

CAROTID ARTERY ELASTICITY:
Describing Elasticity in Healthy Children and Adults, and the Use of Atorvastatin
to Modulate Elasticity in Adult Survivors of Childhood Cancer

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CHAPTER 1. INTRODUCTION

Carotid artery elasticity has become a commonly assessed non-invasive marker of arterial health that is relatively easy to administer (Chirinos et al., 2012; Hoeks et al., 1990; Hoeks et al., 1999; Riley et al., 1992; Tardy et al., 1991) compared to other arterial health measures (Charakida et al., 2010; Corretti et al., 2002; Deanfield et al., 2005; Harris et al., 2010; Thijssen et al., 2011). Indeed, the loss of elastic properties within the larger and more elastic arteries like the carotid artery contributes substantially to the increase in systolic blood pressure as well as pulse pressure, which are known independent risk factors for the development of cardiovascular disease (Menotti et al., 1989; Safar et al., 2003). Such risk factors can lead to compensatory remodeling of the carotid artery and nearby arterial beds, as well as create synergistic detriments to the arterial tree in the presence of other risk independent risk factors (Benetos et al., 2002; Giannattasio et al., 1996; Humphrey, 2002; Lakatta, 2003; Laurent et al., 1993; Nichols et al., 1993; Nichols & O'Rourke, 1998).

Decreased carotid arterial elasticity and increased stiffness has been reported with advanced aging in both men and women (Arnett, Evans, & Riley, 1994; Mitchell et al., 2004). However, little research has examined artery elasticity measures in otherwise healthy adults with special focus on potential sex differences. Additionally, little research on arterial elasticity and stiffness has been conducted in children and adolescents. Specifically, while no studies have examined arterial elasticity throughout puberty, cardiovascular and metabolic changes throughout puberty indeed occur (Amiel et al., 1986; Bloch & Clemons, 1987; Caprio et al., 1994; Caprio & Tamborlane, 1994; Cook et al., 2003; Goran & Gower, 2001; Moran et al., 1999; Richards et al., 1992; Sinaiko et al.,

2001; Travers et al., 1995; Uwaifo et al., 2002) and may influence vascular structure, mechanics, and function. Specifically, increased insulin resistance associated with pubertal onset and subsequent recession following maturation (Moran et al., 1999) may influence arterial elasticity. Based on the available literature, the purpose of the following dissertation was to both perform a cross-sectional examination of the potential influence of sex, pubertal development, and age, on measures of carotid arterial elasticity in otherwise healthy children, adolescents, and adults, to lend additional insight to the already existing body of research surrounding arterial elasticity and stiffness.

Given vascular dysfunction is considered an early manifestation of atherosclerosis (Celermajer et al., 1992; Deanfield et al., 2007), potential therapeutic remedies that dampen cardiovascular risk in populations that are at increased risk for vascular dysfunction are critical to explore. Childhood cancer survivors are one such population at increased risk of cardiovascular disease compared to otherwise healthy individuals, with survivors having reduced carotid artery elasticity compared to healthy sibling controls (Dengel et al., 2014). Furthermore, HMG coenzyme A reductase inhibitors, or statins, are widely used for cardiovascular disease risk reduction (Stone et al., 2013). Specifically, atorvastatin has been shown to improve endothelial function independent of reducing cholesterol (Reriani et al., 2011), as well as reduce arterial stiffness and slow arterial thickening in multiple populations (Orr et al., 2009; Ratchford et al., 2011), yet the effects of atorvastatin have never been evaluated in childhood cancer survivors. Therefore, the therapeutic effect of atorvastatin on carotid arterial elasticity in adult survivors a childhood cancer was further evaluated within the present dissertation.

The specific study aims arising from this dissertation are as follows:

1. Evaluate the influence of pubertal development on carotid artery elasticity in healthy children and adolescents.
 - a. *Hypothesis 1:* Carotid arterial elasticity and stiffness will differ by pubertal stage in accordance with associated changes in cardiometabolic risk factors. Specifically, carotid elasticity will be lowest among Tanner stages II-IV compared to Tanner stages I and V.
2. Evaluate differences related to sex and age on carotid arterial elasticity in healthy children and adults. Also evaluate whether a relationship exists between brachial and carotid arterial elasticity in a subset of participants with both measures.
 - a. *Hypothesis 1:* Carotid arterial elasticity will differ by sex and differences will become more apparent with age. Additionally, an association between brachial and carotid arterial elasticity may exist.
3. Evaluate the ability of 6-months of atorvastatin treatment to improve carotid artery elasticity in a randomized trial of adult survivors of childhood cancer.
 - a. *Hypothesis 1:* Compared to placebo, atorvastatin will increase carotid arterial elasticity and reduce carotid stiffness in adult survivors of childhood cancer.

The second chapter of this dissertation will provide an extensive review of the existing literature on arterial elasticity. Arterial wall properties related to vessel elasticity and stiffness, and methodologies commonly used to quantify carotid artery elasticity and stiffness will be described. Additionally, risk factors associated with carotid arterial elasticity and stiffness will also be discussed.

The third chapter will examine the influence of pubertal development on carotid arterial elasticity in a healthy sample of children and adolescents. Insulin resistance is commonly associated with pubertal onset in childhood and adolescents, with insulin levels returning to near pre-pubertal status following maturation. Moreover, the potential impact of insulin resistance on vascular health is evaluated.

The influence of sex on carotid arterial elasticity in healthy children and adults, and the potential relationship to brachial arterial elasticity, will be examined in chapter four. Few studies have examined differences between the sexes in both children and adults, and no studies have examined the relationship between brachial and carotid arterial elasticity and stiffness. Indeed, systemic versus regional differences in arterial elasticity is important to examine.

The fifth chapter will evaluate whether 6-months of atorvastatin therapy, compared to placebo, can elicit improvements in carotid artery elasticity and stiffness in a population of young adult survivors of childhood cancer. Atorvastatin has been shown to improve endothelial function independent of reducing cholesterol, as well as reduce arterial stiffness and slow arterial thickening in multiple populations, yet has never been studied in childhood cancer survivors. Given childhood cancer survivors are at higher risk for cardiovascular disease compared to an otherwise healthy population, safe and effective methods of improving vascular healthy are explored here.

And finally, a summary of each study and pertinent observations will be reviewed in chapter six. Future research surrounding arterial elasticity will be proposed.

CHAPTER 2. REVIEW OF LITERATURE

Introduction

Over the last several decades, there has been great interest in the early detection of and progression to cardiovascular disease (CVD) in order to prevent future cardiovascular events. Consequently, non-invasive assessment of vascular health is currently employed to assess arterial wall properties to provide a preclinical marker of cardiovascular risk and subclinical atherosclerosis (Barenbrock et al., 2002; Blacher et al., 1998; Laurent et al., 2001; Leone et al., 2008). Indeed, peripheral vascular function as commonly assessed by brachial artery reactivity testing via flow-mediated dilation (FMD) correlates well with coronary artery endothelial function (Anderson et al., 1995; Takase et al., 1998) and quantifies the functional capacity of the vascular endothelium; however, while the principle of FMD appears simple, its application is technically challenging and requires extensive training and standardization (Charakida et al., 2010; Corretti et al., 2002; Deanfield et al., 2005; Harris et al., 2010; Thijssen et al., 2011). Conversely, non-invasive imaging of the common carotid artery is often easier to administer and can lend to the identification of central vascular dysfunction and therefore improve risk stratification (Flammer et al., 2012). Besides being used to diagnose atherosclerotic lesions, imaging of the carotid artery is commonly used to assess intima-media thickness (IMT). Certainly, carotid IMT is a parameter used in epidemiological (Bots et al., 1997; O'Leary et al., 1990) and intervention studies (Boutouyrie et al., 2000) as an indicator of atherosclerotic disease (Simon et al., 2002).

More recently, the focus of non-invasive imaging of the carotid artery has been on the assessment of arterial wall properties related to elasticity and stiffness, specifically

vessel distension, circumferential strain, compliance, distensibility, as well as Young's modulus via conventional ultrasound imaging (Chirinos et al., 2012; Hoeks et al., 1990; Hoeks et al., 1999; Riley et al., 1992; Tardy et al., 1991). Other techniques have been further employed to quantify relative degrees of arterial stiffness, such as pulse wave velocity and aortic pulse wave analysis (Nichols, 2005; O'Rourke et al., 2001). Understanding these dynamic parameters is important because the loss of elastic properties within larger elastic arteries like the carotid artery, as in aging (Reneman et al., 1985; Reneman et al., 1986) and in borderline (Van Merode et al., 1988; Van Merode et al., 1993) and essential hypertension (Laurent et al., 1994; Laurent, 1995), contributes substantially to the increase in systolic blood pressure as well as pulse pressure, which are known independent risk factors (Menotti et al., 1989; Safar et al., 2003). Indeed, arterial stiffness also increases with other disease states, such as diabetes mellitus, obesity, smoking, hypercholesterolemia, and kidney disease (Payne et al., 2010).

The following literature review will describe arterial wall properties related to vessel elasticity and stiffness, and methodologies commonly used to quantify carotid artery elasticity and stiffness. Risk factors associated with carotid arterial elasticity and stiffness will be discussed. Furthermore, this review will identify the need for extended carotid artery elasticity and stiffness research in children and adult populations, as well as present a case in which individuals at higher risk for CVD mortality, specifically childhood cancer survivors, may be ideal candidates for treatment specific therapy that improves structural and mechanical properties of elastic arteries like the carotid artery.

Arterial Wall Properties Related to Elasticity and Stiffness

The Windkessel model of the arterial system is the oldest arterial model to date (Westerhoff et al., 2009). The concept arose from old-fashioned fire engines that transformed pulsatile flow from a steam or hand-activated pump into a steady water stream through the fire hose nozzle. In such a model, the origin of the flow represents the cushioning function of the arteries, particularly the aorta that acts as an elastic reservoir, and the nozzle represented peripheral resistance (Nichols & O'Rourke, 1998). Indeed, the Windkessel effect of an elastic aorta helps to dampen fluctuations in blood pressure (or pulse pressure) over the cardiac cycle, keeping pressure relatively constant despite the pulsatile nature of blood flow, and therefore assists in the maintenance of organ perfusion during diastole when cardiac ejection ceases. Although conceptually useful, the Windkessel model is unrealistic given elastic properties are distributed all along the arterial tree and not just confined to the aorta. Physical properties of arteries are different as well, with different arterial sites responding differently to aging, hypertension, and drugs (Bank & Kaiser, 1998; Boutouyrie et al., 1992). Therefore, the mechanical and arterial properties of the arterial system need to be understood in order to fully comprehend measures of arterial elasticity and stiffness at different arterial sites.

The Stress-Strain Relationship of the Arterial System

The major elastic arteries of the body exhibit a stress-strain relationship that allows them to react to blood flow demands and resist overstretching. The stress-strain relationship of the arterial system (synonymously referred to as the pressure-strain,

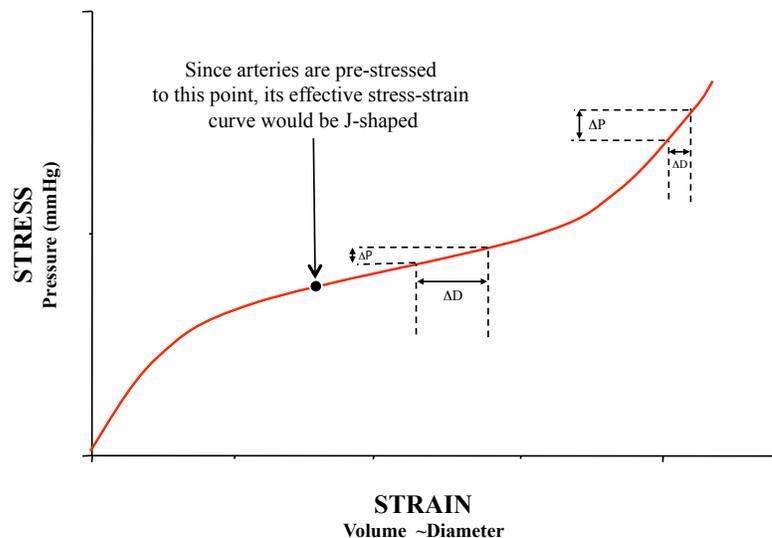
pressure-volume, or pressure-diameter relationship), details the stretch capacity of elastic arteries such as the common carotid artery. Indeed, mechanical forces and the resulting induced deformations on particular materials have been extensively studied for over three centuries. The classic physics of elasticity is said to have started in 1600 with the discovery by Robert Hooke who stated relationships between mechanical stress and strain in isotropic materials, within their elastic limit, are constant. In physics, stress is the internal distribution of forces within a body that balance and react to the external loads applied to it, while strain is defined as the mechanical (fractional) deformation of a physical body under the action of applied forces or deforming stress. The standard definition of Robert Hooke's law implies that bodily exposures to these stresses exhibit a linear-elastic relationship, or "Hookean."

Indeed, the stress-strain relationship is a critical component of arterial elasticity and stiffness and is thus essential to the understanding of pathophysiological mechanisms in the vascular bed. Within an arterial vessel, stress is the intensity of the force applied across a plane (or arterial wall) expressed as units of force per unit area. Hemodynamic conditions inside the blood vessel lead to the development of superficial stresses near the vessel walls. Specifically, three distinct stressors exist within blood vessels: (1) circumferential (direct) stress due to the pulse pressure (PP) variation inside the vessel itself, (2) shear (indirect) stress applied to the vessel wall due to the increase in blood flow through the vessel following ventricular contraction, and (3) axial wall stress that arises during development and persists into maturity due to the long half-life of elastin (Dobrin et al., 1990; Humphrey, 2008; Papaioannou et al., 2005). Conversely, with

respect to an arterial vessel, strain is the relative increase in vessel volume (or the more easily measured diameter change) produced by that force.

Given the diverse molecular and mechanical composition of blood vessels, however, arterial walls are *not* Hookean (Nichols & O'Rourke, 2005). Interestingly, only a few biomaterials truly approximate Hookean behavior, like wood and skeletal bone. Instead, arteries are naturally pre-stressed due to previously noted axial wall stress and therefore even the minimum blood pressure must be sufficiently positive to raise blood to the highest point of the body. The somewhat neutral position of a healthy artery demonstrates the presence of constant low tension. As a result, the stress-strain relationship is usually represented by a continually upward sloping J-shaped curve (Fig. 1). Initially, small increases in stress (or pressure) produce large relative changes in diameter; however, an elastic artery becomes stiffer and more difficult to distend at larger strain levels and thus requires larger stresses to elicit larger strains (Chirinos et al., 2012).

Figure 1. Stress-Strain Relationship of an Elastic Artery



*Adapted from the University of Cambridge DoITPoMS
(Online Tutorial titled 'Elasticity in Biological Materials')*

Stress loading and unloading occurs along the same curve and thus, the loading is completely reversible in a healthy artery with little to no vascular remodeling. A healthy elastic artery maintains that all energy used in dilatation is returned once the load is removed to ensure smooth variations in blood pressure and blood flow. Moreover, normal blood pressure, and subsequently PP, and shear stress are transferred to the heterogeneous arterial wall layers (i.e., intima, media, and adventitia) and regulate blood vessel diameter depending on the elasticity and physiologic function of the individual vessels. Of course, the proportions of these arterial wall layers differ along the arterial tree.

Differences Along the Arterial Tree

Wall Properties

Findings over the past few decades have revealed the larger (elastic) arteries emerging from the heart (i.e. aorta, pulmonary artery, brachicephalic trunk, common carotid artery, and subclavian) have different vessel wall structure compared to the more distal (muscular) branching arteries like the brachial, radial, and femoral arteries, and thus respond markedly different to altered stimuli. Indeed, the proportional layering of wall properties such as smooth muscle, elastin, collagen, and fibroblasts is critical to the homeostasis of the vascular wall and thus, the arterial proportions of these properties along the arterial tree is important to consider when assessing vascular function and subsequently comparing one arterial bed to another.

While the more central (elastic) arteries contain more elastin and less smooth muscle, collagen, and connective tissue components, peripheral arteries tend to contain

just the opposite. Additionally with age, the amount of elastic fibers decreases while collagen increases throughout the arterial tree. Amongst the many effects of aging on arteries, including increased endothelial dysfunction and advanced glycation endproducts (Lakatta et al., 2009; Safar, 2010), it appears that fatigue-type damage to elastic fibers is particularly important (Arribas et al., 2006; O'Rourke & Hashimoto, 2007). Still, the exact pathophysiological and temporality of arterial stiffening is not fully understood (Tsamis & Stergiopoulos, 2007).

Differential remodeling can even occur within vessels of the same general classification and within close proximity to one another. In response to hypertension, all arteries and arterioles tend to thicken, yet the elastic arteries, muscular arteries, and arterioles tend to do so while increasing, maintaining, and decreasing their caliber (or luminal diameter), respectively (Humphrey, 2002). Sparring of the peripheral arteries has been reported previously (Benetos et al., 1993; Gillessen et al., 1995). Specific to the larger, more elastic arteries, an increase in diameter may be due to compensation for decreases in aortic elasticity (Benetos et al., 1993; Reneman et al., 1985) and attempts of the more elastic vessels to maintain relatively low impedance while maintaining pressure and stroke volume. Furthermore, dilatation of the aorta or common carotid artery is often accompanied by geometric changes in the left ventricle indicating the presence of cardiovascular coupling (Boutouyrie et al., 1995).

