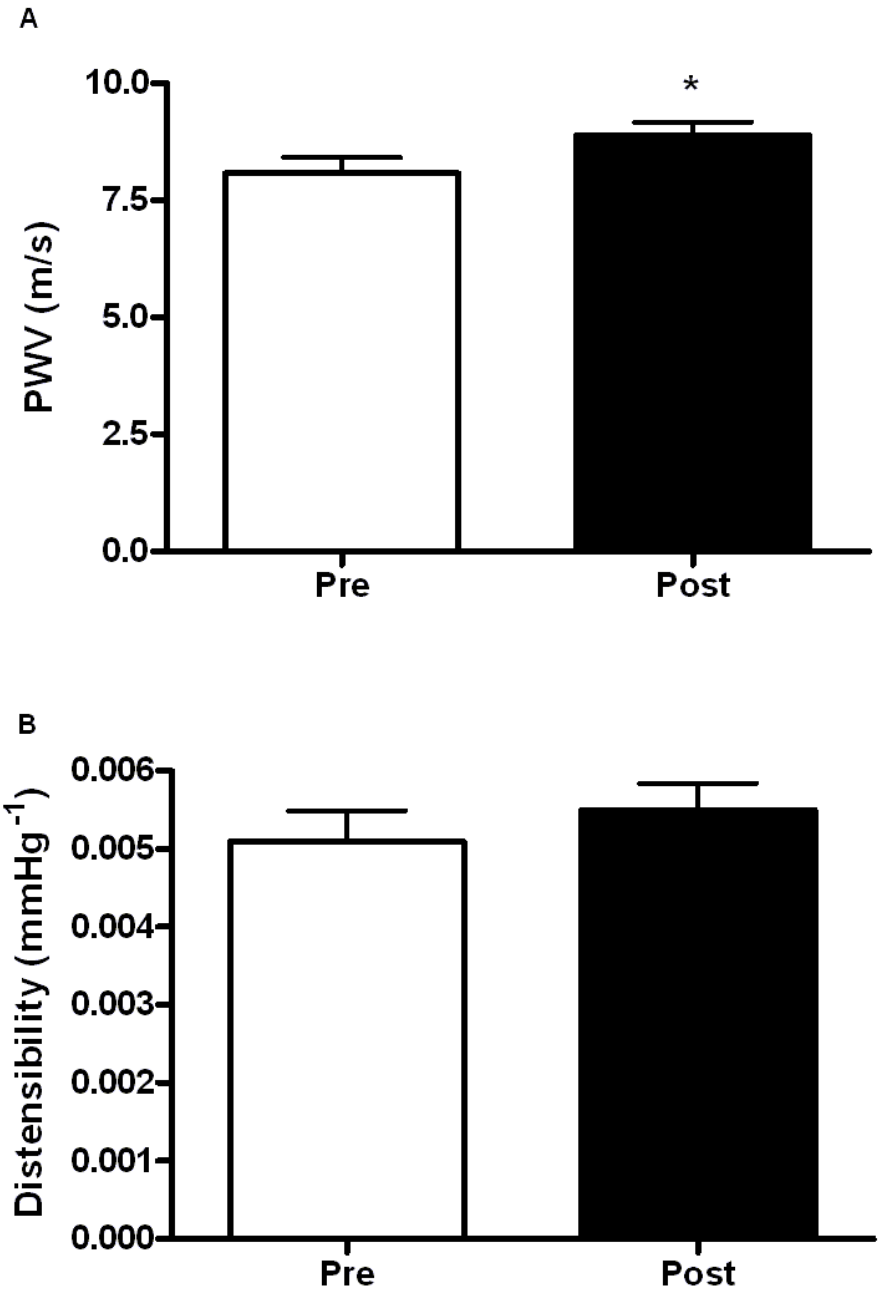
Cardiovascular Data. There were no changes in supine resting heart rate, MAP. SBP or DBP following the intervention **(Table 2).**

Table 2. Resting vascular measures before and following the 16-week intervention.

