THE EFFECTS OF UNI-LATERAL IMMOBILIZATION ON ARTERIAL VASCULAR COMPLIANCE AND ENDOTHELIAL FUNCTION

THE EFFECTS OF 12 DAYS OF UNI-LATERAL IMMOBILIZATION ON ARTERIAL VASCULAR COMPLIANCE AND ENDOTHELIAL FUNCTION IN HEALTHY YOUNG HUMANS

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ABSTRACT

Physical inactivity or deconditioning has been shown to be an independent risk factor for cardiovascular disease. Contrary to the previously demonstrated effects of exercise training on the cardiovascular system, the vascular adaptations that occur with a deconditioned state have not been adequately characterized within a young, healthy population. Thus, it was the interest of the present study to examine vascular adaptations to 12 days of unilateral lower limb immobilization (ULI) in young, healthy humans. Previous studies have used other models to mimic a deconditioned state such as paraplegia, simulated microgravity and bed rest; however, such models are also associated with factors that are not physiologically applicable to normal deconditioning in the able-bodied population. Fifteen young, healthy participants [age: 20.6±0.51 (mean ± SEM)] participated in the 12-day knee-braced immobilization period that consisted of PRE and 12-DAY time point testing sessions. Measurements of supine common carotid, popliteal and common femoral artery cross sectional compliance as well as popliteal artery endothelial function (using flow mediated vasodilation (FMD)) were acquired prior to the 12-day immobilization (PRE) and on the 12th day of the immobilization (12-DAY). Arterial characteristics of the Immobilized legs (IMM) and NON-Immobilized (NIM) legs were assessed by echo Doppler ultrasound and applanation tonometry.

Resting carotid artery cross sectional compliance and blood flow showed no change throughout the 12-day time period, (Compliance: PRE = 0.001209 ± 0.000067 mm^2/mmHg, 12-DAY= 0.001230 ± 0.00085 mm^2/mmHg; Blood Flow: PRE = 242.8±14.2 mL/min, 12-DAY = 226.0±14.27 mL/min). Popliteal artery cross sectional

compliance decreased significantly over the 12 day time period in both legs ($p<0.05$) (IMM PRE = 5.7±0.4 $10^{-4}mm^2/mmHg$, IMM 12-DAY = 3.8±0.4 $10^{-4}mm^2/mmHg$; NIM PRE = 6.7±0.9 $10^{-4}mm^2/mmHg$, NIM 12-DAY = 5.5±0.6 $10^{-4}mm^2/mmHg$). Common femoral artery cross sectional compliance decreased in the immobilized leg but not in the non-immobilized leg ($p<0.05$) over 12 days of immobilization (PRE = 1.2±0.1 $10^{-4}mm^2/mmHg$, 12-DAY =: 0.79±0.1 $10^{-4}mm^2/mmHg$). Neither popliteal nor common femoral artery mean blood flow changed throughout the 12 days of immobilization. Popliteal arterial mean diameter decreased significantly over time in both the IMM and NIM legs showing greater decreases in the IMM leg, while common femoral arterial mean diameter decreased in both the IMM and NIM legs through the 12 days (Popliteal: IMM PRE = 0.57±0.02 cm, IMM 12-DAY = 0.50±0.02 cm; NIM PRE = 0.59±0.02 cm, NIM 12-DAY = 0.55±0.02 cm; Common Femoral IMM PRE = 0.83±0.04 cm, IMM 12-DAY = 0.77±0.03 cm; NIM PRE = 0.81±0.03 cm, NIM 12-DAY = 0.77±0.03 cm). Popliteal artery endothelial function, calculated as both relative FMD and FMD normalized to shear stress, increased ($p<0.05$) throughout the 12 days in the immobilized leg while showing no change in the non-immobilized leg (Relative FMD: IMM PRE 6.0 ± 1.4 %, IMM 12-DAY = 12.6 ± 2.7%; NIM PRE = 5.8 ± 1.4%, NIM 12-DAY = 8.3 ± 1.6 %; Normalized FMD: IMM PRE = 0.023 ± 0.007%/$^{sec-1}$, IMM 12-DAY = 0.037 ± 0.008 %/$^{sec-1}$; NIM PRE = 0.016 ± 0.003 %/$^{sec-1}$, NIM 12-DAY = 0.022 ± 0.004 %/$^{sec-1}$).