The ascending aorta is suggested as being the first central artery to manifest aging related structural changes that affect overall mechanical properties (Redheuil et al., 2010), and has thus surrogate measures of aortic function are valuable indicators of

arterial health. Indeed, both the aorta and common carotid artery are close in proximity and demonstrate similar remodeling techniques compared to the more peripheral arteries (Boutouyrie et al., 1992). Interestingly, there exists the possibility that increased susceptibility of the ascending aorta to an aging related losses of elastic fiber integrity may explain in part the increased in susceptibility of the same region to dilatation and dissection in Marfan syndrome (Pearson et al., 2008), which results from a genetic mutation in the fibrillin-1 gene. While fibrillin-1 appears to help stabilize elastic fibers, mutations result in decreased elastic fiber integrity.

Hemodynamic Stress

The pulse pressure wave following ventricular contraction also changes shape within the different vascular beds. Ventricular contraction creates both arterial blood flow and a pressure wave that propagates a pulse along the arterial tree. Not to be confused with blood velocity, ‘forward moving’ pulse pressure waves relate to the transmission of energy through the arterial wall. Indeed, the speed of pulse wave transmission largely exceeds blood velocity in the aorta to minimize arterial damage due to longitudinal shock waves and turbulent flow if blood flow velocity exceeded pulse wave velocity (O’Rourke, 2006). As the pulse pressure wave form moves distally along the arterial tree, peripheral resistance increases as a result of reduced vessel diameter towards arteries that are comprised of less elastic fibers and, subsequently, an amplification in PP occurring and directly changes the shape of the forward moving pressure wave. Indeed, systolic pressure rises and diastolic pressure falls (thus, increase in PP) as the pressure pulse

travels away from the aorta (O'Rourke et al., 2001).

Conversely, 'reflective' waves, or the forward pulse pressure wave reflections, can be initiated at multiple sites along the arterial tree because of changes in vessel wall properties (e.g. elasticity, stiffness, vasomotor tone) or in the architecture of the artery itself (e.g., branching points, calcifications). In young subjects, the backward (reflective) pressure wave returns from the distal arterial compartment during diastole, making PP higher in peripheral than in central arteries (Nichols & O'Rourke, 1998). The resulting combination of both the forward and reflective waves ('augmented' wave) indeed represents a surrogate aortic measure of structure and mechanics of the vessel wall.

Arterial Elasticity

Given the increased understanding of the distinct wall properties and pressure differences along the arterial tree, arterial elasticity has become an increasing focal point in the past decade and is associated with CVD (Fjeldstad et al., 2008; Havlik et al., 2003). Elasticity, by definition, is the ability of a body or material to return to its previous shape after a deforming force or stress is released. Three common measures of arterial elasticity are compliance, distensibility, and elastic modulus.

Compliance, Distensibility, and Elastic Modulus

Compliance, by definition, is the absolute change in volume (ΔV , strain) induced by a change in pressure (ΔP , stress), or the absolute change in arterial volume that reflects the arterial ability to store volume and thus reducing increases in pressure during left

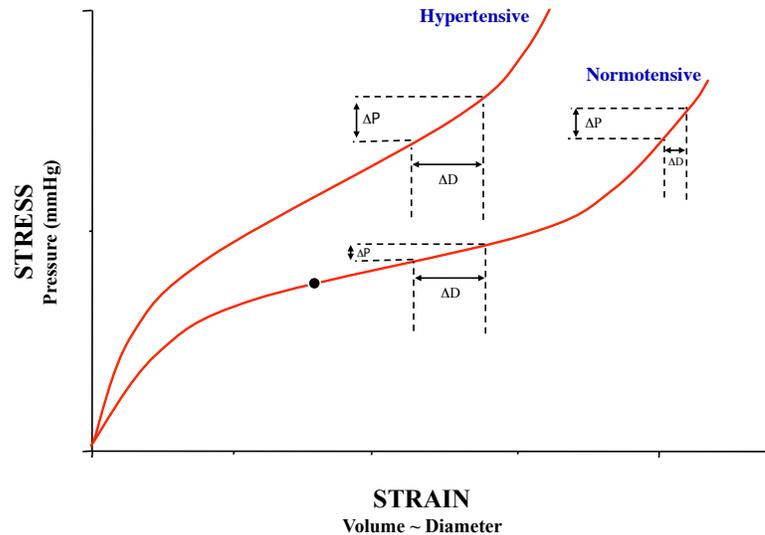
ventricular ejection (Jani & Rajkumar, 2006; Reneman et al., 2005). Given arterial length does not change substantially due to longitudinally tethering of arteries at their *in vivo* length (L'Italien et al., 1994; Patel & Fry, 1966), an artery should distend via increases in diameter with increases in intra-arterial pressure. Since arterial volume is difficult to accurately measure *in vivo*, a change in arterial volume is often substituted for changes in diameter (ΔD) or vessel cross-sectional area. Together with systemic vascular resistance, compliance is considered the major determinant of afterload (Levy et al., 1990).

Although compliance is influenced by the material properties of the arterial wall layers (Bank et al., 1996; Farrar et al., 1978; Roach & Burton, 1957), compliance is not a direct index of wall stiffness because the measure is affected by arterial size and wall thickness. As the law of Laplace states, circumferential wall stress is directly proportional to the vessel radius and the luminal pressure and inversely proportional to the wall thickness and, thus, increased luminal diameter or decreased wall thickness is proportional to circumferential wall stress and luminal pressure. Therefore, variability between arteries (and individuals) via arterial size and wall thickness need to be accounted for because of the influence pressure-strain relations, pressure-volume, and pressure-diameter relationships for any given stiffness of the wall material. Indeed, arteries with thicker walls will have lower compliance than those with thinner walls, provided identical elastic properties of the wall material. Similarly, larger arteries will have larger compliance for identical wall material properties and relative geometry (wall thickness-to-wall ratio) than smaller arteries. As a result, compliance should be normalized for initial arterial volume when comparing arteries of a different size.

Distensibility incorporates the relative change in arterial volume ($\Delta V/V$) against the change in pressure (ΔP), or the mechanical load or stress placed on the arterial wall (Reneman et al., 2005), and thus reflects normalized compliance. Distensibility tends to relate more closely to wall stiffness due to its relativeness to initial volume (V).

Another measure of arterial elasticity is an incremental elastic modulus (E_{inc}), or synonymously referred to as Young's modulus. Because the stress-strain relationship of an elastic artery is a non-linear J-shaped curve, the tangential slope of the curve provides direct information on the intrinsic elastic properties of the arterial wall independent of vessel geometry. Whereas small PP increases initially produce a larger relative increase in vessel diameter in a healthy elastic artery, the non-linear J-shaped stress-strain curve tends to shift up and left in an adapted hypertensive state (Fig. 2); thus, a greater tangential slope demonstrates that a greater PP is necessary to produce the same relative change in vessel diameter – the hallmark of a stiffer artery (Tsamis & Stergiopulos, 2007). The elastic modulus is inversely proportional to cross-sectional area as it relates to ΔP . Indeed, a more elastic vessel will lend to a decreased tangential slope.

Figure 2. Effect of Hypertension on the Stress-Strain Relationship



*Adapted from the University of Cambridge DoITPoMS
(Online Tutorial titled 'Elasticity in Biological Materials')
with reference to Tsamis & Stergiopoulos (2007)*

Non-Invasive Ultrasound Imaging of Arterial Elasticity

Two critical measures are required to compute arterial elasticity. First, the change in arterial diameter (ΔD), or the surrogate measure of arterial volume, is required. And second, local blood pressure at the assessed artery is needed to calculate ΔP (or PP). Changes in arterial diameter (distension) can be assessed via conventional ultrasound imaging and wall-tracking techniques, tissue Doppler imaging, or magnetic resonance imaging (MRI) (Herment et al., 2010; Meinders & Hoeks, 2004; Redheuil et al., 2010; Vermeersch et al., 2008). While MRI imaging is particularly useful to assess the distension of deeper arteries (i.e. aorta), conventional ultrasound imaging technology is often used in subclinical populations as a well-accepted non-invasive measure. Blood pressure, and subsequently PP, is also required to compute measures of arterial elasticity. Specifically, ΔP should be determined at the same site at which arterial elasticity

measurements are performed.

Standard practice of collecting carotid artery images is to concurrently measure carotid artery diameter as well as supine systolic and diastolic blood pressure and PP with subjects in the supine position following a bout of quiet rest. Over a 10-sec period, 20 frames per second are collected (200 frames) at a fixed position approximately 1 cm proximal from the carotid artery bulb. Mean luminal systolic and diastolic diameters, as well as a concurrent brachial artery blood pressure, are recorded during the 10-sec carotid assessment and then used to calculate measures of carotid compliance and distensibility. Due to variability of PP amplification between the central carotid artery and the brachial artery, brachial PP is not equivalent to carotid, aortic, or radial PP (or PP in any other distant artery) (Nichols & O'Rourke, 2005). Indeed, using brachial PP as a surrogate measure of carotid PP is a distinct limitation; however, it is common limitation among non-invasive assessment of carotid elasticity.

Within the context of the following dissertation, we will be referring to the following formulas for the quantification of arterial elasticity parameters:

Table 1. Measures of Arterial Elasticity		
<i>Measurements</i>	<i>Formula</i>	<i>Comments</i>
Diameter Compliance (DC, mm/mmHg)	$\Delta D / \Delta P$	↑DC ~ ↑Elasticity
Cross-Sectional Compliance 1 (CSC1, mm ² /mmHg)	$[(\pi*(D_{max}/2)^2 - \pi*(D_{min}/2)^2)/(\Delta P)]$	↑CSC1 ~ ↑Elasticity
Cross-Sectional Compliance 2 (CSC2, 1/mmHg)	$[(\pi*(D_{max}/2)^2 - \pi*(D_{min}/2)^2)/(\pi*(D_{min}/2)^2*\Delta P)]$	↑CSC2 ~ ↑Elasticity
Diameter Distensibility (DD, %)	$[\Delta D / D_{min}] \times 100\%$	↑DD ~ ↑Elasticity
Cross-Sectional Distensibility (CSD, %)	$[(\pi*(D_{max}/2)^2 - \pi*(D_{min}/2)^2)/\pi*(D_{min}/2)^2] \times 100\%$	↑CSD ~ ↑Elasticity

Incremental Elastic Modulus (IEM or E, mmHg)	$\frac{3 \{ 1 + [\pi^*(\maxDiamM/2)^2 / \pi^*(\minDiamM/2)^2] \}}{CSC1}$	↑IEM ~ ↓Elasticity
--	--	-----------------------

ΔD, change/difference in arterial diameter at systolic and diastolic pressures; ΔP, difference between systolic and diastolic pressures; Dmax, maximum diameter; Dmin, minimum diameter.

While distensibility is the relative change in diameter ($\Delta D/D$) relative to the ΔP during the cardiac cycle, the following dissertation describes diameter distensibility as the relative change in diameter without the influence of ΔP , and thus describes simply the ‘distention’ of the artery. Additionally, the variations in elasticity calculations should be carefully considered when comparing the results of different studies.

Arterial Stiffness

Arterial stiffness, another measure of vascular wall mechanics, directly impacts the ability of the arteries to accommodate the blood ejected by the ventricles without imposing an excessive afterload (Chirinos & Segers, 2010a; Chirinos & Segers, 2010b; Nichols & O’Rourke, 2005; Segers & Verdonck, 2002). Stiffness is resistance offered by an elastic body to deformation, and can be measured with several methods depending on the clinical use or experimental situation. In clinical practice, arterial stiffness can be non-invasively estimated by three principal methodologies: (1) pulse transit time for evaluation of the pulse wave velocity, (2) the analysis of the arterial pressure wave contour, and (3) the direct stiffness estimation using measurements of diameter or arterial luminal cross-sectional area and distending pressure measured at the site of diameter changes, as previously described as arterial elasticity (Karamanoglu et al., 1993; Laurent & Boutouyrie, 2006; Van Bortel & Segers, 2006). Indeed, the two most frequently used

stiffness methods are measurement of pulse wave velocity (PWV) and central (aortic or common carotid artery) pulse wave analysis.

Pulse Wave Velocity

Pulse wave velocity is often regarded as the *in vivo* “gold standard” index of arterial stiffness (Laurent et al., 2006). Due to its independence of both the stress-strain relationship and wave reflection, PWV is also considered a very robust index of stiffness (Asmar, 2000; Bramwell & Hill, 1922; Nichols & O’Rourke, 1998) yet is strongly dependent on current blood pressure and heart rate. As PWV increases from the aorta towards the peripheral arteries, this rapidly propagated pressure gradient moves distally along the arterial tree resulting in almost immediate downstream mobilization of blood in the arterial system (Vlachopoulos et al., 2010), and therefore is a functional parameter directly affected by arterial wall stiffness, as well as other factors including geometry (i.e. thickness h , radius r), the intrinsic elastic properties of the arterial wall (i.e. incremental elastic modulus, E), and blood density (ρ). The first relationship was formulated by Moens and Korteweg that $PWV = \sqrt{Eh/2r\rho}$ (O’Rourke, 2006). Later, Bramwell and Hill described the association in terms of distensibility (D), which is determined by the blood vessel’s compliance (C): $PWV = 1/\sqrt{\rho DC}$, and moreover that higher PWV corresponds to lower vessel distensibility and compliance, and therefore to a higher arterial stiffness.

The forward traveling pulse wave is commonly assessed between two recorded (paired) sites in the line of pulse travel, and the delay between the ventricular ejection and initial upstroke of the pulse wave at each site is the point of reference for PWV

quantification (Asmar, 2000). While PWV can be measured at the level of peripheral arteries, carotid-femoral ('aortic') PWV is the most relevant because the aorta is the principal 'cushioning' artery and is responsible for the pathophysiological effects of arterial stiffening and its association with cardiovascular morbidity and mortality in hypertensive patients, type 2 diabetes, end-stage renal disease and in elderly populations (Hansen et al., 2006; Kullo et al., 2006; Laurent et al., 2006; Pannier et al., 2005; Tillin et al., 2007). Yet while aortic PWV is considered the gold standard of the paired sites (Laurent et al., 2006), femoral measurement can be tough to administer due to the physical location and ability to generate a confident pulse wave index. As such, carotid-radial techniques are often employed in substitution (as in the proposed dissertation) albeit usual limitations (Salvi, 2012).

Aortic Pulse Wave Analysis (PWA)

Recent studies have shown that central aortic blood pressure, the pressure exerted on the heart, brain, and kidneys, is a better predictor of CV risk than brachial blood pressure (Chirinos et al., 2005). Consequently, analysis of aortic waveform and amplitude, or aortic pulse wave analysis, is another frequently used method to assess large artery properties and eventually arterial stiffness. As a pressure wave moves through the arterial tree, it encounters impedance resulting in a reflected wave that moves back toward the heart, and thus augments (i.e. amplifies) peak systolic pressure. The amplification of the PP is due to greater stiffness of peripheral arteries and enhanced wave reflection amplitude, which depends on the difference between elastic moduli of the

respective arteries and distance to major reflecting sites (Nichols & O'Rourke, 2005). As a result, these two pulsatile blood pressure components are greater in arteries of the extremities than in the central aorta. As stiffness increases, reflected wave amplitude increases and augments pressure in late systole, or in worse cases early systole, producing an increase in afterload and myocardial oxygen demand. The term augmentation index (AI) has been coined to showcase the relationship in aortic amplitude.

Applanation Tonometry Assessment of PWV and Aortic PWA

Recently, several technologies have been proposed to record non-invasive pressure waveforms and apply signal processing to derive arterial wave contour parameters. One technique is the reliable recording of pressure waves by arterial applanation tonometry. Indeed, applanation tonometry has been widely validated and allows for the estimation of systolic and diastolic blood pressures, of form factor (an indicator of pulse wave shape), and of the AI, an index of all systemic reflection waves at the site of assessment.

The forward traveling pulse wave is commonly assessed between two recorded sites in the line of pulse travel, and the delay between the R-wave and initial upstroke of the pulse wave at each site is the point of reference for PWV quantification (Asmar, 2000). Subsequently, the pressure gradient transit time (t) and distance (D) as measured over the body surface between paired pulse wave sites is determined. Paired comparisons of the forward traveling pulse wave are assessed to provide a D/t relationship at the carotid-femoral, carotid-radial, or (less so) at the carotid-ankle sites (Laurent et al., 2006;

Sugawara et al., 2005; Tillin et al., 2007). Regardless of paired site, subtracting the proximal D and t site measurements from distal measurements, respectively, and then generating the composite PWV for the paired sites can be used to calculate PWV.

Conversely, aortic pulse wave analysis can be quantified at both the carotid and radial artery sites, resulting in separate aortic AI measures at each site, via the transmitted pulse wave at the relevant site. Given mean arterial pressure is relatively constant throughout the arterial tree, applanation tonometry uses brachial blood pressure to calibrate and non-invasively estimate ascending aortic pressure wave forms from both the carotid and radial arteries via a patented transfer function (Karamanoglu et al., 1993). The ascending aortic waveforms are then averaged into a single calibrated wave whose different features can be identified automatically with clinically important measures of pressure and time intervals. Indeed, aortic AI estimation at the radial site is known to be a more reliable measure than AI assessment at the carotid artery (Kelly et al., 1990). Apart from some uncertainty of calibrating carotid artery blood pressure from mean pressure via the brachial pressures, carotid tonometry requires considerable expertise due to the artery being deep in the neck. Additionally, the carotid artery is not easily applanated due to frequent respiration and swallowing artifact given the technique is often uncomfortable (O'Rourke & Adji, 2010). Conversely, radial applanation is easier to administer given the superficial nature of the radial artery as well as the bones and tendons that lay posterior that aid in the applanation of the artery.