	Pre	Post
Resting Supine HR (bpm)	65 ± 1	62 ± 1
Resting Supine MAP (mmHg)	81 ± 2	81 ± 3
Resting Supine SBP (mmHg)	116 ± 2	114 ± 3
Resting Supine DBP (mmHg)	63 ± 2	63 ± 3

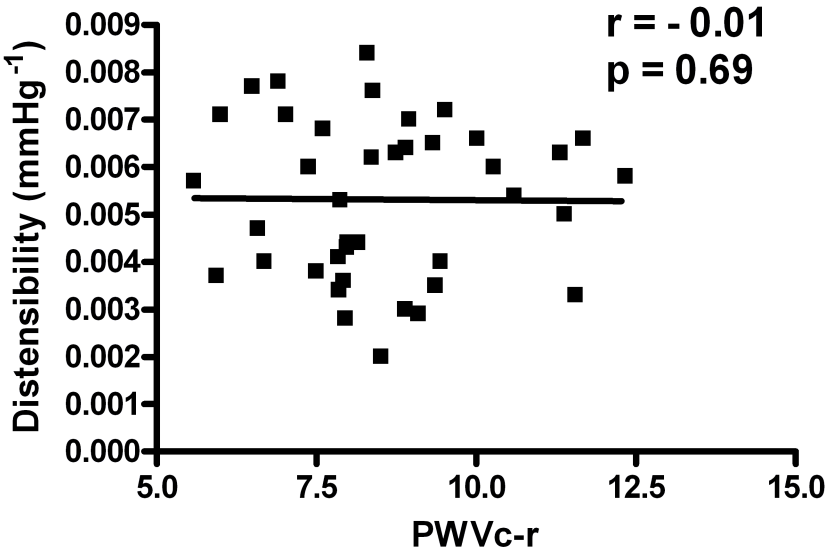
Arterial Stiffness. $PWV_{c\text{-}r}$ (Figure 1A) increased after the 16-week diet and exercise intervention (Pre: 8.1 ± 0.3 m/s vs. Post: 8.9 ± 0.3 m/s, $p<0.05$). However, carotid artery distensibility (Figure 1B) was unchanged with the intervention (Pre: $5.1 \times 10^{-3} \pm 3.9 \times 10^{-4}$ vs. Post: $5.5 \times 10^{-3} \pm 3.5 \times 10^{-4}$, $p = 0.26$).

Figure 1: Changes in PWV_{c-r} and carotid artery distensibility from Week 0 to Week 16.



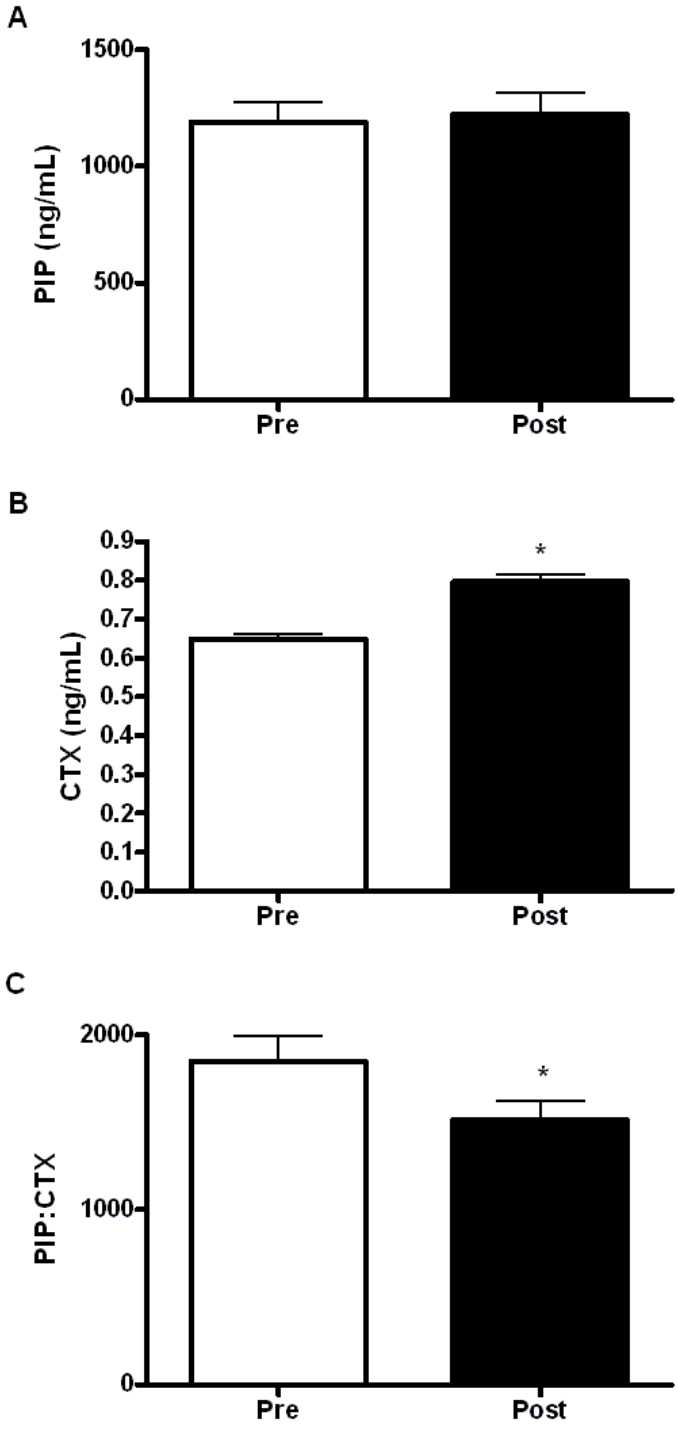
Relationship between peripheral and central artery stiffness. There was no relationship observed between measures of PWV_{c-r} and carotid artery distensibility (Figure 2).

Figure 2: Relationship between peripheral and central artery stiffness.



Type I collagen markers. As a marker of collagen synthesis PIP was unchanged (Pre: 1188 ± 91 ng/mL vs. Post: 1222 ± 94 ng/mL, p = 0.69; Figure 3A) while a marker of collagen degradation, CTX, increased after the 16-week intervention (Pre: 0.65 ± 0.01 ng/mL vs. Post: 0.80 ± 0.02 ng/mL, p<0.001; Figure 3B). When analyzed as a ratio (PIP:CTX), used previously to represent collagen turnover, there was a decrease as a result of the intervention (Pre: 1851.6 ± 140.8 vs. Post: 1514.7 ± 111.6, p = 0.008; Figure 3C).

Figure 3: Changes in serum type I collagen markers from Week 0 to Week 16



Relationship between type I collagen markers and arterial stiffness. There were no relationships observed between either the central or peripheral artery stiffness measures and the type I collagen markers or the ratio of those markers.

6.5 Discussion

The main finding from our study was that the impact of a combined diet and exercise intervention on arterial stiffness in overweight and obese pre-menopausal women may depend on the location of the arterial segment examined. This regional variation in changes in arterial stiffness may explain the variability in the relationships between arterial stiffness and type 1 collagen markers observed in the literature and the variability in the impact of different exercise/weight loss interventions on arterial stiffness. Central artery stiffness is an accepted predictor for CVD risk [25], while peripheral artery stiffness may provide valuable information about the mechanisms regulating vascular remodeling and may be an acceptable surrogate for central stiffness in some populations [25].

In the current study, $PWV_{c\text{-}r}$ increased after the 16-week diet and exercise intervention, indicating increased upper limb arterial stiffness. A marker of collagen synthesis (PIP) was unchanged, however, collagen degradation (CTX) increased following the intervention. When expressed as a ratio of PIP:CTX, thought to be representative of type I collagen turnover, there was no change after 16-weeks. In this study we measured both central artery stiffness, using carotid artery distensibility and peripheral artery stiffness using $PWV_{c\text{-}r}$. We were interested in determining if $PWV_{c\text{-}r}$ would be a good surrogate for central artery

stiffness in this population since the gold standard measure of central PWV involves exposure of the inguinal region for the femoral site measurement. In our population of overweight and obese women, measurements of arterial pressure at the femoral site generally are difficult due to the distribution of body fat and the discomfort of the participants. Our results demonstrate that despite the positive weight loss outcomes, this multi-faceted exercise and diet-based intervention lead to diverse arterial structural changes that were dependent on the arterial segment examined and the technique used.