Calculated from baseline resting pulse waves, site-specific AI values represent the relative contribution of augmented pressure due to wave reflection to the pressure

waveform. Proprietary software automatically identifies inflection points distinguishing the systolic peak (P1) and the reflected peak (P2) for the calculation of this ratio and converts it into a percentage $(P1-P2/P1*100)$. Because AI is inversely related to heart rate, values are sometimes mathematically adjusted to represent arterial stiffness at a standard heart rate of 75 beats per minute (AI@75); however, standard unadjusted AI is often sufficient when heart rate is not different across or between multiple measurements. As such, AI and PWV are not interchangeable indices as determined by cross-sectional and longitudinal studies (Cecelja et al., 2009; Cecelja et al., 2012). AI depends not only on arterial stiffness per se (reflected by PWV) but also on other parameters, such as the pattern of ventricular ejection, the vascular tone, or the absolute systole duration (Lantelme et al., 2002).

Limitations of Arterial Elasticity and Stiffness Assessment

There are indeed limitations to using non-invasive assessment for quantifying arterial elasticity. First, using changes in vessel diameter (and not changes in volume) when calculating elasticity assumes that vascular distention is due to radius changes and not arterial lengthening, as well as that the vascular cross section is perfectly circular. Second, brachial PP is not necessarily equivalent to carotid, aortic, or radial PP, and thus using brachial PP as a surrogate measure of carotid PP is not ideal. Third, there are indeed intrinsic problems with characterizing arterial elasticity due to the complexity of the arterial system, including the non-linear nature of an artery and the gradual shift in wall parameters that make identifying the same physiologic point in all study participants

rather difficult. Thus, measurement of arterial properties at one segment of the arterial tree does not necessarily assist in the interpretation of changes at other points (Giannattasio et al., 1996; Nichols & O'Rourke, 1998). Additionally, characterizing patients with distended or dilated aortas (as in Marfan syndrome), or even patients whose central arteries have dilated via compensatory remodeling, may have greater compliance relative to a much lower distensibility (Nichols & O'Rourke, 1998). And finally, while not necessarily a limitation per se, differing formulas are used throughout the literature when calculating measures of elasticity, making direct comparisons across studies more difficult. Indeed, while non-invasive assessment showcases these limitations, such non-invasive assessments are critical for early detection of vascular dysfunction whilst more invasive techniques are more cumbersome and indeed more expensive.

Several limitations exist when quantifying vessel stiffness as well. One obvious limitation of PWV is the determination of actual arterial distance between recording sites via measurements above the skin surface. Another PWV limitation, specific to carotid-radial quantification, is the fact that the carotid and radial arteries are not in the direct line from the aorta and thus incorporate more diversified vascular properties (O'Rourke & Mancia, 1999; O'Rourke et al., 2002). Subsequently, using brachial artery blood pressure to calibrate radial and carotid pulse waves for quantification of augmentation index is also not ideal (Smulyan et al., 2003). Use of a generalized transfer function assumes that properties of the arterial system between the two sites are the same in all persons under all conditions (O'Rourke et al, 2001). Indeed, the output error (at the aorta) is associated with the input errors at the radial artery attributable to both the under- or over-estimation

of actual (intraarterial) systolic and diastolic pressures, depending on which noninvasive method is applied (Smulyan et al., 2008), as well as the presence of brachial-to-radial pressure amplification (Nichols & O'Rourke, 2005; Verbeke et al., 2005).

Risk Factors Associated with Arterial Elasticity and Stiffness

Despite the large number of studies indicating that elevated levels of cardiovascular risk factors are related to decreased vessel elasticity and increased stiffness, the pathophysiological and temporal mechanism of stiffening of the arteries is not fully understood. There are several risk factors, specifically age and hypertension (Benetos et al., 2002; Cecelja & Chowienczyk, 2009), which are more confidently associated with arterial elasticity and stiffness.

Age

Increasing age is associated with worsening arterial elasticity properties in both men and women, and may be an independent cardiovascular risk factor (Benetos et al., 1997; Franklin et al., 1999; Laurent et al., 2001; Madhavan et al., 1994; Smulyan et al., 2001). While some research suggests a linear relationship between arterial stiffness and age (Avolio et al., 1983; Avolio et al., 1985), others have found accelerated stiffening between 50 and 60 years of age (Benetos et al., 2002; Franklin et al., 1997; Lajemi et al., 2001; McEniery et al., 2005). Indeed, the effects of aging are different on proximal, predominantly elastic arteries compared with distal, predominantly muscular arteries (Avolio et al., 1985; Benetos et al., 1993; Nichols & O'Rourke, 1990; Nichols et al.,

2008), which may lead to differences in age-related effects. More central arteries like the aorta and common carotid arteries stiffen progressively with age, whereas stiffness of muscular arteries changes little with age (Benetos et al., 1993; Mitchell et al., 2004; O'Rourke et al., 2002; Reneman et al., 1985). Additionally, larger artery compliance likely decreases due to both a pronounced decrease in arterial distensibility and with an increase in diameter (Benetos et al., 1993; Reneman et al., 1985). Again, increases in diameter with aging (9% increase in central artery luminal diameter per decade from 20 to 60 years in the ascending aorta) might be due to compensation for the decrease in elasticity in order to limit a decrease in compliance (Lakatta, 2003). More distal arteries like the radial and femoral artery in otherwise healthy subjects have been reported to not decrease in elasticity with age, possibly due to compensatory loss of elasticity in the more elastic arteries (Laurent et al., 1993).

As previously discussed, the aorta and common carotid arteries are also subject to age-related structural remodeling in both the tunica media and intima layers. Within the media specifically, fracturing of elastin and increases in collagen and calcium deposits occur with age likely as the result of repeated cyclic stress (Johnson et al., 2001; O'Rourke & Hashimoto, 2007). Predominant changes within the intima include increase in intima-media thickness and prevalence of atherosclerosis. Interestingly, histological examination of the intima of stiffened vessels reveals abnormal and disarrayed endothelial cells, increased collagen, frayed and broken elastin molecules, infiltration of vascular smooth muscle cells, macrophages and mononuclear cells, and increased matrix metalloproteinases, transforming growth factor (TGF)- β , intracellular cell adhesion

molecules, and cytokines (Lakatta, 2003). Remodeling of both the intima and media layers result in decreased elasticity and increases in stiffness via PWV (Avolio et al., 1983; Cecelja & Chowienczyk, 2012).

Interestingly, infants have remarkably similar arterial pulse wave contours (and amplitudes) as the elderly. Specifically, amplitude in both central and peripheral arteries is low with no secondary wave in diastole and the peak wave is seen in late rather than early systole (Hsieh et al., 1989; Nichols & O'Rourke, 1998; O'Rourke et al., 1992). With progression to adolescence, however, peripheral pulse amplifies compared to central pulse, with the peak moving into early systole with the secondary diastolic wave apparent in early diastole, and is accounted for by the marked increase in peripheral PP and systolic pressure during adolescence (Uiterwaal et al., 1997), as well as the relatively long left ventricular ejection period despite small body size and faster heart rate (Hsieh et al., 1989; Nichols & O'Rourke, 1998). The short body length in infants, despite the aorta being very distensible and having relatively low PWV, showcases a similar amplitude and wave contour as elderly, whose higher PWV is due to increased stiffness.

Hypertension

Increasing systolic blood pressure paralleled with a lack of change or decrease in DBP is another common culprit of elasticity deterioration and increased stiffness with aging (Benetos et al., 2002; Kannel et al., 1981; Liu et al., 1989; Safar et al., 1984; Staessen et al., 1990) with the subsequent increases in PP and increased afterload (due to early reflective pulse waves) likely affecting cardiovascular risk factors and contributing

to vascular damage over time as well as reduced perfusion of the coronary arteries in diastole (Darne et al., 1989; Fang et al., 1995; Madhavan et al., 1994; Nichols & O'Rourke, 1998). Sustained, chronic hypertension is strongly related to increased arterial stiffness (Amar et al., 2001; Benetos et al., 1993; Liao et al., 1999; Safar et al., 1987) by induction of structural changes, among which include doubling or tripling of the intima-media thickness, hypertrophy of the vascular smooth muscle layer, derangements in extracellular matrix, and excessive collagen production (Glagov et al., 1992; Nagai et al., 1998; O'Leary et al., 1999; Salonen & Salonen, 1991; Virmani et al., 1991; Xu et al., 2000). Indeed, the elastic modulus of excised arterial segments increased non-linearly with increasing pressure, and that, at any given pressure (Fig. 2), the modulus was higher in peripheral than central arterial segments (Bergel, 1961).

Interestingly, central pressure waves have been shown to be only augmented in young adults with high arterial pressure, further increasing after the 50 years of age and becoming markedly augmented in subjects with isolated systolic hypertension (Benetos et al., 2002; Nichols et al., 1993; Nichols & O'Rourke, 1998).

Other Associated Risk Factors

Other risk factors have less confidently been associated with arterial stiffening. While these risk factors may not be the direct cause of arterial stiffening, the addition of such risk factors on top of an already stiffening artery likely exacerbates stiffening. Among the other cardiovascular risk factors, the presence of dyslipidemia (Lehmann et al., 1995), diabetes (Emoto et al., 1998), high heart rate (Sa Cunha et al., 1997), and

tobacco smoking (Stefanadis et al., 1997) are often associated with increased stiffness, but the impact of these risk factors in the development of arterial stiffness remains unclear. A few of the commonly described risk factors that may influence stiffening are gender, heart rate, cigarette smoking, diabetes mellitus.

Gender. Change in aortic elasticity is similar in both males and females for the first 10 years of life (Laogun & Gosling, 1982). Thereafter, greater declines in aortic distensibility and stiffness (Laogun & Gosling, 1982; Sonesson et al., 1993) have been reported in males compared to females. By approximately 60 years of age, both sexes are comparable with menopause and loss of estrogen being the contributing factor for evening out measures of aortic elasticity (Rajkumar et al., 1997).

Heart rate. Using atrial pacing, it has been shown that heart rate changes were able to influence arterial distensibility (Mangoni, et al., 1996). Because the viscous component of the elastic wall is highly frequency dependent, it can be expected that higher heart rate values are associated with reduced distensibility. The shortening of the time available for recoil may be a plausible, although not an exclusive, explanation for decreased distensibility; however, a shortened recoil period may lend itself to increases in pulse wave propagation speed. Conversely, decreased augmentation is observed with increased heart rate likely due to a decreased ventricular ejection period resulting in late diastole augmentation versus systole (O'Rourke et al., 2001; Wilkinson et al., 2000). Indeed, chronic increases in heart rate contribute to arterial fatigue and to atherosclerotic lesions (Bassiouny et al., 1994) and are a significant determinant of arterial stiffness in hypertensives (Albaladejo et al., 2000; Sa Cunha et al., 1997).

Cigarette smoking. Smoking is associated with acute decreases in arterial distensibility in both normotensive and hypertensive individuals, and is independent of increases in blood pressure (Failla et al., 1997; Giannattasio et al., 1994; Kool et al., 1993). Furthermore, women tend to be more vulnerable to the vascular decrements from smoking than men, despite the higher aortic elasticity in women prior to menopause (Sonesson et al., 1997).

Diabetes Mellitus. Previous studies have shown that aortic PWV is greater in subjects with diabetes than in controls (Cruickshank et al., 2002; Dabelea et al., 2013). While acute hyperglycemia in non-diabetic individuals likely does not influence carotid artery elasticity (Lambert et al., 1997), chronic hyperglycemia in diabetics is associated with reduced aortic elasticity independent of atherosclerosis or other clinical complications of diabetes (Oxlund et al., 1989). Indicators of glucose intolerance have been related to arterial stiffness (Amar et al., 2001; Emoto et al., 1998; Lehmann et al., 1992). Among type I diabetic subjects, arterial stiffening may be found in the absence of any diabetic-related complication, suggesting that in diabetes stiffening may be an early marker of vascular damage (Lacolley et al., 2001). The association may be due to high levels of insulin, or directly, by having elevated glucose levels leading to collagen cross linkage due to non-enzymatic glycation and its end-products deposited in the arterial wall (Airaksinen et al., 1993; Sims et al., 1996). Arterial stiffness has also been reported in pre-diabetic states (Salomaa et al., 1995). A 25% increase in fasting glucose is associated with increased artery stiffness. Nonetheless, determination of aortic stiffness or measurement of pulse wave contour have not been shown to aid in the

diagnosis of diabetes mellitus, nor definitely proven to assist in assessment of its severity (O'Rourke et al., 2001).

Cardiovascular Outcomes Associated with Arterial Elasticity and Stiffness

Stiffening of the larger central arterial system significantly contributes to CVD outcomes in older individuals and is positively associated with systolic hypertension (Franklin et al., 1997), end-stage renal disease mortality (Blacher et al., 1998), chronic kidney disease (Blacher et al., 2003; London et al., 1998; London et al., 2001), coronary artery disease and mortality (Benetos et al., 1998; Sutton-Tyrrell et al., 2005), stroke (Sutton-Tyrrell et al., 2005), heart failure (Chae et al., 1999), and atrial fibrillation (Mitchell et al., 2007). Indeed, the elastic properties of the aorta are related to the degree of coronary artery disease (Hirai et al., 1989; Stefanadis et al., 1990). Stroke patients also have less abdominal aorta distensibility (Lehmann et al., 1995). Additionally, hemodialysis patients have reduced aortic and carotid compliance despite compensatory aortic dilatation (London et al., 1990). Indeed, dialysis is strongly associated with reduced carotid compliance in renal transplant patients (Barenbrock et al., 1993). Furthermore, arterial stiffness is said to synergistically increase in proportion to the number of cardiovascular risk factors and clinical events (Lehmann et al., 1998).

Summary

While there appears to be a reliable relationship between risk factors such as advanced aging and hypertension with decreased arterial elasticity and increased

stiffness, studies of carotid arterial elasticity in otherwise healthy populations are lacking. Specifically in young adolescents, carotid artery elasticity differences throughout pubertal development have never been adequately evaluated. Additionally, sex differences in otherwise healthy individuals have not been extensively evaluated. Possible decrements in vascular mechanics in an otherwise healthy and young population may support usage of therapeutic remedies to offset further vascular dysfunction. Indeed, the following dissertation further evaluates whether a therapeutic dose of atorvastatin modulates vascular dysfunction in childhood cancer survivors, a population known to have an increased risk of cardiovascular disease.

**CHAPTER 3. IMPACT OF PUBERTAL DEVELOPMENT ON
ENDOTHELIAL FUNCTION AND ARTERIAL ELASTICITY**

Impact of Pubertal Development on Endothelial Function and Arterial Elasticity

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SUMMARY

Background: Cardiovascular and metabolic risk factor levels differ by pubertal stage. However, little is known about the relation of pubertal development on endothelial function and arterial elasticity in children and adolescents. **Objective:** We compared brachial artery flow-mediated dilation (FMD) and carotid artery elasticity across Tanner (pubertal) stages in children and adolescents. **Methods:** Blood pressure, fasting lipids, glucose, and insulin, body fat, insulin sensitivity (Mlbm), brachial FMD (percent dilation and area-under-the-curve), endothelium-independent dilation (EID peak dilation and area-under-the-curve), and carotid artery elasticity were evaluated across pubertal stages (Tanner I vs. Tanner II-IV vs. Tanner V) in 344 children and adolescents (184 males, 160 females; ages 6 to 21 years). **Results:** 124 subjects (mean age 8.23 ± 0.15 years; 52 females) were Tanner stage I; 105 subjects (mean age 13.19 ± 0.17 years; 47 females) were Tanner stages II-IV; and 115 subjects (mean age 17.19 ± 0.16 years; 61 females) were Tanner stage V. There were no significant differences for any of the measures of vascular structure and function across pubertal stages. **Conclusion:** Results of the current study indicate smooth muscle and endothelial function, as well as carotid artery elasticity, do not differ throughout pubertal development and that accounting for pubertal stage when reporting vascular data in children and adolescents may be unnecessary.

INTRODUCTION

Throughout puberty, transient changes in cardiometabolic risk factors occur as a normal part of development. The relationship between puberty and cardiometabolic factors, such as body fatness, body mass index (BMI), lipids, and insulin resistance has been well documented (Amiel et al., 1986; Bloch & Clemons, 1987; Caprio et al., 1989; Caprio et al., 1994; Caprio & Tamborlane, 1994; Cook et al., 2003; Goran & Gower, 2001; Gower, 1999; Moran et al., 1999; Richards et al., 1992; Sinaiko et al., 2001; Travers et al., 1995; Uwaifo et al., 2002). Specifically, increased insulin resistance is often associated with pubertal onset, with levels returning to near pre-pubertal status following maturation (Moran et al., 1999). Although insulin resistance is associated with adiposity throughout childhood and adolescence (Moran et al., 1999; Richards et al., 1992; Sinaiko et al., 2001), differences in body fatness do not entirely explain the development of insulin resistance during puberty (Goran & Gower, 2001; Moran et al., 1999; Sinaiko et al., 2001). Although not firmly established, it is possible that insulin resistance influences vascular health during pubertal development in children and adolescents.