Aerobic exercise training has been reported to decrease arterial stiffness, as measured by central augmentation index and central and peripheral PWV in a variety of populations, including overweight women [9, 26]. We did not, however, see a decrease in arterial stiffness, which may have been due to the added stimulus of resistance training in our study population [8, 10, 11, 27, 28]. For example, Rakobowchuk *et.al.* (2005) [10] observed no change in carotid artery compliance after 6 and 12 weeks of whole body resistance training in young healthy men. However, Collier et al. observed an increase in central pulse wave velocity and lower limb peripheral PWV after 4 weeks of resistance training in pre- and stage 1 hypertensives [8] and moderately active middle-aged men and women [11]. In direct contrast to our results, Yang reported a decrease in brachial-ankle PWV in overweight women (30-60 years) after three months of combined aerobic and resistance training [12]. Our measure of peripheral artery stiffness was limited to the upper limb, and to our knowledge no studies have compared upper and lower

limb peripheral artery and central artery stiffness in pre-menopausal women. Interestingly, in this study, PWV_{c-r} was affected by the intervention but it is not clear if that is an acceptable indication of changes in cardiovascular risk as risk prediction is only linked to central artery stiffness [25], which in our study remained unchanged.

In an effort to determine mechanisms associated with changes in arterial stiffness, we measured the effects of our intervention on blood markers of type I collagen synthesis and degradation, as others have done [13-16], and looked at their relationship to the measures of both central and peripheral artery stiffness before and after the intervention. Pro-collagen type I, an indicator of type I collagen synthesis was unchanged after the intervention and this finding is consistent with other reports [17]. To our knowledge measures of type I collagen synthesis following a long-term (16-week) diet and combined aerobic and resistance – exercise program have not been made in pre-menopausal women. Studies have, however, also shown that long-term low and high intensity aerobic training do not result in changes in markers of type I collagen degradation (CTX or ICTP) in post-menopausal women [17, 29]. In contrast, we observed an increase in type I collagen degradation following the 16-week intervention.

The difference in total collagen content between healthy elastic and unhealthy stiff arteries is a topic of debate [30-33]. McNulty et al. discussed the possibility of alterations in the proportions of arterial collagen subtypes affecting vascular stiffness, despite no changes in total collagen content. Dellegrottaglie et al.

observed an increase in type III collagen with increasing stiffness as measured by central PWV [34]. It is possible that the relative proportion of different types (I, III and V) of collagen may have an impact on the structure of the arterial wall. An increase in type I degradation may permit increases in other types of collagen without a concurrent increase in total collagen and thus any change in the structural components of the arterial wall. In our study we observed an increase in the marker of type I collagen degradation following the intervention that may be linked to increased peripheral artery stiffness. This finding is congruent with that of McNulty et al. [15] who reported a positive correlation between ICTP (type I collagen degradation) and central PWV in a mixed group of hypertensive and normotensive middle-aged men and women.

Conflicting observations of the relationships between arterial stiffness and type I collagen degradation have been reported in the literature [13-15]. We did not find a relationship between either type I collagen synthesis or degradation and either central or peripheral artery stiffness. It is possible our sample size of 25 was not large enough to see this relationship, as other studies have had larger sample sizes [13-15], however, we did not even observe noticeable trends in our data. It is also possible that the degree of vascular remodeling, if it occurred, was relatively small in our young obese, but otherwise healthy, women who may not have had extensive arterial dysfunction to begin with. It is also possible that circulating markers of collagen synthesis/degradation reflect turnover of pools of collagen distinct from that present only in arteries (i.e., bone and muscle) and thus

cannot, particularly in an exercise intervention, be used as a reflection of solely arterial remodeling.

In conclusion, we observed an increase in peripheral artery stiffness as measured by $PWV_{c\text{-}r}$; however, we saw no change in measures of central artery stiffness as measured by carotid artery distensibility. We also saw an increase in CTX, a marker of type I collagen degradation. Our study demonstrates that despite weight loss following a 16-week diet and combined aerobic and resistance training intervention there may be negative effects on the vascular system. It is important to consider the duration and mode of exercise and diet employed and the overall balance of cardiovascular risk outcomes when assessing the total benefits of lifestyle interventions. This study does not support the use of type 1 collagen turnover as an index over either central or peripheral artery stiffness. Further research is warranted to better understand these relationships and the mechanisms responsible for the observed changes.

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7.0 CHAPTER 7:

GENERAL DISCUSSION AND CONCLUSIONS

7.1 Summary of Major Findings

A better understanding of the mechanisms responsible for the regulation of vascular structure and function may lead to the design of more effective strategies targeted at reducing CVD risk. The first study of this thesis investigated the potential associations between circulating blood markers of type I collagen turnover and arterial stiffness and between markers of vasoconstriction and inflammation and endothelial function. A series of populations were included, to address the limitation in the current literature, regarding collagen turnover and arterial stiffness, whereby previous studies were limited to older clinical populations [1-5]. Thus, the individuals included in our observational study represented a large range of both arterial stiffness and endothelial function. We hypothesized that altered collagen turnover would be associated with measures of central artery stiffness, including central PWV and carotid artery distensibility. We found that over a span of vascular health, increased arterial stiffness is associated with less type I collagen blood markers and potentially less collagen turnover. Specifically, central PWV was negatively associated with serum concentrations of CTX and PIP. Correspondingly, carotid artery distensibility, the inverse of stiffness was positively associated with both CTX and PIP. These results suggest that increased collagen turnover is associated with healthier, less stiff arteries.

With respect to the regulation of endothelial function, we hypothesized that measures of FMD would be negatively associated with circulating markers of both inflammation and vasoconstriction. Indeed circulating ET-1, a vasoconstrictor, was inversely correlated with FMD. These results suggest that circulating ET-1 may be influencing endothelial function in a wide range of vascular function. In contrast, systemic inflammation, represented by IL-6, was not correlated with measures of FMD in any of the populations tested; despite the large range of endothelial health represented, suggesting this circulating inflammatory marker may not be a useful surrogate or regulator of endothelial function.

With the knowledge of these relationships between blood markers and functional measures of vascular structure and function, studies #2 and #3 were designed to investigate the effects of a lifestyle intervention on these measures and the relationships previously observed. Therefore, in the second study of this thesis, it was hypothesized that a 16-week diet and exercise intervention would increase endothelial function, as measured by FMD and decrease systemic inflammation and circulating vasoconstrictors, in pre-menopausal overweight women. As expected, the 16-week diet and exercise intervention led to an increase in endothelial function. However, there were no observed changes in either IL-6 or ET-1 and neither factor was correlated with relative, absolute or normalized FMD before or after the intervention. These results suggest that the chosen diet and exercise intervention successfully increased endothelial function,

however may not have been robust enough to target vasoconstriction or inflammatory markers. It is also possible the sample size in this study alone was not sufficient to replicate our previous observation of relationships between these ET-1 and FMD.

In the third study of this thesis it was hypothesized that a 16-week diet and exercise intervention would improve arterial stiffness. Specifically, it was hypothesized that both peripheral and central artery stiffness would decrease over time and that changes would be associated with markers of type 1 collagen as previously demonstrated. However, contrary to the hypotheses, peripheral PWV increased and carotid artery distensibility was unchanged following the intervention, while our marker of type I collagen degradation increased over time. Neither collagen synthesis nor degradation was found to be associated with our measures of arterial stiffness. These findings suggest that the chosen diet and exercise intervention may have led to increased peripheral artery stiffening, but unchanged central elasticity. It is possible that the inclusion of resistance training had a negative effect on the peripheral vascular measures or that changes in upper limb vessel diameter and wall thickness influenced the measurement of peripheral artery stiffness. Contrary to the findings in the first study of this thesis, collagen degradation increased over time, despite the observed increase in peripheral stiffness. In the first study of this thesis, we observed increased serum concentration of collagen markers with decreased arterial stiffness. However, in the first study we measured central arterial stiffness and not peripheral and also

observed a coinciding positive relationship between healthier arteries and greater collagen synthesis. In Study 3 we observed no change in PIP (collagen synthesis) after the intervention and there were no relationships observed between either marker of collagen and the measures of vascular stiffness. These apparent internal inconsistencies in our findings may be due to the small sample size included in study 3 and the potentially confounding effects of the resistance exercise program in particular on collagen type I turnover from bone and muscle.