A number of studies among children and adolescents have also reported that endothelial function, as measured by flow-mediated dilation (FMD), is associated with obesity (Tounian et al., 2001), dyslipidemia (Järvisalo, Lehtimäki, & Raitakari, 2004), blood pressure (Farpour-Lambert et al., 2009), insulin resistance (Tounian et al., 2001; Kapiotis et al., 2006), and oxidative stress (Järvisalo, Lehtimäki, & Raitakari, 2004). Limited studies have measured arterial elasticity among children and adolescents

(Chalmers et al., 2011; Reed et al., 2005). Among adults, BMI and elements of metabolic syndrome have been reported to have an inverse relationship with large and small artery compliance (Acree, Montgomery, & Gardner, 2007; Fjeldstad et al., 2007). Although it has been well-documented that cardiovascular and metabolic risk factors differ by pubertal stage, to our knowledge, no studies have examined whether measures of vascular function and stiffness differ among pubertal stages among children and adolescents. Therefore, the purpose of this study was to evaluate the influence of Tanner stage on endothelial function and arterial elasticity among children and adolescents. We hypothesized that vascular variables would differ by pubertal stage in accordance with associated changes in cardiometabolic risk factors. Specifically, we hypothesized that FMD and arterial elasticity would be lowest among Tanner stages II-IV compared to Tanner stages I and V.

MATERIALS AND METHODS

The study protocol was approved by the University of Minnesota Institutional Review Board (IRB). The study procedures adhered to the University of Minnesota's IRB and the Health Insurance Portability and Accountability Act (HIPAA) guidelines. All parents and subjects provided informed consent and assent, respectively, for study participation.

Study Population

Three hundred and forty-four subjects (184 males, 160 females), who had participated in a study evaluating cardiovascular risk among families, were included in this cross-sectional study. Enrolled children and adolescents were between the ages of 6 and 21 years of age (mean age 12.7 ± 4.1 years). Subjects had been fasting for at least eight hours prior to the vascular assessment and were asked to abstain from caffeine ingestion on the morning of testing and to avoid strenuous exercise or physical activity for 24 hours prior to the study visit. Subjects who were greater than 18 years of age were excluded from BMI-percentile, SBP-percentile, and DBP-percentile calculations.

Measurements

Physical Assessments

Measurements for height and weight were obtained with a standard stadiometer (Ayrton, Model S100, Prior Lake, MN, USA) and electronic scale (ST Scale-Tronix, Serial No. 5002-8893, White Plains, NY, USA), respectively. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters-squared. Body composition was obtained using dual energy X-ray absorptiometry (DXA) (Lunar Prodigy, Software version 10.5, GE Healthcare Lunar, Madison, WI, USA). Blood pressure percentile data was classified based on The National Heart, Lung, and Blood Institute (NHLBI) guidelines.

Clinical Assessments

Seated blood pressure was measured by a random-zero sphygmomanometer on the right arm, and the average of two systolic blood pressure (SBP) measurements and fifth phase Korotkoff diastolic blood pressure (DBP) measurements were analyzed. Tanner staging of pubertal development was performed by trained providers and was based on breast and pubic hair development in girls and pubic hair development in boys. Participants were classified as pre-pubertal (Tanner I), pubertal (Tanner II-IV) and post-pubertal (Tanner V).

Insulin sensitivity, adjusted for lean body mass (MIbm), was determined by euglycemic hyperinsulinemic clamp as previously described (Sinaiko et al., 2001). Fasting blood samples were obtained for serum insulin and lipids, as well as plasma glucose. Insulin levels were determined using a chemoluminescence immunoassay (Immulin Insulin DPC, Los Angeles, CA). Samples for serum lipids were analyzed with standard procedures at the Fairview-University Medical Center clinical laboratory.

Vascular Assessments

Testing was performed in the Vascular Biology Laboratory in the University of Minnesota Clinical and Translational Science Institute. All the vascular studies were performed in a quiet, temperature-controlled environment (22-23°C). Vascular images were measured by a non-invasive ultrasound with subjects in the supine position. Images were digitized and stored on a personal computer for later off-line analysis with an electronic wall-tracking software program (Vascular Research Tools 5, Medical Imaging Application, LLC, Iowa City, IA, USA).

FMD and EID. Following 15 minutes of quiet rest in the supine position, vascular images of the brachial artery were obtained using a conventional ultrasound scanner (Acuson, Sequoia 512, Siemens Medical Solutions USA, Inc., Mountain View, CA, USA) with a 7.5 MHz linear array probe held at a constant distance from the skin and at a fixed point over the imaged artery. Assessment of FMD was performed by imaging the left brachial artery at the distal third of the upper arm using techniques previously described (Celermajer et al., 1992; Kelly et al., 2004). After a 15-minute break following FMD assessment, endothelium-independent dilation (EID) was assessed using 0.3 mg sublingual nitroglycerin (NTG), the dose considered appropriate by the University of Minnesota Institutional Review Board for subjects <18 years old, and 0.4 mg sublingual NTG for subjects 18+ years old. Brachial artery diameter was assessed continuously for 5 minutes post-NTG administration. Peak dilation during the study was defined as the greatest percent change from resting brachial artery diameter, while area under the curve (AUC) was defined as the total relaxation of the brachial artery from resting baseline following reactive hyperemia or sublingual nitroglycerin administration.

Carotid arterial elasticity. Carotid artery images, as well as supine systolic and diastolic blood pressure and pulse pressure, were concurrently measured by a non-invasive ultrasound with subjects in the supine position. Following 15-min of quiet rest in the supine position, luminal systolic and diastolic diameters were obtained at a fixed point over the left common carotid artery, approximately 1 centimeter proximal from the carotid bulb. Images were collected at 20 frames per second for 10 seconds (200 frames) to ensure the capture of full arterial diameter change during a cardiac cycle. Systolic and

diastolic blood pressures were recorded with an automated blood pressure sphygmomanometer during the 10-second carotid measurement. The mean diameter through the 10-second cycle was used to calculate measures of compliance and distensibility. The ultrasound scanning system was interfaced with a standard personal computer equipped with a data acquisition card for attainment of radio frequency ultrasound signals from the scanner. Digital image analysis was performed by the same trained reader blinded to group assignments.

Pulse pressure (ΔP) was calculated as the difference between systolic and diastolic pressures. Additionally, maxDiamM denotes maximum diameter measurement, and minDiamM denotes minimum diameter measurement.

Statistical Analysis

SAS Software Package (Version 9.2, 2009, SAS Inc., Cary, NC, USA) was used for statistical analyses. Our study data was segmented into Tanner stages I, II-IV, and V groupings due to the equal-size, homogenous clusters within each stage of our study population. Results are expressed as mean \pm standard deviation (SD), unless otherwise stated. A one-way analysis of variance (ANOVA) was used to compare demographic characteristics by pubertal stage groups, as well as to compare vascular measures between-groups of pubertal stage groups, adjusting for age, sex, race, and baseline brachial artery diameter. An alpha value of 0.05 was used to signify statistical significance.

RESULTS

Tanner stage I included 124 children (mean age 8.23 ± 0.15 years; 52 females); Tanner stages II-IV included 105 children and adolescents (mean age 13.19 ± 0.17 years; 47 females); and Tanner stage V included 115 adolescents (mean age 17.19 ± 0.16 years; 61 females).

Table 1 shows the descriptive and clinical characteristics of the three pubertal groups. Percent body fat and systolic blood pressure were significantly ($P < 0.001$) lower among Tanner stage I, while diastolic blood pressure was significantly ($P < 0.002$) higher among Tanner stage V. SBP-percentile ($P = 0.48$) was not significantly different among pubertal groups. DBP-percentile was significantly ($P = 0.003$) lower in Tanner I than in Tanner II-IV; however, no significant difference was observed between Tanner II-IV and Tanner V. Fasting glucose and insulin were significantly different among the pubertal groups, with Tanner stage I having significantly ($P < 0.0001$) lower fasting glucose and insulin levels than both Tanner stages II-IV and Tanner stage V. Tanner stages II-IV and Tanner stage V trended towards being significantly ($P = 0.095$) different, with Tanner stages II-IV reporting higher values. MIbm was significantly different between all pubertal stage groups, with Tanner stage I exhibiting the highest values, followed by Tanner stages II-IV, then Tanner stage V (all $P < 0.05$). Total cholesterol and LDL-cholesterol were significantly ($P < 0.05$) higher among Tanner stage I, while HDL-cholesterol was significantly ($P < 0.005$) lower among Tanner stage V. Furthermore, triglycerides increased with pubertal progression, with Tanner stage V observed to be significantly ($P < 0.0003$) larger than Tanner stage I. Overall, mean descriptive and

clinical measurements were observed to be within normal reference ranges for children and adolescents.

Table 2 shows the vascular response measures of the three pubertal groups. Baseline brachial artery diameter significantly increased ($P=0.012$) with pubertal progression. Specifically, significant differences between Tanner I and Tanner II-IV ($P=0.014$), as well as Tanner II-IV and Tanner V ($P=0.028$) were observed. Vascular measures, following adjustments by age, sex, race, and resting diameter, revealed no significant differences by pubertal stage groups for FMD ($P=0.68$), FMD-AUC ($P=0.91$), EID ($P=0.88$), EID-AUC ($P=0.92$), diameter distensibility (DD) ($P=0.66$), cross-sectional distensibility (CSD) ($P=0.66$), diameter compliance (DC) ($P=0.18$), or cross-sectional compliance (CSC) ($P=0.41$); these findings did not change with additional adjustment for BMI-percentile. A separate analysis was also performed with adjustments for age, sex, and race (excluding resting diameter), and overall conclusions were the same; moreover, FMD ($P=0.33$), FMD-AUC ($P=0.68$), EID ($P=0.46$), EID-AUC ($P=0.17$), DD ($P=0.86$), CSD ($P=0.86$), DC ($P=0.29$), and CSC ($P=0.36$) were not significantly different by pubertal stage groups.

A gender-stratified analysis was also performed with adjustments for age, race, baseline diameter, and both with and without adjustment for BMI-percentile, revealing no significant difference between the Tanner stage groups for male and female children and adolescents separately.

DISCUSSION

The findings of the current study indicate that adjusting for pubertal stage might not be necessary when reporting vascular data among children and adolescents. The vascular findings are consistent with previously reported studies (Celermajer et al., 1994; van der Heijden-Spek et al., 2000), which have suggested that resting brachial artery diameter was higher in each respective pubertal stage group. However, this appeared to have no impact on endothelial function across groups. In contrast to our findings, however, Bhangoo et al. (2011) reported that endothelial peripheral arterial tonometry (EndoPAT) index, a measure of small artery endothelial function, increased with pubertal progression (Tanner stage I to Tanner stages IV-V) and was significantly correlated with age in healthy children and adolescents. The divergent findings may be explained by the inherent differences in the vascular beds evaluated (conduit versus small artery). Arterial dilation in the conduit arteries is mostly dependent on nitric oxide whereas multiple factors contribute to dilation in the small arteries. In addition, smooth muscle is much more abundant in the conduit arteries. Finally, it is also possible that the “one size fits all” finger probes used with the EndoPAT device assessment provide less accurate reactive hyperemic index values in younger children. Future research should investigate the differences in conduit versus small artery endothelial function across pubertal stages in healthy children and adolescents, as well as diseased populations.

A notable observation of the current study was the lack of difference in smooth muscle function (EID) throughout pubertal development. This finding is important since the use of NTG in children is controversial and the lack of difference in EID across the

pubertal groups in this study suggests that evaluation of smooth muscle function in children may be unnecessary. Additionally, future studies looking at smooth muscle function in children may not need to account for pubertal development.

Although not statistically significant, the current study showed higher carotid artery compliance and endothelial function, specifically FMD and FMD-AUC, among the Tanner stage I group. It is unclear whether these differences are clinically meaningful; however, this should be considered in studies where pre-pubertal children are compared to pubertal children.

The Tanner staging method used within the present study has been the standard for assessing the degree of pubertal development for more than 30 years (Marshall & Tanner, 1969; Marshall & Tanner, 1970), and it is the method used in previous studies of insulin resistance during puberty (Amiel et al., 1986; Travers et al., 1995; Cook et al., 1993; Amiel et al., 1992). Inherent in this method is a discordance between pubic hair growth and breast development in girls (Marshall & Tanner, 1969). We scored both pubic hair and breast development in girls and used the greater of the two values for statistical analysis so that pubertal maturation would not be underestimated. While pubic hair staging in boys often corresponds with testicular volume, pubic hair staging is not always the most accurate measure of gonadal maturation. Nonetheless, exclusion of genital examination was found to improve patient compliance with the study protocol.

Strengths of the current study include a relatively large sample size, balanced Tanner stage groups and the use of the euglycemic, hyperinsulinemic clamp, the gold standard technique for measuring insulin sensitivity. The primary limitation of the study

was its cross-sectional nature. A longitudinal design would be preferred in order to better address the temporal association of pubertal development and arterial health.

In summary, the results from this cross-sectional study indicate that neither vascular function nor arterial compliance differs across pubertal stages in children and adolescents even though cardiometabolic risk factor levels vary. While it is important to report Tanner stage in studies involving children and adolescents, the current findings suggest that adjustments for pubertal stage might not be necessary when reporting vascular data among children and adolescents. Future prospective longitudinal studies should assess whether transient changes in cardiometabolic risk factor levels during puberty influence vascular health to confirm the results of the current study.

TABLE LEGENDS

Table 1. Mean (\pm SD) Demographic and Clinical Characteristics

Table 2. LSmeans(SE) Vascular Response Measures

Table 1. Mean(SD) Demographic and Clinical Characteristics				
	Tanner I (n=124)	Tanner II-IV (n=105)	Tanner V (n=115)	Overall P-value
Age (yrs)	8.23(0.15)	13.19(0.17) ^a	17.19(0.16) ^{b,c}	<0.0001
Male, n(%)	58.1(4.5)	55.2(4.9)	46.9(4.7)	0.21
Race, n(%)				
White	77.4(3.9)	81.9(4.3)	48.7(4.1) ^{b,c}	<0.0001
Black	16.9(3.6)	11.4(3.9)	35.7(3.7) ^{b,c}	<0.0001
Height (cm)	133.4(0.88)	159.8(0.96) ^a	169.7(0.92) ^{b,c}	<0.0001
Weight (kg)	31.3(1.4)	54.9(1.5) ^a	73.6(1.4) ^{b,c}	<0.0001
BMI-Percentile [†]	59.3(2.5)	65.7(2.7)	70.3(2.6) ^b	0.011
Percent Body Fat, DXA, %	20.9(1.0)	26.0(1.0) ^a	28.2(1.0) ^b	<0.0001
SBP, mmHg	100.2(0.89)	108.0(0.97) ^a	110.5(0.92) ^b	<0.0001
SBP-percentile [§]	47.4(2.4)	47.7(2.5)	43.3(3.1)	0.48
DBP, mmHg	56.9(0.69)	57.4(0.75)	60.7(0.72) ^{b,c}	0.0002
DBP-percentile [§]	40.3(1.9)	32.1(2.0) ^a	29.8(2.5) ^b	0.0008
Fasting Glucose (mg/dL)	70.1(1.1)	86.7(1.1) ^a	84.9(1.0) ^b	<0.0001
Fasting Insulin (mU/L)	4.0(0.62)	11.2(0.62) ^a	9.78(0.59) ^b	<0.0001
Mlbm (mg/kg lbm/min)	15.8(0.74)	13.1(0.39) ^a	11.8(0.38) ^{b,c}	<0.0001
Total Cholesterol (mg/dL)	156.0(2.6)	145.9(2.6) ^a	147.3(2.5) ^b	0.011
LDL-Cholesterol (mg/dL)	91.0(2.1)	81.6(2.1) ^a	84.6(2.1) ^b	0.007
HDL-Cholesterol (mg/dL)	52.3(1.1)	50.7(1.1)	46.4(1.0) ^{b,c}	0.0003
Triglycerides (mg/dL)	63.3(3.9)	73.4(3.9)	83.5(3.8) ^b	0.0014

Standard Error of the Mean (SEM) used, unless otherwise noted. P-values <0.05 demonstrate significant differences between Tanner stages I, II-IV, and V. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; Mlbm, insulin resistance (mg per kilogram of lean body mass); LDL, low-density lipoproteins; HDL, high-density lipoproteins.

[†] Denotes Tanner group size (n=124, 105, 110). [§] Denotes Tanner group size (n=123, 105, 72).