7.2 Collagen

Collagen is the most abundant protein in humans [6]. It is most commonly found in tendons, ligaments, skin, the cornea, cartilage, bone, blood vessels, the gut, and intervertebral discs [7]. Despite the fact that collagen is the most abundant protein in the body and found in many different tissues, our findings suggest that circulating markers may be indicative of vascular health. Specifically, in our observational study (Study 1) we observed increased type I collagen turnover in association with less stiff arteries. Predominant in the vessel wall, collagen is the major fibre-forming species and contributes to the mechanical function of vessel wall tensile strength [8]. The current study did not measure any other sub-type of collagen, besides type I, however, past literature has shown a weak positive relationship between serum type III collagen synthesis and increased PWV [2] and type III collagen is thought to be associated with extensibility of the vessel wall [8].

Studies suggest that a relationship exists between markers of type I collagen and measures of vascular structure, including central [1-5] and peripheral PWV [1, 3] and augmentation index [1, 4, 5]. The data from previous research has lead to conflicting outcomes. Specifically, McNulty et al. [4] and Ishikawa et al. [3] concluded that collagen type I degradation is increased with increasing arterial stiffness. Conversely, other studies have demonstrated decreased degradation with increasing arterial stiffness [1]. Alternatively, some literature suggests increased collagen synthesis is associated with increased arterial stiffness [3]. Despite the clear conflict in the literature with regards to the nature and direction of the relationships between collagen turnover and arterial stiffness, all of these studies suggest that an altered collagen environment exists during stiffening. The findings of the current study demonstrate that increased collagen turnover is associated with healthier arteries, suggesting that more type I collagen turnover exists in healthy arteries. Past literature has suggested that total collagen in the vessel wall decreases with age, however, until the age of 40-50 years, total collagen remains constant [9]. The current study, in conjunction with past literature, suggests that alterations in collagen subtypes, i.e. type I vs. type III may also be associated with vascular structure regardless of total collagen content.

7.3 Inflammation

Serum markers of inflammation have been shown to predict CVD [10]. Impaired flow-mediated dilation is potentially, in part, a result of inflammation and may be a mechanistic link in the established relationships between CVD and

inflammation [11] through a reduction of the bioavailability of endothelium-derived vasodilators. Specifically, cytokines can induce synthesis of endothelin-1, inducing vasoconstriction [11] and also increase the production of ROS, which will reduce bioavailability of endothelial NO, and thus endothelial dilation [11]. Production of ROS can also decrease eNOS expression and activity [12]. Endothelial dysfunction and elevated levels of pro-inflammatory cytokines have been previously observed in various CVD states, including congestive heart failure, atherosclerosis, diabetes and hypertension [13, 14] as well as with healthy aging [15]. Recent data suggests that there may be a weak relationship between circulating levels of inflammatory markers and FMD [15-17]. In healthy individuals Verma *et al.* found no relationship, however when they studied a subset of individuals with severe endothelial dysfunction and risk factors for CVD, a weak relationship was observed between CRP and FMD [18]. Similarly, Vita *et al*. reported a modest unadjusted correlation between CRP and IL-6 and FMD. However, this relationship disappeared after adjustment for traditional CVD risk factors [15]. Thus, CRP has emerged as one of the most important predictors of CVD, however, it seems that the association with endothelial function is weak, at least in healthy subjects. The current series of studies sought to investigate a different inflammatory marker with the potential to be linked to endothelial dysfunction, however, no associations were observed between IL-6 and FMD in our cohort of both clinical and healthy individuals spanning a large range of vascular health.

7.4 Endothelin-1

ET-1 was positively associated with endothelial dysfunction in the current study population across a wide range of vascular health. The underlying mechanisms responsible for this relationship are not entirely understood, however, a few hypotheses exist. It is suggested that ET-1 can decrease NO bioavailability in two ways by both decreasing NO production and increasing NO degradation. Specifically, endothelin-1 can decrease NO production through the interaction of eNOS with caveolin-1 [19]. Caveolae are cholesterol- and glycosphingolipid-rich membrane microdomains that function as mobile signaling platforms in the plasma membrane by coating the cytoplasmic surface of these specialized microdomains [19]. ET-1 causes an up-regulation of calveolin-1 expression, leading to a reduction in eNOS activity [19]. The opening of calcium-activated potassium-channels followed by membrane hyperpolarization and calcium-mediated activation of eNOS, stimulates the release of NO from the endothelium into the surrounding smooth muscle leading to vasodilation [20]. Elevated ET-1 also leads to an increase of ROS production, which thereby acts to decrease BH_4 bioavailability [21, 22]. BH_4 couples with eNOS in the pathway to release NO from the vascular smooth muscle. Decreased BH_4 bioavailability leads to uncoupling with eNOS and thus endothelial dysfunction. The first study of this thesis observed a negative relationship between endothelial function and circulating serum ET-1. The exact mechanistic pathway linking these measures