Table 2. LSmeans(SE) Vascular Response Measures				
	Tanner I (n=124)	Tanner II-IV (n=105)	Tanner V (n=115)	Overall P-value
Baseline Diameter (mm)	2.96(0.07)	3.17(0.04) ^a	3.34(0.07) ^{b,c}	0.012
FMD (%)	8.6(0.59)	7.96(0.37)	7.88(0.58)	0.68
FMD-AUC, %-s ⁻¹	805.4(74.5)	772.9(46.5)	786.1(73.5)	0.91
EID (%)	23.7(1.40)	24.4(0.67)	24.5(0.77)	0.88
EID-AUC, %-s ⁻¹	3870.3(264.8)	3768.7(126.1)	3758.0(141.1)	0.92
DD (%)	14.5(0.59)	15.1(0.37)	15.4(0.58)	0.66
CSD (%)	31.2(1.4)	32.7(0.9)	33.2(1.3)	0.66
DC, mm-mmHg ⁻¹	0.013(0.002)	0.018(0.001)	0.018(0.002)	0.18
CSC, mm ² -mmHg ⁻¹	0.18(0.008)	0.16(0.005)	0.16(0.008)	0.41

LSmeans(SE) adjusted for Age, Sex, Race, and Baseline Diameter. P-values <0.05 demonstrate significant differences among Tanner stages I, II-IV, and V. Baseline diameter (baseline brachial artery diameter at rest); FMD, flow-mediated dilation; FMD-AUC, flow-mediated dilation area-under-the-curve; EID, endothelium-independent dilation; EID-AUC, endothelium-independent dilation area-under-the-curve; DD, diameter distensibility; CSD, cross-sectional distensibility; DC, diameter compliance; CSC, cross-sectional compliance.

**CHAPTER 4. THE INFLUENCE OF GENDER ON
CAROTID ARTERY COMPLIANCE AND DISTENSIBILITY IN
CHILDREN AND ADULTS**

The Influence of Gender on Carotid Artery Compliance and Distensibility in Children and Adults

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SUMMARY

Background: Given its association with the development of cardiovascular disease, compliance and distensibility measures of carotid arterial elasticity have been commonly used over the last decade as predictors of cardiovascular risk. However, the gender differences for these measures are unknown. **Objective:** The purpose of this study was to evaluate the impact of gender on carotid arterial elasticity in a large sample of children and adults. **Methods:** Arterial elasticity measures of the carotid artery were obtained with ultrasound imaging in 294 children (157 males, 137 females; 6 to 18-yrs) and 604 adults (291 males, 311 females; 18-49 yrs) previously recruited for a study investigating cardiovascular risk factors. An independent sample t-test was used to compare demographic and carotid artery elasticity measures by age and gender. **Results:** No significant gender differences in carotid arterial compliance and distensibility were observed in children. Adult females had significantly greater cross-sectional compliance compared to adult males (0.004 ± 0.000 vs. 0.003 ± 0.000 mm²/mmHg, $P=0.041$). **Conclusions:** A significant gender difference in carotid compliance was present in adults, but not in children. Thus, data from this study suggests that gender differences in arterial stiffness are not present early in life, but emerge later in adulthood.

INTRODUCTION

Arterial elasticity has become an increasing focal point in the past decade because of its association with cardiovascular disease (CVD) (Fjeldstad, Montgomery, & Gardner, 2008; Havlik et al., 2003). The two most common measures of arterial elasticity are compliance and distensibility. By definition, compliance is the unit change in volume induced by a unit change in pressure, or the absolute change in arterial volume that reflects the arterial ability to store volume and reduce pressures (Jani & Rajkumar, 2006; Reneman, Meinders, & Hoeks, 2005), and distensibility is the relative change in arterial volume against the change in pressure and reflects the mechanical load placed on the arterial wall (Reneman, Meinders, & Hoeks, 2005).

Lower arterial compliance (i.e., the ability to expand and recoil), and/or increased arterial stiffness, is common with advancing age in both men and women (Arnett, Evans, & Riley, 1994; Mitchell et al., 2004). Increased arterial stiffness is also associated with CVD risk factors, such as hypertension, hypertriglyceremia, type 2 diabetes mellitus, and aging (Fjeldstad, Montgomery, & Gardner, 2008; Jani & Rajkumar, 2006; Avolio et al., 1985; Liao et al., 1999; Olinic et al., 2003; Prisant, Resnick, & Hollenberg, 2001; Snijder et al., 2004; van Popele et al., 2001). Moreover, arterial stiffening impairs the ability of the arterial system to handle the spontaneous elevation in blood pressure at systole, which leads to increases in systolic blood pressure and left ventricular mass, as well as decreases in diastolic blood pressure and diastolic coronary perfusion (Westerhof & O'Rourke, 1995).

Assessment of arterial compliance and distensibility within larger conduit arteries, such as the carotid artery, is a widely-used technique to examine vascular elasticity and

arterial stiffness (Avolio et al., 1983; Benetos et al., 1993; Boutouyrie et al., 1992; Kawasaki et al., 1987; Länne et al., 1992; Laogun & Gosling, 1982; Reneman et al., 1986; Sonesson et al., 1993; Tseke et al., 2006) given the abundance of elastic and collagen fibers within these arteries compared to smooth muscle fibers largely abundant in the peripheral vasculature. One may suspect there would exist a relationship between brachial and carotid arterial compliance and distensibility if arterial stiffness was systemically induced rather than occurring regionally.

The primary objective of this study was to evaluate the differences related to gender in carotid arterial elasticity measures in children and adults. We also examined, in a subset of participants, whether a relationship exists between brachial and carotid arterial elasticity measures. Gender differences relative to arterial stiffness, as well as potential arterial stiffness associations among different vascular beds, is critical to comprehensively understand the progression of atherosclerotic diseases and may showcase arterial differences with aging. We hypothesized that carotid artery elasticity will differ by gender and that differences will become more apparent with age (i.e., better identifiable in the adults compared to children). Additionally, we hypothesized that an association between brachial and carotid arterial elasticity may exist.

MATERIALS AND METHODS

The study protocol was approved by the University of Minnesota Institutional Review Board (IRB). The study procedures adhered to the University of Minnesota's IRB and the Health Insurance Portability and Accountability Act (HIPAA) guidelines. All subjects submitted written informed consent and assent for study participation.

Study Population

Eight hundred and ninety-eight subjects (448 males, 450 females) between the ages of 6 and 49-yrs (mean age 28.6 ± 0.5 yrs; males: 28.1 ± 0.7 yrs, females: 29.5 ± 0.6 yrs) were included in the study. Subjects were recruited from a community-based sample and all subjects were healthy. These individuals were participants in a study investigating the early development of obesity, insulin resistance, and their interaction with associated cardiovascular risk factors. Subjects were stratified into 6 to 18-yrs and 18-49 yrs age group to separate the children from adults. Prior to vascular testing, subjects were asked to fast for 12-h and abstain from caffeine ingestion. Subjects were instructed to withhold morning medications until after vascular ultrasound testing, and refrain from strenuous physical activity 12-h prior to testing. A study physician and/or certified nurse practitioner was present to review study procedures and evaluation plans, prescription medications, and conduct comprehensive medical examinations including current and past medical history, review of systems (with particular attention to cardiovascular and endocrine issues), family history (with particular attention to cardiovascular disease and diabetes), and a physical examination.

All 898 subjects had carotid compliance and distensibility measures and a smaller subset of 93 individuals (55 males, 38 females) also had brachial artery measures. Data from this subset were further analyzed to determine if there is an association between brachial and carotid artery compliance and distensibility measures.

Measurements

Testing was performed in the Vascular Biology Laboratory in the University of Minnesota Clinical and Translational Science Institute. All the vascular studies were performed in a quiet, temperature-controlled environment (22-23°C).

Anthropometric and Blood Pressure Assessments

Measurements for height and weight were taken at the start of the visit using a digital stadiometer. Body Mass Index (BMI) was calculated as weight in kilograms (kg) divided by height in meters-squared (m²). Seated blood pressure was obtained on the control arm using an automatic blood pressure monitor (Model BP-8800C; Colin Press-Mate, San Antonio, TX, USA).

Vascular Assessments

Brachial and carotid artery images, as well as supine systolic and diastolic blood pressure and pulse pressure, were concurrently measured by a non-invasive ultrasound with subjects in the supine position. Both brachial and carotid artery images were digitized and stored on a personal computer for later off-line analysis of arterial compliance and distensibility. Electronic wall-tracking software was used for the analysis (Vascular Research Tools 5, Medical Imaging Application, LLC, Iowa City, IA, USA).

Carotid artery measurements. Following 15-min of quiet rest in the supine position, vascular images were obtained of the carotid artery using the conventional ultrasound scanner (Acuson, Sequoia 512, Siemens Medical Solutions USA, Inc., Mountain View, CA, USA) with a 7.5 MHz linear array probe held at a constant distance from the skin and at a fixed point over the imaged artery. The transducer was held at a

constant distance from the skin and at a fixed point over the common carotid artery, approximately 1-cm proximal from the carotid bifurcation bulb, to capture the left common carotid artery's lumen diastolic and systolic diameters. Images were collected at 20 frames per second for 10-s (200 frames) to ensure the capture of full arterial diameter change during a cardiac cycle. The mean diameter through the 10-s cycle was used to calculate measures of compliance and distensibility.

Brachial artery measurements. Following 15-min of quiet rest in the supine position, vascular images of the brachial artery in a smaller subset of 93 individuals were obtained using a conventional ultrasound scanner as previously described for carotid measurements. The transducer probe was held at a constant distance from the skin and the brachial artery was scanned in a fixed, longitudinal section 5 to 15-cm above the elbow with the assistance of a stereotactic arm. Depth and gain settings were set to optimize images taken of the lumen/arterial wall interface. The following procedures ensured consistency throughout subject analysis. Systolic and diastolic blood pressures were recorded with an automated blood pressure sphygmomanometer during both the 10-s carotid and brachial measurements. The ultrasound scanning system was interfaced with a standard personal computer equipped with a data acquisition card for attainment of radio frequency ultrasound signals from the scanner. Brachial artery image collection measurements were conducted similar to carotid artery measures.

Measurement Characteristics. In order to measure the brachial and carotid elasticity properties, the following formulas for distensibility and compliance were used:

- **Diameter distensibility** (DD, %) is defined as $[(\text{maxDiamM} - \text{minDiamM}) / \text{minDiamM}] \times 100\%$.

- **Cross-sectional distensibility** (CSD, %) is defined as $[(\pi*(\text{maxDiamM}/2)^2 - \pi*(\text{minDiamM}/2)^2)/\pi*(\text{minDiamM}/2)^2] \times 100\%$.
- **Diameter compliance** (DC, mm/mmHg) is defined as $[(\text{maxDiamM} - \text{minDiamM})/\Delta P]$.
- **Cross-sectional compliance 1** (CSC1, mm²/mmHg) is defined as $[(\pi*(\text{maxDiamM}/2)^2 - \pi*(\text{minDiamM}/2)^2)/(\Delta P)]$.
- **Incremental elastic modulus** (IEM, mmHg) is defined as $3\{1 + [\pi*(\text{maxDiamM}/2)^2 / \pi*(\text{minDiamM}/2)^2]\} / \text{CSC1}$.

Pulse pressure (ΔP) is calculated as the difference between systolic and diastolic pressures. Additionally, maxDiamM denotes maximum diameter measurement, and minDiamM denotes minimum diameter measurement. Furthermore, although multiple technicians were involved in the data collection, the software program utilized for vascular image assessment and interpretation was automated and operator-independent, minimizing any variability among technicians.

Statistical Analysis

Stata/SE 12.0 (StataCorp, College Station, TX, USA) was used for statistical analyses. Results are expressed as mean \pm standard error of the mean (SEM). An independent sample t-test was used to compare demographic characteristics, as well as carotid artery compliance and distensibility measures by gender within the 6 to 18-yrs and 18-49 yrs age groups. A multiple linear regression model was additionally used to adjust for age, gender, and BMI or BMI Percentile where these risk factors were

significantly different between genders in both age groups. Brachial versus carotid arterial compliance and distensibility measures were compared by Pearson's Correlation analysis within the smaller subset. An alpha value of 0.05 was used to signify statistical significance.

RESULTS

Carotid Artery Elasticity Assessment

Mean demographic data among both the male and female study population, stratified into two age groups (6 to 18-yrs, and 18-49 yrs) are presented in Table 1. For subjects 6 to 18-yrs of age, males were significantly taller ($P=0.043$), and had significantly higher seated systolic blood pressure ($P<0.0001$) and pulse pressure ($P<0.0001$) than females. Age ($P=0.823$), weight ($P=0.309$), BMI ($P=0.895$), BMI Percentile ($P=0.957$), and seated diastolic blood pressure ($P=0.286$) were not significantly different between males and females. For subjects 18-49 yrs of age, a significant difference in height ($P<0.0001$), weight ($P<0.0001$), as well as seated systolic blood pressure ($P<0.0001$), diastolic blood pressure ($P=0.0001$), and pulse pressure ($P<0.0001$) were reported between genders, with males reporting significantly higher values. Age ($P=0.559$) and BMI ($P=0.270$) were not significantly different between males and females.

Carotid artery measures of compliance and distensibility are displayed in Table 2. The letter 'c' is used to denote 'carotid' classifications when referencing all compliance and distensibility measures. Within the 6 to 18-yrs age group, females reported significantly greater supine diastolic blood pressure ($P=0.033$) compared to males, while

males reported significantly greater supine pulse pressure ($P=0.012$) than females. No significant gender differences were reported among supine systolic blood pressure, diameter distensibility (cDD), cross-sectional distensibility (cCSD), diameter compliance (cDC), cross-sectional compliance 1 (cCSC1), and incremental elastic modulus (cIEM) measurements in children. Within the 18-49 yrs age group, males reported significantly greater supine diastolic blood pressure ($P=0.0002$) and supine pulse pressure ($P=0.0001$) compared to females, while females reported significantly greater cCSC1 ($P=0.041$) compared to males. Following adjustment for supine pulse pressure, cCSC1 was no longer significantly different between males and females ($P=0.072$). No significant gender differences were reported for cDD, cCSD, cDC, or cIEM measurements within the 18-49 yrs age group.

Additionally, adjustments for age, gender, and BMI Percentile in children or BMI in adults showed age was a significant negative predictor for adult cDD, cCSD, cDC, and cCSC1, yet was a significant positive predictor of cIEM for both children and adult age groups ($P<0.05$). BMI Percentile was a significant positive predictor of childhood cDD and cCSD, while BMI was a significant negative predictor of adult cDD, cCSD, cDC, and cCSC1 and a significant positive predictor of adult cIEM. Gender was not a significant predictor of any of the evaluated arterial elasticity measures.

Brachial and Carotid Artery Elasticity Assessment

A subset of 93 individuals was further analyzed to assess the relationship between brachial and carotid artery compliance and distensibility measures. Mean data for this subset are presented in Table 3. The letter ‘b’ is used to denote ‘brachial

classifications when referencing all compliance and distensibility measures. Among the 6 to 18-yr age group, 39 subjects were available for analysis (22 males, 17 females). Among the 18-49 yr age group, 54 subjects were available for analysis (33 males, 21 females). Age was not significantly different between males and females in the 6 to 18-yr age group (12.0 ± 0.7 vs. 12.5 ± 0.8 yrs, $P=0.639$). Additionally, no significant differences among carotid or brachial measures of compliance and distensibility were reported within the 6-18 yr age group. Within the 18-49 yr age group, age was not significantly different between males and females (34.3 ± 1.3 vs. 32.3 ± 2.0 yrs, $P=0.359$). Adult males did report significantly larger cDD ($P=0.004$), cCSD ($P=0.004$), cCSC1 ($P=0.026$) compared to adult females, while adult females reported significantly higher cIEM ($P=0.043$) compared to adult males. There were no significant differences present among bDD, bCSD, cDC, bDC, bCSC1, and bIEM compliance and distensibility measures.

Brachial and Carotid Artery Elasticity Relationship. A significant correlation between brachial and carotid measures of compliance and distensibility was observed for DD ($r=0.334$, $P=0.038$), CSD ($r=0.337$, $P=0.036$), DC ($r=0.367$, $P=0.025$), and CSC1 ($r=0.391$, $P=0.017$) among the 6 to 18-yr age group. No significant correlation between compliance and distensibility measures was observed for IEM ($r=0.113$, $P=0.499$). Among the 18-49 yr subjects, a significant correlation between brachial and carotid measures of compliance and distensibility was observed for IEM ($r=0.300$, $P=0.028$). No significant correlation was observed between brachial and carotid measures of DD ($r=0.191$, $P=0.166$), CSD ($r=0.190$, $P=0.168$), DC ($r=0.094$, $P=0.501$), and CSC1 ($r=0.243$, $P=0.090$).

DISCUSSION

To our knowledge, this is the first study to evaluate the influence of gender on carotid arterial elasticity measures in a large sample of children and adults. Most arterial stiffness studies to date have been conducted in adult populations given arterial stiffness is common within the aging process of the arterial wall (Hickler, 1990; Hodes, Lakatta, & McNeil, 1995; O'Rourke, 1971). Furthermore, arterial stiffness is an important early marker of atherosclerotic disease identification. This is the first study, to our knowledge, to examine compliance and distensibility differences between genders in children.

The present study demonstrated that adult females had significantly greater carotid artery compliance compared to adult males, whereas in children compliance among males and females was not significantly different. Decreased arterial compliance has been shown to correlate with increased age (Avolio et al., 1985; Laogun & Gosling, 1982; Avolio et al., 1983; Benetos et al., 2001; deSimone et al., 1997; Franklin et al., 1999; Kelly et al., 1989), as well as gender and obesity (Liao et al., 1999; Johnson et al., 1986; Riley et al., 1986; Safar, 2001). Studies also have shown that sex hormones play an important role in vasomotion and vascular remodeling. Indeed, vascular function has been shown to change throughout the menstrual cycle (Williams et al., 2001). Specifically, early luteal (EL) has been reported to significantly reduce flow-mediated dilation relative to early follicular (EF), late follicular (LF), and late luteal (LL) cycle stages, while whole body arterial compliance (WBAC) has been shown to significantly increase during the LF than in the EF and EL phases. Conversely, pulse wave velocity (PWV), a measure of regional compliance, did not vary over the four phases of the

menstrual cycle (Williams et al., 2001). Moreover, estradiol has been shown to promote nitric oxide (NO)-mediated vasodilatation, reduce vascular oxidative stress, and retard atherosclerosis (Lieberman et al., 1996; Skafar et al., 1997). Sherwood et al. (2007) also reported that the effects of estrogen on receptors in vascular smooth muscle may be age-related; showing greater sensitivity in young, mature subjects as opposed to postmenopausal women. Furthermore, arterial stiffness and pulse pressure measures are often mitigated by sex steroids both pre- and post-puberty (Rossi et al., 2011). Therefore, the higher compliance of adult females compared to adult males may be due to the protective effects of estrogen.

The present study is also the first to examine the relationship between brachial and carotid arterial elasticity measures. A significant correlation between brachial and carotid measures of compliance and distensibility was observed for DD, CSD, DC, and CSC1 among the children. The adult group, on the other hand, displayed a significant correlation between brachial and carotid measures of IEM only. A clearer association between brachial and carotid measures of compliance and distensibility within the younger age group may also be specific to the effects of aging; further lending support to the negative relationship between aging and vessel compliance observed in the present study. Furthermore, it is possible that the high BMI values in adults, in relation to the children, may have explained some of the differences in arterial elasticity and vascular bed correlations. It is also possible that aging may play a roll in the mechanical changes in the vasculature.

Study strengths included the large sample size and wide age-range of the participants. A limitation of the study is that data on sexual development was not

available in all children; therefore, a potential relation between pubertal development and differences in carotid artery compliance could not be assessed. Another limitation within this study is that physical activity level was not assessed. Studies suggest that increased physical activity improves arterial elasticity (Arnett, Evans, & Riley, 1994; Tanaka et al., 2000); therefore, future studies assessing arterial elasticity in both children and adults should account for physical activity levels. Within the present study, participants were instructed to avoid strenuous exercise 24-h prior to vascular imaging to help minimize variability from such physical activity effects. And finally, vascular assessments were not timed around the menstrual cycle in females, which can be considered a limitation since cycle stage likely influences vascular function (Williams et al., 2001).

In summary, in this study of subjects between the ages of 6 and 49-yrs, adult females had significantly greater carotid artery compliance than adult males. No significant gender difference was observed within children suggesting that gender differences in arterial stiffness are not present early in life. A significant positive association also existed between brachial and carotid measures of DD, CSD, DC, and CSC1 group in the children, while a significant positive association between brachial and carotid IEM existed within the adult group. These findings suggest that arterial compliance and distensibility are somewhat similar among vascular beds during childhood but that differences emerge in adulthood. The clinical implications are not entirely clear; however, it is reasonable to speculate that arterial stiffening may occur earlier in certain vascular beds compared to others. Future research directed toward understanding the age at which arterial stiffening begins will have clinical and epidemiologic implications for early CVD prevention.

TABLE LEGENDS

Table 1. Mean (\pm SEM) Demographic Characteristics

Table 2. Mean (\pm SEM) Carotid Artery Compliance and Distensibility

Table 3. Subset of Mean (\pm SEM) Brachial and Carotid Artery Compliance and Distensibility

Table 1. Mean (\pmSEM) Demographic Characteristics				
	6 to 18-yr		18-49 yrs	
	Male (n=157)	Female (n=137)	Male (n=291)	Female (n=313)
Age (yrs)	10.9 \pm 0.3	11.0 \pm 0.3	37.4 \pm 0.4	37.1 \pm 0.3
Height (cm)	149.8 \pm 1.6	145.3 \pm 1.5*	178.5 \pm 0.4	164.6 \pm 0.4*
Weight (kg)	48.8 \pm 2.0	46.0 \pm 1.9	91.5 \pm 1.1	79.4 \pm 1.3*
BMI (kg/m ²)	20.5 \pm 0.5	20.6 \pm 0.5	28.6 \pm 0.3	29.3 \pm 0.5
BMI Percentile (%)	65.7 \pm 2.4	65.5 \pm 2.4	--	--
Seated SBP (mmHg)	105.2 \pm 0.8	100.4 \pm 0.8*	119.1 \pm 0.7	109.9 \pm 0.8*
Seated DBP (mmHg)	56.9 \pm 0.6	57.8 \pm 0.7	74.2 \pm 0.6	70.5 \pm 0.7*
Seated PP (mmHg)	48.3 \pm 0.7	42.5 \pm 0.7*	44.9 \pm 0.4	39.4 \pm 0.4*

*BMI, body mass index; Seated SBP, seated systolic blood pressure; Seated DBP, seated diastolic blood pressure; Seated PP, seated pulse pressure; *P-values <0.05 demonstrate significant differences between genders of the same age group (6 to 18-yr, 18-49 yr).*

Table 2. Mean (\pmSEM) Carotid Artery Compliance and Distensibility						
	6 to 18-yrs			18-49 yrs		
	Male (n=157)	Female (n=137)	<i>P</i> -value	Male (n=291)	Female (n=313)	<i>P</i> -value
Supine SBP	109.7 \pm 1.0	108.9 \pm 1.1	0.598	126.6 \pm 0.8	121.9 \pm 1.0	0.0002
Supine DBP	55.2 \pm 0.7	57.3 \pm 0.7*	0.033	72.9 \pm 0.6	69.5 \pm 0.6*	0.0001
Supine PP	54.4 \pm 0.8	51.6 \pm 0.8*	0.012	53.7 \pm 0.5	52.4 \pm 0.6*	0.089
cDD	15.2 \pm 0.3	14.9 \pm 0.3	0.536	8.3 \pm 0.2	8.4 \pm 0.1	0.542
cCSD	32.9 \pm 0.7	32.3 \pm 0.8	0.536	17.3 \pm 0.3	17.5 \pm 0.3	0.533
cDC	15.8 \pm 0.5	15.8 \pm 0.4	0.963	10.6 \pm 0.2	10.4 \pm 0.3	0.587
cCSC1	6.1 \pm 0.2	6.2 \pm 0.2	0.940	3.2 \pm 0.1	3.6 \pm 0.2*	0.041
cIEM	963.7 \pm 42.6	918.7 \pm 26.6	0.386	1795.7 \pm 34.3	1760.7 \pm 49.5	0.566

Notation “c” denotes carotid artery measures; SBP, systolic blood pressure (mmHg); Supine DBP, supine diastolic blood pressure (mmHg); Supine PP, supine pulse pressure (mmHg); DD, diameter distensibility (%); CSD, cross-sectional distensibility (%); DC, diameter compliance (mm/mmHg $\times 10^{-3}$); CSC1, cross-sectional compliance 1 (mm²/mmHg); IEM, incremental elastic modulus (mmHg). **P*-values <0.05 denote significant differences between genders of the same age group (6 to 18-yrs, 18-49 yrs).

Table 3. Subset of Mean (\pmSEM) Brachial and Carotid Artery Compliance and Distensibility						
	6 to 18-yr			18-49 yrs		
	Male (n=22)	Female (n=17)	<i>P</i> -value	Male (n=33)	Female (n=21)	<i>P</i> -value
cDD	15.0 \pm 0.6	15.3 \pm 0.8	0.756	11.0 \pm 0.6	8.5 \pm 0.4*	0.004
bDD	3.0 \pm 0.2	2.6 \pm 0.3	0.320	2.5 \pm 0.2	2.7 \pm 0.3	0.572
cCSD	32.3 \pm 1.4	33.0 \pm 1.8	0.772	23.4 \pm 1.3	17.8 \pm 0.9*	0.004
bCSD	6.1 \pm 0.4	5.4 \pm 0.6	0.323	5.1 \pm 0.4	5.5 \pm 0.6	0.562
cDC	16.2 \pm 0.1	17.6 \pm 0.1	0.262	13.1 \pm 0.7	11.4 \pm 0.7	0.089
bDC	2.2 \pm 0.3	2.7 \pm 0.1	0.628	1.8 \pm 0.1	2.3 \pm 0.4	0.104
cCSC1	6.0 \pm 0.3	6.5 \pm 0.3	0.215	4.3 \pm 0.3	3.5 \pm 0.2*	0.026
bCSC1	1.4 \pm 0.3	2.2 \pm 0.1	0.423	1.4 \pm 0.3	1.7 \pm 0.3	0.792
cIEM	898.5 \pm 34.6	814.9 \pm 40.9	0.125	1324.4 \pm 77.1	1574.3 \pm 90.6*	0.043
bIEM	5617.5 \pm 429.1	6170.9 \pm 866.2	0.544	6838.3 \pm 502.3	6091.5 \pm 515.9	0.326

Notation “b” denotes brachial artery measures and “c” denotes carotid artery measures; DD, diameter distensibility (%); CSD, cross-sectional distensibility (%); DC, diameter compliance, mm/mmHg $\times 10^{-3}$; CSC1, cross-sectional compliance 1, mm²/mmHg $\times 10^{-3}$; IEM, incremental elastic modulus, mmHg. **P*-values <0.05 denote significant differences between brachial and carotid arterial measures of the same age group (6 to 18-yr, 18-49 yr).

**CHAPTER 5. PILOT STUDY OF STATIN THERAPY IN YOUNG
ADULT SURVIVORS OF CHILDHOOD CANCER**

**The Effect of Atorvastatin on Vascular Function and Structure
in Young Adult Survivors of Childhood Cancer:
a Randomized, Placebo-Controlled Pilot and Feasibility Clinical Trial**

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SUMMARY

Background: Many adult survivors of childhood cancer are at high risk of developing cardiovascular disease (CVD). Research suggests chemotherapy and radiation may cause damage to the vascular endothelium, thereby initiating atherosclerosis and progression to CVD. Atorvastatin has been shown to improve endothelial function independent of reducing cholesterol, as well as reduce arterial stiffness and slow arterial thickening in multiple populations, yet has never been studied in childhood cancer survivors (CCS). The objective of this pilot clinical trial was to evaluate the effects of atorvastatin on vascular health in young adult CCS. **Methods:** Twenty-seven young adult (mean age 26.8 ± 6.2 years; 14 male, 13 female) survivors of childhood acute lymphoblastic leukemia (ALL) or Non-Hodgkin's lymphoma (NHL) were randomly assigned (1:1) to receive 40 mg of atorvastatin once daily or placebo for 6 months. Participants had been treated for cancer before 21-yrs of age and were ≥ 5 -yrs post-treatment. The primary endpoint was brachial artery flow-mediated dilation (FMD). Secondary endpoints included small artery reactive hyperemia index (RHI), arterial stiffness, and carotid artery elasticity and thickness. **Results:** Fifteen participants completed the trial and had vascular outcome measures available at follow-up. There was not a significant treatment effect on the 6-month change from baseline for any primary or secondary vascular outcome measure. Specifically, treatment effect on peak FMD and RHI was 2.3 [95% CI: -0.4, 5.0] and 0.37 [95% CI: -0.05, 0.80], respectively. No meaningful changes in arterial stiffness, carotid arterial elasticity or thickness were observed. **Conclusion:** Six months of atorvastatin treatment did not significantly improve endothelial function or arterial stiffness in young adult CCS. However, these findings should be interpreted with caution owing to the small

number of evaluable participants. Future trials will require much larger sample sizes to sufficiently study this question. This trial was registered at *clinicaltrials.gov* as NCT01733953.

INTRODUCTION

The prevalence of childhood cancer survivorship has increased steadily over the past three decades (Ries et al., 1999). Progressively more effective surgical interventions, radiotherapy, and risk-stratified chemotherapeutic approaches have led to dramatic improvements in survival rates for many childhood cancers (Smith & Ries, 2002; Ries, Eisner & Kosary, 2003; Linabery & Ross, 2008). These same cancer therapies are also thought to be responsible for many neurocognitive, metabolic, and cardiovascular complications experienced by survivors post-treatment (Oeffinger et al., 2006).

Although free of cancer, many childhood cancer survivors (CCS) are plagued by a higher incidence of abnormal growth and development (Gurney et al., 2003), endocrine disorders (Nandagopal et al., 2008), premature cardiovascular disease (CVD) and cerebrovascular disease (Mertens et al., 2001; Oeffinger et al., 2001; Bowers et al., 2006; Mulrooney et al., 2009), as well as declines in neurocognitive function (Moleski, 2000; Kaemingk et al., 2004; Anderson & Kunin-Batson, 2009). Indeed, many adult survivors of childhood cancer are at seven times the risk of dying from CVD compared to the general population (Mertens et al., 2001; Mertens, 2007). Most of the increased risk is thought to be the result of the therapies used to treat the cancer, such as chemotherapy and radiation. These therapies likely cause damage to the endothelial cells, which line the arterial wall and, when functioning properly, offer protection from atherosclerosis (Lüscher & Barton, 1997; Kinlay, Libby, & Ganz, 2001; Drexler, 1998; Halcox & Deanfield, 2004). Research has demonstrated that young adult survivors of childhood acute lymphoblastic leukemia (ALL) have endothelial dysfunction compared to healthy controls approximately 20-years after receiving cancer treatment (Dengel et al., 2008;

Dengel et al., 2014). Additionally, adult survivors of childhood ALL have reduced carotid compliance and distensibility, indicators of arterial stiffness, compared to healthy sibling controls (Dengel et al., 2014). Endothelial dysfunction and arterial stiffness are considered early manifestations of atherosclerosis and therefore may be ideal targets of therapy to reduce CVD risk (Celermajer et al., 1992; Deanfield et al., 2007).

HMG coenzyme A reductase inhibitors, or statins, are widely used for cardiovascular disease risk reduction because of their ability to lower circulating low-density lipoprotein (LDL) cholesterol and triglycerides (Stone et al., 2013). However, there are multiple cholesterol-independent beneficial pleiotropic effects of statins on vascular health (Reriani et al., 2011), including: (1) up-regulation of endothelial nitric oxide synthase resulting in increased production of nitric oxide (Matsuno et al., 2004; Ota et al., 2010); (2) inhibition of arterial smooth muscle cell proliferation (Axel et al., 2000); (3) reduction of arterial stiffness (Orr et al., 2009; Ratchford et al., 2011); (4) inflammation (Ridker et al., 2008); and (5) oxidative stress (Suzumura et al., 1999; Rikitake et al., 2001; Singh et al., 2008). Therefore, statin treatment, independent of cholesterol lowering, may improve endothelial function and other aspects of vascular health in young adult CCS, which could mitigate the medium- and long-term risk of developing CVD.

Despite the potential for improved vascular health and reduced CVD risk, to our knowledge, statin therapy has never been evaluated in young adult CCS. Therefore, the primary objective of this pilot clinical trial was to assess the ability of atorvastatin to improve brachial artery endothelial function (primary endpoint) and other aspects of vascular health including small artery endothelial function, regional arterial stiffness, and

carotid artery elasticity and thickening in young adult CCS over a 6-month treatment period. The focus of the trial was on survivors of hematologic malignancies, ALL and Non-Hodgkin's lymphoma (NHL), as studies have shown ALL to be associated with endothelial impairments and because both ALL and NHL survivors share common treatment exposures (chemotherapy and radiation). We hypothesized that, compared to placebo, atorvastatin would significantly increase brachial artery endothelial function, small artery endothelial function, and carotid artery elasticity, as well as reduce arterial thickness, in young adult survivors of ALL and NHL.

MATERIALS AND METHODS

The study protocol was approved by the University of Minnesota Institutional Review Board (IRB) and the Children's Hospitals and Clinics of Minnesota IRB. All participants provided informed consent for study participation. All testing was performed at the University of Minnesota Clinical and Translational Science Institute (NCT01733953).

Study Population

Twenty-seven young adult survivors of childhood ALL or NHL were recruited to participate in the randomized, double-blind, placebo-controlled, pilot clinical trial. Due to historical treatment heterogeneity among Hodgkin's lymphoma and NHL survivors, adult survivors of childhood Hodgkin's lymphoma were not recruited in the present trial. Inclusion criteria included being between the ages of 18 and 39 years old, having been treated for cancer before 21 years of age, and being ≥ 5 -yrs post-treatment.

Randomization was stratified by receipt of hematopoietic stem cell transplantation (i.e. bone marrow transplant). Initial exclusion criteria included: prior bone marrow transplant (BMT); Type 1 or Type 2 diabetes mellitus; LDL-cholesterol ≥ 130 mg/dL; alanine transaminase (ALT), aspartate transaminase (AST), or creatine kinase (CK) values $>2x$ the upper limit of normal; current or recent (within 6 months) use of lipid-lowering medication; recent initiation (within 6 months) of anti-hypertensive medication (participants on stable therapy were allowed to enroll); current or recent (within 6 months) use of fibric acid derivatives, lipid-modifying doses of niacin, cyclosporine or strong CYP3A4 inhibitors (i.e. clarithromycin, HIV protease inhibitors, and itraconazole); and females who were pregnant or planning to become pregnant. Individuals with elevated LDL-cholesterol were referred for clinical management of dyslipidemia. Exclusion criteria were relaxed approximately halfway through enrollment to include both survivors treated with a BMT in attempts to combat low enrollment. To further combat low recruitment, LDL exclusion at baseline was also relaxed to ≤ 160 mg/dL (originally ≤ 130 mg/dL) to reflect the 2013 ACC/AHA Guidelines on the Treatment of Blood Cholesterol to Reduce Atherosclerotic Cardiovascular Risk in Adults (Stone et al., 2014).