cannot be confirmed with the results of this study, however, measurement of resting serum levels of ET-1 may be indicative of overall vascular function.

7.5 Lifestyle Interventions

Obesity is identified as an independent risk factor for CVD [23]. Strong evidence exists suggesting aerobic exercise leads to improvements in vascular health, however the effects of resistance training and the combination of aerobic and resistance training on vascular health are less clear.

Central artery stiffness, assessed by arterial compliance, stiffness index and carotid-femoral PWV, is a strong predictor of CVD risk in healthy [24] and at risk individuals [25, 26]. Studies have shown a decrease in arterial stiffness following aerobic [27-31] and some combined aerobic and resistance training programs [32, 33], however the literature is less clear with regards to resistance training on its own [34, 35]. Specifically, aerobic exercise has been shown to increase central arterial compliance and decrease carotid-femoral PWV and augmentation index and thus the risk of CVD [27-31]. Yang et al. reported a decrease in brachial-ankle PWV in obese women (30-60 years) after three months of combined aerobic and resistance training [32]. Similarly, Figueroa et al. observed a decrease in brachial-ankle PWV following a 12-week moderate-intensity combined circuit of resistance and endurance exercise training [33]. Ho et al. observed a decrease in augmentation index after 12 weeks of combined aerobic and resistance training [36]. Contrary to these results, the results of Study 3 of this thesis indicated an increase in peripheral PWV. There are a few possible

explanations for these findings. First, none of the existing literature measured upper limb PWV or carotid artery distensibility after a combined aerobic and resistance training program. It is possible that improvements in arterial stiffness with combined aerobic and resistance training may be segment specific. Brachial-ankle PWV is an indication of whole body PWV, while $PWV_{c\text{-}r}$, as measured in Study 3 of this thesis is only representative of upper limb arterial stiffness. Central PWV estimates are expected to be dominated by the stiffness of the descending aorta, whereas carotid artery distensibility, as measured in Study 3 measures the inverse of stiffness in the carotid artery, which is also considered to be a central elastic artery. Secondly, the introduction of resistance training may have lead to increased stiffness in the peripheral segment measured. The existing literature demonstrates conflicting results with regards to the effects of resistance training on vascular structure. Some studies have observed an increase in both central and peripheral arterial stiffness [30, 34, 37], whereas others have seen no difference in artery stiffness, as measured by carotid artery compliance with resistance training [35].

Aerobic exercise training increases brachial FMD [38]. The literature is less clear with regards to resistance training and FMD. Kwon and colleagues observed no changes in FMD in individuals with type 2 diabetes following 12 weeks of resistance training [39]. Similarly, Rakobowchuk *et al.* observed no change in FMD in young healthy men following 12 weeks of whole body resistance training [40]. However, Cohen *et al.* saw improvements in endothelial

function with 14-months of a progressive resistance exercise program in obese individuals with type 2 diabetes [41]. In the current study, FMD was increased with 16-weeks of combined aerobic and resistance training in overweight and obese women. It is possible that in the current study and in the Cohen study, the larger decreases in body weight following the intervention were related to the observed improvements in endothelial function. In both studies the starting weights of the participants were much higher than those of the Kwon and Rakobowchuk studies, suggesting that the individuals in those studies may have reached a ceiling and had no room for further improvement, despite the form of exercise training. In addition, Phillips *et al.* observed a decrease in subclinical inflammation, as measured by TNF∝ and CRP, following 12-weeks of resistance training in obese, post-menopausal women in the absence of changes in body composition [42]. However, similar to the findings in the current thesis, they observed no change in circulating IL-6 with resistance training [42].