Eligible participants were recruited from the childhood Cancer Survivorship Program (cCSP) at the University of Minnesota and Children's Hospitals and Clinics of Minnesota from 2013-2014 with follow-up lasting until early 2015. Enrollment was ceased once recruit goal and study timeline were met.

Experimental Design and Protocol

Baseline measurements were conducted following initial screening of inclusion and exclusion criteria. Family and personal history, current medical status, and current medication usage were assessed, along with anthropometrics, urine pregnancy test (females), metabolic panel, and vascular function, structure, and mechanical measurements. Cancer diagnosis and specific treatment details were obtained from medical chart extraction.

Following baseline measurements, the Investigational Drug Services (IDS) at the University of Minnesota used the Web site Randomization.com to generate the permuted-block randomization scheme (blocks of 4 and 1:1 allocation ratio). Participants were randomized in a double-blind manner to receive 40 mg of atorvastatin once daily or matched placebo for 6 months. Participants returned at 1 month and 3 months for safety assessments (blood draw and adverse event review) and at 6 months for assessment of safety and follow-up vascular assessment. Anthropometric measurements were obtained at each visit. Participants were asked to fast for a minimum of 12 hours prior to the baseline and 6-month visits. Phone calls were conducted at 2, 4, and 5 months to review interim medical history, changes in concomitant medication, as well as assess adverse events. Participants were instructed to take each dose in the evening along with food. Drug compliance was assessed by counting the number of pills returned at each study visit.

A data and safety review was conducted after approximately half of participants were enrolled, and at the end of the trial. Also, at any given time throughout the study,

the trial was to be stopped if more than 25% of subjects were withdrawn due to elevated enzyme levels. Trial stoppage due to withdrawal did not occur.

Anthropometric and Clinical Assessments

Measurements for height and weight were obtained with an electronic scale (ST Scale-Tronix, Model 5002, White Plains, NY, USA) with participants in light clothing, without shoes. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters-squared. Seated blood pressure and heart rate were measured three consecutive times via automated sphygmomanometer at approximately 3-minute intervals on the right arm following 10-minute of quiet rest. The final two measurements were averaged and used for analysis. Fasting blood samples were obtained for lipids and plasma glucose (analyzed with standard procedures at the Fairview-University Medical Center clinical laboratory).

Vascular Assessments

Testing was performed in the Vascular Biology Laboratory in the University of Minnesota Clinical and Translational Science Institute. All the vascular studies were performed in a quiet, temperature-controlled environment (22-23°C). Participants had fasted for a minimum of 12 hours prior to both baseline and 6-month vascular assessments and were also asked to abstain from caffeine ingestion or cigarette smoking. Baseline vascular assessment was prioritized for the morning hours (between 8:00 AM and 11:00 AM), and 6-month testing was required to be within ± 2 hours of the time of day when baseline testing was assessed.

Endothelial Function

Flow-Mediated Dilation (FMD). Following 15 minutes of quiet rest in the supine position, vascular images of the left brachial artery at the distal third of the upper arm was obtained using a conventional ultrasound scanner (Siemens, Sequoia 512, Mountain View, CA, USA) with a 10.0 MHz linear array probe held at a constant distance from the skin and at a fixed point over the brachial artery by a stereotactic arm (Celermajer et al., 1992). Brachial artery blood flow was determined by pulse-wave Doppler ($HSS = 8 \times 0.035 \times \text{systolic flow velocity} / \text{diameter}$). Following baseline measurements of the brachial artery, a blood pressure cuff was placed on the forearm (distal to the imaged area) and inflated to a suprasystolic level for 5 minutes. The change in brachial artery blood flow and diameter was measured by B-mode ultrasound imaging for 3 minutes after cuff release. Peak dilation during the study was defined as the greatest percent change from resting brachial artery diameter, while area under the curve (AUC) was defined as the total relaxation of the brachial artery from resting baseline following reactive hyperemia or sublingual nitroglycerin administration. Intra-individual reproducibility of FMD within our laboratory has been demonstrated as a mean difference of $0.53 \pm 0.28\%$. Images were digitized and stored on a personal computer for later off-line analysis with an electronic wall-tracking software program (Vascular Research Tools 5, Medical Imaging Application, LLC, Coralville, IA, USA).

Digital Reactive Hyperemia Index (RHI). Reactive hyperemia (EndoPAT2000, Itamar Medical, Caesarea, Israel) was simultaneously measured alongside FMD assessment. After 10 minutes of quiet rest in the supine position (and 5 minutes prior to

FMD), one peripheral arterial tonometry (PAT) finger probe was placed on the index finger of the left hand undergoing hyperemia testing, and a second PAT probe was placed on the contralateral index finger (right). Both probes inflate to apply a uniform pressure on the fingers (10 mmHg less than diastolic blood pressure) and detect small pulse volume changes throughout the cardiac cycle. Following 5 minutes of baseline data collection, a blood pressure cuff on the upper left forearm (just below the elbow) was inflated to a suprasystolic level for 5 minutes. Following cuff release, the change in pulse amplitude during reactive hyperemia was measured for 5 minutes. The ratio of the hyperemic and the baseline pulse amplitude (corrected for the same ratio on the control finger, thereby controlling for autonomic tone due to vasoconstriction and transient environmental effects) was calculated and expressed as the reactive hyperemic index (RHI).

Artery Elasticity and Thickness

Carotid artery images were obtained by a non-invasive ultrasound scanner (Siemens, Sequoia 512, Mountain View, CA, USA) with a 10.0 MHz linear array probe. Following 15 minutes of quiet rest in the supine position, luminal systolic and diastolic diameters were obtained at a fixed point over the left common carotid artery, approximately 1 centimeter proximal from the carotid bulb. Images were collected at 20 frames per second for 10 seconds (200 frames) to ensure the capture of full arterial diameter change during a cardiac cycle. Systolic and diastolic blood pressures were recorded with an automated blood pressure sphygmomanometer during the 10-second carotid measurement. The mean diameter through the 10-second cycle was used to

calculate measures of elasticity, specifically carotid compliance and distensibility. Carotid artery intima-media thickness (cIMT) measures were also obtained using the same technique. The ultrasound scanning system was interfaced with a standard personal computer equipped with a data acquisition card for attainment of radio frequency ultrasound signals from the scanner. Digital image analysis was performed by the same trained reader blinded to group assignments. The following formulas for carotid artery compliance and distensibility were used:

- **Diameter distensibility** (DD, %): $[\Delta D / D_{min}] \times 100\%$
- **Cross-sectional distensibility** (CSD, %): $[(\pi \cdot (D_{max}/2)^2 - \pi \cdot (D_{min}/2)^2) / \pi \cdot (D_{min}/2)^2] \times 100\%$
- **Diameter compliance** (DC, mm/mmHg): $\Delta D / \Delta P$
- **Cross-sectional compliance 1** (CSC1, mm²/mmHg): $[(\pi \cdot (D_{max}/2)^2 - \pi \cdot (D_{min}/2)^2) / (\Delta P)]$
- **Cross-sectional compliance 2** (CSC2, 1/mmHg): $[(\pi \cdot (D_{max}/2)^2 - \pi \cdot (D_{min}/2)^2) / (\pi \cdot (D_{min}/2)^2 \cdot \Delta P)]$
- **Incremental elastic modulus** (IEM, mmHg): $3 \{1 + [\pi \cdot (\maxDiamM/2)^2 / \pi \cdot (\minDiamM/2)^2]\} / CSC1$

Pulse pressure (ΔP) was calculated as the difference between systolic and diastolic pressures. Diameter change (ΔD) was calculated as the difference in arterial diameter at systolic and diastolic pressures. Additionally, D_{max} denotes maximum diameter, and D_{min} denotes minimum diameter.

Regional arterial stiffness was also measured non-invasively by carotid-radial pulse wave velocity (PWV) and aortic augmentation index (AI) (SphygmoCor[®] system,

Sydney, Australia). Carotid-radial PWV and aortic AI were derived by automated algorithms from pressure waveforms obtained by lightly applying a tonometer to the skin. AI is a measure of the relative magnitude of the reflected (or retrograde) pulse wave early in the cardiac cycle. PWV is calculated as distance (m) / transit time (s). The distance was measured between the carotid and radial sites and the sternal notch. The SphygmoCor® system measures the time between the R-wave of the electrocardiogram (ECG) and the feet of the pressure and distension wave respectively at the site of measurement. Higher values of AI and PWV represent increased arterial stiffening.

Sample Size Determination

Our goal was to collect preliminary data to determine the treatment effect and variability estimates to inform the design of a larger clinical trial. Our choice of sample size (n=26) was based upon the number of participants that could be feasibly enrolled in a one-year period and the amount of funding available.

Statistical Analysis

SAS Software Package (Version 9.2, 2009, SAS Inc., Cary, NC, USA) was used for statistical analyses. Baseline results are expressed as mean±standard deviation (SD), unless otherwise stated. The primary analysis was performed on a per-protocol population, which included participants with a treatment compliance level of $\geq 70\%$ of expected doses. An intent-to-treat analysis was also performed on all randomized participants who completed all 6 months of the study according to treatment assignment.

A generalized linear model was used to assess per-protocol treatment effect on

vascular health outcome changes at 6 months (i.e. difference between 6-month and baseline), adjusted for individual baseline values (Frison & Pocock, 1992; Senn, 2006). Mean differences (with 95% confidence intervals) from baseline were also calculated for each treatment group. Brachial artery peak FMD was the primary vascular outcome of interest. Secondary vascular outcome measures were RHI, carotid-radial PWV, aortic AI measured via carotid and radial sites, and carotid artery elasticity and thickness. An alpha value of 0.05 was used to signify statistical significance.

RESULTS

Twenty-seven participants (mean age 26.8 ± 6.2 years; 14 male, 13 female) were randomized. Figure 1 shows participant flow and disposition (CONSORT diagram). Mean baseline demographic, clinical, and vascular characteristics are presented in Tables 1 and 2 for all randomized participants.

Per-protocol treatment effects on vascular outcome changes at 6 months, along with mean differences from baseline by treatment group, are displayed in Table 3. No significant treatment effects were observed for any primary or secondary vascular outcome measures. Specifically, treatment effect on peak FMD and RHI was 2.3 [95% CI: -0.4 to 5.0; $P=0.090$] and 0.37 [95% CI: -0.05 to 0.80; $P=0.083$], respectively. Similar results were observed in the intent-to-treat analysis.

Of the 14 participants randomized to atorvastatin, 5 participants were not used in the per-protocol analysis. Specifically, physician withdrawals ($n=3$), loss to follow-up ($n=1$), and drug compliance $<70\%$ ($n=1$) resulted in 9 atorvastatin-randomized participants analyzed. Of the 13 participants randomized to placebo, 7 participants were

not used in the analysis with physician withdrawals (n=3), loss to follow-up (n=1), unrelated injury (n=1), and self-withdrawal (n=2) resulting in 6 placebo-randomized participants analyzed. Additionally, participant movement during FMD assessment and/or poor ultrasound image quality due to difficult brachial artery anatomy or poor circulation led to some unusable data and thus lowered the analyzable sample size.

A summary of cumulative adverse events by treatment group is displayed in Table 4. Abnormal safety labs included all instances where an elevated safety blood draw (ALT, AST, or CK) required additional follow-up beyond protocol requirements. Miscellaneous adverse events included reports of headache, insomnia, dizziness, rash, urinary tract infection, etc. Total drug compliance was not significantly different between atorvastatin and placebo groups (86.1 ± 11.4 vs. $82.9 \pm 10.7\%$, $P=0.587$), respectively.

DISCUSSION

To our knowledge, this is the first trial to evaluate the effect of atorvastatin on arterial health in adult survivors of childhood cancer. While the vascular benefits of statin therapy in different patient populations are well documented, atorvastatin did not significantly improve arterial health compared to placebo in this trial of young adult survivors of childhood cancer. Unfortunately, the very small number of evaluable participants who completed follow-up limits our ability to draw confident and meaningful conclusions regarding the effect of atorvastatin on artery health in young adult CCS. Indeed, conservative management of adverse events by the study physician led to higher withdrawal than originally anticipated. Additionally, loss to follow-up, self-withdrawal due to time commitment, and poor ultrasound image quality due to participant movement,

difficult brachial artery anatomy, or poor circulation further contributed to a smaller analyzable sample size.

A recent meta-analysis of 46 randomized controlled trials has demonstrated that statin therapy improves endothelial function, as assessed by FMD [standardized mean difference 0.68 (95% CI: 0.46 to 0.90; $P < 0.001$)], as well as venous occlusion plethysmography and coronary infusion of acetylcholine, in a wide array of populations (Reriani et al., 2011). Additional studies in overweight and obese adults have also reported arterial de-stiffening with 80 mg/day of atorvastatin following 30 days (Ratchford et al., 2011) and 12-weeks (Orr et al., 2009) of treatment. In the present study, however, we did not observe such an effect.

Aside from a small analyzable sample size, another possible factor that may have influenced our findings was the inconsistent time of day that vascular testing was performed. Early morning testing was not always a viable option for participants due to conflicting work schedules or longer commutes. Endothelial function is influenced by diurnal variation. Indeed, both in asymptomatic participants and those with established cardiovascular disease, peak FMD is lower in the morning compared with other times of day (Bau et al., 2008; Kollias et al., 2009; Otto et al., 2004), which may be the result of acute increases in augmented sympathetic activation (Muller et al., 1995; Hijmering et al., 2002), hemodynamic changes (Panza et al., 1991), neuro-hormonal factors and increases in coagulation (Tofler et al., 1987) shortly after waking. Similar findings of increased arterial stiffness via pulse wave analysis have been reported in the morning hours (Papaioannou et al., 2006; Bodlaj et al., 2007).

There were several limitations of the present trial. While we exceeded our

recruitment goal, the number of participants with complete data in each treatment group was much smaller than expected due to early withdrawal/termination, loss to follow-up, and inability to analyze some of the vascular images due to poor image quality (e.g. arterial nodules, poor circulation). Second, the time of day when the daily dose was taken was not always in the evening (as recommended). While a meta-analysis of 164 trials examining the effect of statins on LDL-lowering reported average LDL-cholesterol reduction of 3.6 mg/dL smaller with morning vs. evening dosing (Law et al., 2003), statins like atorvastatin and rosuvastatin have longer half-lives and avoid the timing of peak synthesis problem. Specifically, Cilla et al. (1996) reported similar lipid changes with atorvastatin in both morning and evening doses. Third, we did not measure statin concentration in the blood or urine directly to assess drug compliance. And finally, given that CVD outcomes among CCS vary by cancer severity, treatment modalities, sex, age at treatment and number of years since treatment (Shankar et al., 2008; Buizer et al., 2005; van der Pal et al., 2010), future trials should strive to achieve a sufficient sample size to allow for stratification by these factors.

CONCLUSION

Within the context of this small pilot clinical trial, 6 months of atorvastatin treatment did not significantly improve endothelial function, regional arterial stiffness, or carotid elasticity and structure, compared to placebo. However, the present trial results should be interpreted with caution owing to the small sample size. A larger pilot clinical trial is warranted before moving to a full-scale study.