Additionally, Maeda *et al.* observed a decrease in plasma ET-1 following 8-weeks of aerobic training in healthy young [43] and old individuals [44] and following 8 weeks of resistance training in healthy young humans [45]. Conversely, 16-weeks of combined aerobic and resistance training in overweight women led to no changes in serum ET-1 in Study 2 of this thesis.

7.6 Future Directions

Further research is necessary to better understand the relationships identified in this thesis both before and after interventions designed to reduce

CVD risk. It is crucial to understand the specific relationships of these blood markers and functional measures within different populations. The studies in this thesis were designed to identify relationships across a large range of vascular health. Larger scale studies investigating each different population is necessary to better understand population specific relationships. It is possible these relationships become stronger or alternatively disappear in more clinical populations as compared to healthier individuals.

It is also important to further investigate the relationships of these potential mechanisms and their role in lifestyle interventions. Applying specific diet, exercise and pharmacological interventions targeting specific blood markers would provide information on effective strategies for decreasing CVD risk. Studies 2 and 3 of this thesis were designed to investigate the effects of a combined diet and exercise intervention on both the functional tests of vascular health and blood markers potentially associated with those tests. Further interventions designed with the intention of targeting these specific blood markers may lead to decreased CVD risk.

We are aware that collagen in the arterial wall exists in different subtypes. Studying the other subtypes of collagen existing in the arterial wall is necessary to advance the current state of knowledge. Changes in the ratio of type I and III collagen turnover may provide information on the structural changes occurring in stiffer or more elastic arteries. Type III collagen is thought to be associated with extensibility of the vessel wall, whereas changes in type I collagen may be

specifically associated with arterial stiffening [8]. Unfortunately, there is little known about the change in ratio of collagen subtypes in the arterial stiffening process and before and after interventions specifically targeting these structural proteins. Evidence exists suggesting that changes in the composition of the collagen subtypes occur in hypertensive rats with stiffer arteries [46, 47] but applying this research in human models is necessary.

7.7 Limitations

The series of studies included in this thesis had some limitations, which may have affected the observed results. Specifically, Study 1 was comprised of data pooled from a variety of different studies. Although, care was given to ensure that techniques were kept consistent between the studies, there were different ultrasound and tonometry technicians involved in each study potentially affecting the functional vascular tests. Despite the variation in ultrasound operators, the same person analyzed all of the blood samples, carotid distensibility, PWV and FMD involved in Study 1 of this thesis. The data collection and analyses involved in Study 1 of this thesis were completed over a three-year time period. Sometimes, in long-term studies, techniques change from the beginning to the end of a study and analysis techniques are developed over time. As such, extra effort was made to ensure consistent techniques and analyses were used in all data collection and analysis involved in this study.

Studies 2 and 3 of this thesis may have been limited by the small sample size. It is possible changes in the blood markers measured in both Study 2 and 3

may have been observed with a larger sample size. Additionally, there was no control group in this study, and therefore it is difficult to truly conclude that the results were due to the intervention. However, past research has demonstrated that combined aerobic and resistance training leads to various effects on vascular health [32, 33, 36, 48-51], suggesting the observed results are potentially due to the intervention. Finally, for all of the studies involved in this thesis, it is possible that the functional vascular tests in the clinical populations were compromised by the fact that data collection and analysis is difficult in overweight and clinical populations.

7.8 Conclusions

The series of studies contained in this thesis demonstrated that a relationship exists between various functional tests of vascular structure and function and blood markers of collagen and vasoconstriction in individuals with a range of vascular health. In a population of overweight women, 16-weeks of combined aerobic and resistance training and a hypocaloric diet, resulted in a mix of vascular results. Specifically, upper limb PWV was increased, carotid artery distensibility was unchanged and brachial artery FMD was increased with the intervention. Despite the results from Study 1, there were no associations observed between any of the measured blood markers and the functional vascular tests in Study 2 or 3. However, CTX increased after the 16-week intervention. The results of the studies involved in this thesis increase the comprehensive understanding of factors associated with the regulation of vascular structure and

function in a spectrum of populations spanning a large range of vascular health. The information provided by these studies may assist with focusing on direct targets, such as type I collagen turnover and vasoconstrictors to assist with lifestyle interventions set to decrease CVD risk.

7.9 References

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