TABLE LEGENDS

Table 1. Baseline Demographic and Clinical Characteristics for All Randomized Participants

Table 2. Baseline Vascular Characteristics for All Randomized Participants

Table 3. Per-Protocol Treatment Effect of Atorvastatin Therapy on Vascular Health

Table 4. Intent-to-Treat Summary of Cumulative Adverse Events (AE) by Treatment Group for All Randomized Participants

Table 1. Baseline Demographic and Clinical Characteristics for All Randomized Participants					
	Mean (SD)				
	Overall (N=27)	Randomized		Completed 6-Month Follow-Up	
		Atorvastati n (n=14)	Placebo (n=13)	Atorvastatin (n=9)	Placebo (n=6)
Age (years)	26.8 (6.2)	26.6 (5.7)	26.9 (6.9)	28.0 (5.7)	26.4 (7.5)
Sex (Male), <i>n</i> (%)	14 (51.9%)	6 (42.9%)	8 (61.5%)	3 (33.3%)	3 (50.0%)
White Race, <i>n</i> (%)	27 (100%)	14 (100%)	13 (100%)	9 (100%)	6 (100%)
Latino/Hispanic, <i>n</i> (%)	1 (3.7%)	0 (0%)	1 (7.7%)	0 (0%)	1 (16.7%)
Current Smokers, <i>n</i> (%)	1 (3.7%)	1 (7.1%)	0 (0%)	1 (11.1%)	0 (0%)
Diagnosis Age (years)	7.2 (5.2)	8.0 (4.6)	6.2 (5.8)	7.8 (3.9)	5.0 (3.2)
Years Cancer Free (years)	17.1 (7.3)	16.1 (6.6)	18.2 (8.1)	17.9 (7.3)	18.4 (7.6)
Cancer Diagnosis, <i>n</i> (%)					
ALL	19 (70.4%)	10 (71.4%)	9 (69.2%)	6 (66.7%)	5 (83.3%)
NHL	8 (29.6%)	4 (28.6%)	4 (30.8%)	3 (33.3%)	1 (16.7%)
Cancer Treatment, <i>n</i> (%)					
Chemotherapy	17 (63.0%)	9 (64.3%)	8 (61.5%)	5 (55.6%)	4 (66.7%)
Chemo + Rad	9 (33.3%)	4 (28.6%)	5 (38.5%)	4 (44.4%)	2 (33.3%)
BMT	1 (3.7%)	1 (7.1%)	0 (0%)	0 (0%)	0 (0%)
Weight (kg)	88.3 (22.1)	89.5 (22.8)	87.0 (22.1)	91.6 (21.5)	72.5 (12.3)
Height (cm)	169.1 (11.7)	168.1 (10.0)	170.2 (13.6)	165.5 (10.9)	169.6 (12.8)
BMI (kg/m ²)	31.0 (7.5)	31.8 (7.9)	30.1 (7.3)	33.6 (7.9)	25.4 (4.8)
SBP (mmHg)	124.2 (14.5)	119.4 (13.3)	129.4 (14.4)	119.0 (14.9)	121.3 (14.2)
DBP (mmHg)	69.7 (11.9)	67.5 (10.1)	72.2 (13.7)	66.8 (11.6)	69.2 (12.3)
Heart Rate (bpm)	67.8 (10.0)	68.8 (7.6)	66.8 (12.3)	69.4 (8.4)	64.7 (11.3)
Fasting Glucose (mg/dL)	79.9 (16.7)	83.9 (10.7)	75.1 (20.9)	84.0 (10.5)	69.5 (30.5)
TC (mg/dL)	174.0 (34.9)	175.9 (40.8)	171.9 (28.9)	190.6 (33.1)	167.3 (26.9)
LDL (mg/dL)	104.0 (31.5)	105.2 (34.0)	102.8 (29.8)	119.4 (25.3)	97.5 (36.1)
VLDL (mg/dL)	23.1 (9.0)	25.4 (9.7)	20.6 (7.9)	28.0 (8.6)	18.5 (6.8)
HDL (mg/dL)	46.8 (10.8)	45.2 (10.2)	48.5 (11.6)	43.0 (9.1)	51.2 (13.8)
Triglycerides (mg/dL)	115.6 (44.6)	127.0 (48.0)	103.3 (38.8)	140.3 (43.3)	93.5 (33.1)
TC/HDL Ratio	3.9 (1.1)	4.0 (1.2)	3.7 (1.1)	4.5 (1.0)	3.6 (1.3)
ALT (U/L)	38.7 (16.2)	36.9 (15.3)	40.7 (17.5)	34.6 (11.6)	27.5 (8.2)
AST (U/L)	29.5 (10.4)	27.4 (11.8)	31.9 (8.5)	26.9 (9.4)	24.8 (2.4)
CK (U/L)	107.4 (53.9)	104.2 (61.6)	110.9 (47.7)	93.7 (54.6)	99.7 (15.9)

Results expressed as Mean (SD) or *n* (%) where indicated. Abbreviations: ALL, acute lymphoblastic leukemia; NHL, Non-Hodgkin's lymphoma; BMT, bone marrow transplant; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; LDL, low-density lipoproteins; VLDL, very low-density lipoproteins; HDL, high-density lipoproteins; ALT, alanine transaminase; AST, aspartate aminotransferase; CK, creatine kinase.

Table 2. Baseline Vascular Characteristics for All Randomized Participants						
	All Randomized (n=27)		Atorvastatin (n=14)		Placebo (n=13)	
	<i>n</i>	Mean(SD)	<i>n</i>	Mean (SD)	<i>n</i>	Mean(SD)
<i>Brachial Artery Ultrasound</i>						
Peak FMD (%)	22	6.7(3.7)	12	6.7(3.4)	10	6.7(4.2)
<i>EndoPAT</i>						
RHI (%)	25	1.90(0.47)	12	2.05(0.47)	13	1.76(0.45)
AI (%)	25	-7.64(9.76)	12	-6.62(11.72)	13	-8.59(7.92)
<i>Carotid Artery Ultrasound</i>						
cIMT (mm)	23	0.55(0.05)	13	0.56(0.04)	10	0.55(0.06)
cDD (%)	25	9.58(2.28)	14	9.58(2.65)	11	9.58(1.84)
cCSD (%)	25	20.13(5.02)	14	20.15(5.83)	11	20.11(4.05)
cDC (mm/mmHg x 10 ⁻³)	25	11.3(2.5)	14	11.7(3.0)	11	10.8(1.6)
cCSC1 (mm ² /mmHg x 10 ⁻³)	25	120.7(28.5)	14	124.2(35.2)	11	116.2(17.3)
cCSC2 (1/mmHg x 10 ⁻³)	25	3.7(0.9)	14	3.8(1.1)	11	3.5(0.6)
cIEM (mmHg)	25	1527.4(421.6)	14	1484.1(444.3)	11	1582.6(404.9)
<i>SphygmoCor</i>						
PWV (m/s)	26	7.9(2.0)	14	8.1(2.5)	12	7.6(1.0)
Carotid AI (P2/P1)	18	116.8(19.2)	9	121.8(23.0)	9	111.8(14.3)
Radial AI (P2/P1)	25	119.0(25.4)	13	118.8(32.4)	12	119.2(16.5)

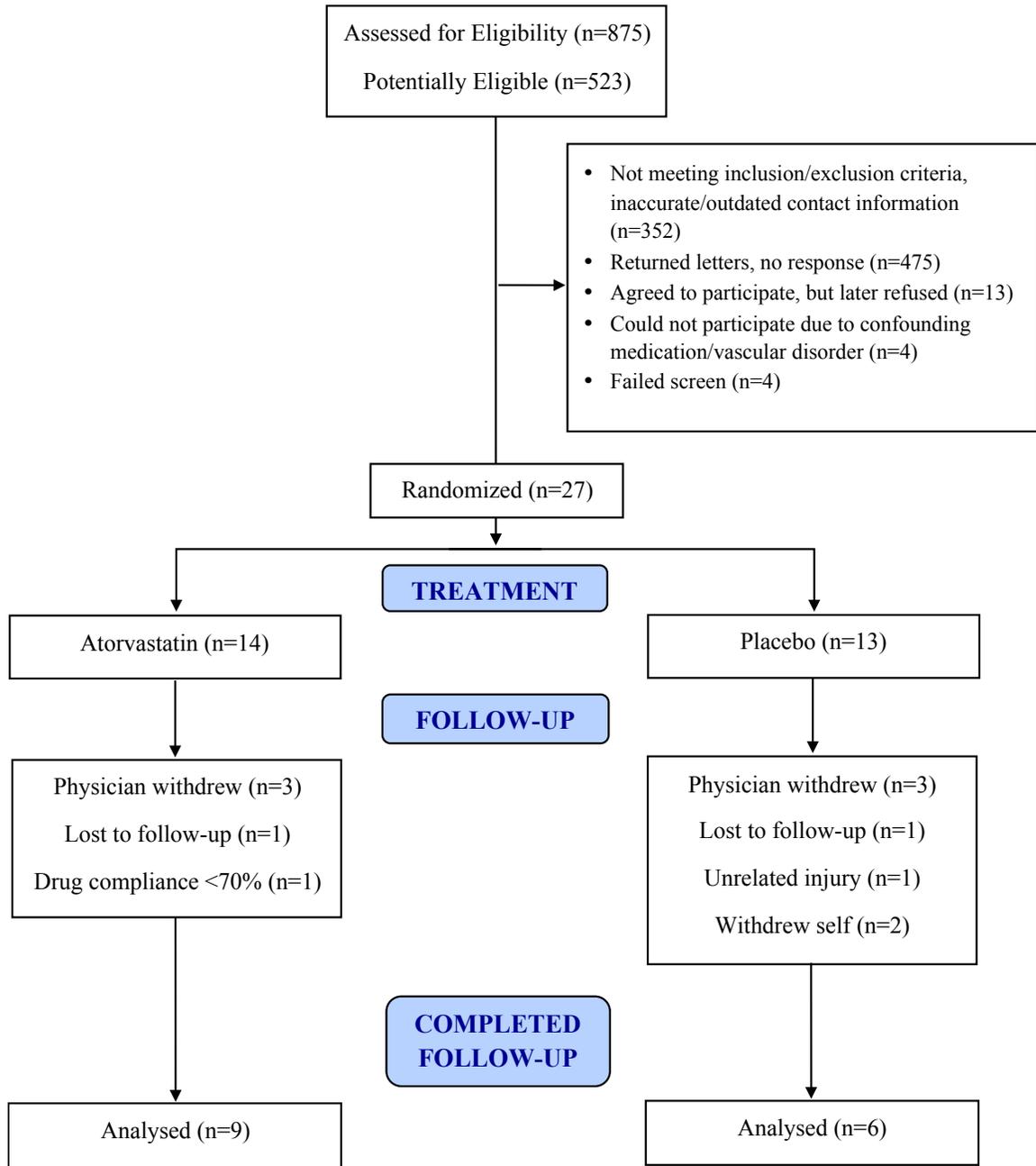
Results expressed as Mean (SD) or *n* (%) where indicated. All reported brachial and carotid artery measurements performed on the left brachial artery and left common carotid artery. “c” denotes carotid artery assessment. Abbreviations: FMD, flow-mediated dilation; RHI, reactive hyperemia index; AI, augmentation index; IMT, intima-media thickness; DD, diameter distensibility; CSD, cross-sectional distensibility; DC, diameter compliance; CSC1, cross-sectional compliance 1; CSC2, cross-sectional compliance 2; IEM, incremental elastic modulus; PWV, pulse wave velocity.

Table 3. Per-Protocol Treatment Effect of Atorvastatin Therapy on Vascular Health									
Outcome Variables	Mean Difference [95% CI] from Baseline				Treatment Effect				
	n	Atorvastatin	n	Placebo	n	Difference (Ref=Placebo)	95% CI		P-value
							LL	UL	
Brachial Artery Ultrasound									
FMD Peak Dilation (%)	8	-0.5 [-2.9, 1.9]	3	-3.4 [-7.3, 0.6]	11	2.3	-0.4	5.0	0.090
EndoPAT									
RHI (%)	9	-0.12 [-0.52, 0.28]	6	-0.01 [-0.51, 0.48]	15	0.37	-0.05	0.80	0.083
AI (%)	9	-2.92 [-10.44, 4.60]	6	7.56 [-1.65, 16.77]	15	-7.12	-18.77	4.52	0.230
Carotid Artery Ultrasound									
cIMT (mm)	9	-0.01 [-0.03, 0.01]	5	0.01 [-0.02, 0.04]	14	0.002	-0.038	0.042	0.922
cDD (%)	9	0.14 [-0.60, 0.87]	5	0.14 [-0.84, 1.12]	14	0.052	-1.137	1.240	0.932
cCSD (%)	9	0.31 [-1.28, 1.90]	5	0.33 [-1.81, 2.47]	14	0.116	-2.469	2.702	0.930
cDC (mm/mmHg x10 ⁻³)	9	-0.34 [-1.4, 0.7]	5	0.57 [-0.8, 2.0]	14	-0.9	-2.66	0.858	0.315
cCSC1 (mm ² /mmHg x10 ⁻³)	9	-1.6 [-14.01, 10.81]	5	8.94 [-7.71, 25.59]	14	-9.71	-31.07	11.65	0.373
cCSC2 (1/mmHg x 10 ⁻³)	9	-0.00222 [-0.39, 0.382]	5	0.18 [-0.34, 0.695]	14	-0.19	-0.82	0.454	0.570
cIEM (mmHg)	9	146.4 [-103.2, 396.0]	5	99.6 [-235.3, 434.4]	14	46.6	-372.4	465.6	0.827
SphygmoCor									
PWV (m/s)	9	-0.52 [-2.06, 1.02]	5	-0.30 [-2.36, 1.76]	14	0.76	-0.871	2.397	0.360
Carotid AI (P2/P1)	6	-0.66 [-15.38, 14.06]	4	-6.01 [-24.16, 12.15]	10	5.35	-18.43	29.13	0.659
Radial AI (P2/P1)	9	6.74 [-5.64, 19.13]	5	-8.34 [-25.29, 8.61]	14	15.09	-6.56	36.73	0.172

Per-Protocol Analysis ($\geq 70\%$ drug compliance) was performed on both treatment groups. Participant data was not included as part of per-protocol analysis if participant did not finish the trial or had insufficient or uninterpretable data. All reported brachial and carotid artery measurements performed on the left brachial artery and left common carotid artery. “c” denotes carotid artery assessment. FMD, flow-mediated dilation; RHI, reactive hyperemia index; AI, augmentation index; IMT, intima-media thickness; DD, diameter distensibility; CSD, cross-sectional distensibility; DC, diameter compliance; CSC1, cross-sectional compliance 1; CSC2, cross-sectional compliance 2; IEM, incremental elastic modulus; PWV, pulse wave velocity.

Table 4. Intent-to-Treat Summary of Cumulative Adverse Events (AE) by Treatment Group for All Randomized Participants			
AE Categories	1-Month Visit	3-Month Visit	6-Month Visit
	Atorvastatin (n=14)		
Musculoskeletal	3 (21%)	5 (36%)	6 (43%)
Gastrointestinal	3 (21%)	3 (21%)	3 (21%)
Fatigue	0 (0%)	1 (7%)	1 (7%)
Cognitive	0 (0%)	0 (0%)	0 (0%)
Abnormal Safety Labs	3 (21%)	3 (21%)	3 (21%)
Respiratory	3 (21%)	7 (50%)	8 (57%)
Miscellaneous	4 (29%)	8 (57%)	11 (79%)
Placebo (n=13)			
Musculoskeletal	0 (0%)	3 (23%)	4 (31%)
Gastrointestinal	2 (15%)	2 (15%)	3 (23%)
Fatigue	1 (8%)	2 (15%)	2 (15%)
Cognitive	1 (8%)	2 (15%)	2 (15%)
Abnormal Safety Labs	4 (31%)	4 (31%)	4 (31%)
Respiratory	2 (15%)	5 (38%)	6 (46%)
Miscellaneous	2 (15%)	4 (31%)	6 (46%)

Figure 1. Consort Diagram



CHAPTER 6. CONCLUSION

Research Results and Implications

Non-invasive assessment of carotid arterial elasticity and stiffness has been extensively studied over the past several decades. While there appears to be a reliable relationship between risk factors such as advanced aging and hypertension with decreased arterial elasticity and increased stiffness, studies of carotid arterial elasticity in otherwise healthy populations are lacking. The present dissertation offers a more detailed understanding of arterial elasticity and stiffness and how to non-invasively assess carotid artery mechanics detailed in three separate studies with one common theme. The main findings of the three studies presented in this dissertation demonstrate the ease and feasibility of accurately assessing carotid artery elasticity and stiffness, as well as extends previous knowledge age and sex differences in healthy adolescents and adults.

First, carotid artery elasticity in children and adolescents was assessed and differences between pubertal stages were evaluated. Results indicated that carotid artery elasticity did not differ significantly throughout pubertal development and that accounting for pubertal stage when reporting vascular data in children and adolescents may be unnecessary. Vascular elasticity and stiffness impairment is more prevalent with increasing age and thus, evaluating differences in carotid artery elasticity and stiffness in otherwise healthy youth may not be a sensitive enough measure for detecting vascular dysfunction. Indeed, pubertal stage and the cardiometabolic risk factors associated with puberty (specifically insulin resistance mid-puberty) did not seem to affect vascular mechanics in our study.

Second, carotid artery elasticity in both adolescents and adults was assessed and

examined for sex differences. Results indicated that adolescent males and females did not significantly differ with respect to carotid artery elasticity measures. Conversely, adult females had significantly greater cross-sectional compliance compared to males. While the exact mechanisms for the sex difference observed in adults is not clear, a greater supine pulse pressure observed in males in addition to a possible greater overall vessel distention in females may explain part of the observation. Interestingly, significant correlations between brachial and carotid artery measures of elasticity were more common among youth than in adults. Indeed, vascular remodeling increases with age and may explain the divergent relationship between nearby vascular beds in adults.

And finally, the effect of 6-months of atorvastatin on carotid artery elasticity and stiffness was assessed in a group of adult survivors of childhood cancer in the third and final study. While the results of this study indicate that a low-dose of atorvastatin does not improve carotid elasticity and stiffness compared to placebo, results should be interpreted with caution due to the small number of evaluable participants. Nonetheless, this was a groundbreaking study to examine the feasibility of conducting such a trial in adult survivors of childhood cancer. Indeed, recruitment and execution of studies in cancer survivors are particularly difficult due to low patient retention and general lack of willingness to participate.

This dissertation establishes an argument that measures of carotid artery elasticity lacks differentiation throughout development in youth, yet sex differences in carotid elasticity may become more apparent in adulthood. While inconclusive, atorvastatin did not improve carotid artery elasticity compared to controls as previously hypothesized.

Future Research

While this dissertation demonstrates that sex and pubertal stage differences may not be different otherwise healthy youth, future studies should continue to gather these measures to confirm these findings. Specifically, longitudinal studies examining carotid artery elasticity should be employed in youth to determine possible risk factors that contribute to possible differences seen in adulthood. Additionally, future studies in higher risk youth should also be engaged to better understand physiologic differences in vascular remodeling under such conditions.

While findings in the atorvastatin trial were difficult to confidently interpret, the primary goal of the study was to assess trial feasibility and estimate a treatment effect for future studies wanting to expand on the effect of statin therapy on vascular function. Indeed, our treatment effect indicates that a much larger sample size is needed to adequately answer the question of whether atorvastatin can improve vascular function, structure, and mechanics.

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