In conclusion, 12 days of deconditioning by ULI was able to cause structural and functional changes in the arteries of the immobilized leg, but not the central elastic artery in the neck in healthy young humans. Specifically in the legs, a decrease in arterial

compliance, increases in mean blood velocity and increases in endothelial function were noted, with no change in volumetric blood flow. Surprisingly, our results suggest, with regards to endothelial function, that the vascular effects of deconditioning are not simply the inverse of exercise training which also shows increases in endothelial function. Thus the present study concludes that there exists a very short time course to arterial adaptations in healthy young humans with significant changes within the vasculature occurring within 12 days of deconditioning.

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Best of luck to all of you!
Sincerely,
Jenn C

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CVD	Cardiovascular Disease
SBP	Systolic blood pressure
DBP	Diastolic blood pressure
AC	Arterial Compliance
PP	Pulse Pressure
VSMC	Vascular smooth muscle cell
NO	Nitric Oxide
PWV	Pulse wave velocity
ULI	Unilateral Lower-limb Immobilization
ULLS	Unilateral Lower-limb Suspension
EF	Endothelial function
eNOS	Endothelial nitric oxide synthase
FMD	Flow mediated dilation
CAD	Coronary artery disease
MBV	Mean blood velocity
BV	Blood velocity
d	Diameter
r	Radius
v	Velocity
0	Angle of insonation
c	Speed of sound in tissue

f_d	Frequency difference
f_t	Transmitted frequency
f_r	Received frequency

1.0 INTRODUCTION AND HYPOTHESIS

1.1 Introduction

A sedentary lifestyle has been established as an independent risk factor for atherosclerosis and cardiovascular disease (CVD), and both have been correlated to each other through numerous epidemiological investigations (Blair *et al.*, 1995). Some forms of CVD have been reported to be related to the inner most layer of the vasculature, the endothelium, and a high correlation has been found between endothelial dysfunction and cardiovascular risk factors such as hypertension (Laurent, 1990; Taddei *et al.*, 1995), hypercholesterolemia (Steinberg *et al.*, 1997), cigarette smoking (Celermajer *et al.*, 1994), diabetes (Cosentino & Luscher, 1998), and aging (Celermajer *et al.*, 1994; Muller-Delp *et al.*, 2002). Inadequate functioning of the endothelium, or endothelial dysfunction, plays a critical role in the pathogenesis of cardiovascular disease and is related to cardiovascular mortality, (Landmesser *et al.*, 2004).

Studies have shown that exercise training is associated with several changes in vascular structure (Masuda *et al.*, 1989). Arterial compliance (AC) is a measure of the ability of an artery to store energy from volume and is important when conditions of pulsatile flow exist. In extreme states of inactivity, such as paraplegia, compared to sedentary and endurance trained athletes, paraplegic subjects demonstrate decreased arterial compliance in the affected limbs while athletes show the highest (Schmidt-Trucksass *et al.*, 2000). Studies have also shown that central thoracic artery compliance decreases with advancing age (Cameron & Dart, 1994) and that decreases in arterial

compliance are correlated to hypertension and cardiovascular disease (Rush *et al.*, 2005). A decrease in compliance of arteries has been identified as an independent risk factor for the development of cardiovascular disease (Rush *et al.* 2005). Since compliance of the arteries is determined by both the intrinsic elastic properties of the and by the tone of smooth muscle cells, it is speculated that structural changes within the vasculature, as well as changes in sympathetic tone of the smooth muscle cells that surround the artery, are responsible for any alterations in compliance (Langille *et al.*, 1993; Masuda *et al.*, 1989). Compliance can also be modified by changes in blood flow to the limb and thus a decrease in the vasoactive substances (nitric oxide) that allow a more distensible and compliant state (Langille *et al.*, 1993).

Studies have shown that the arterial structural and functional alterations are correlated to changes in the blood flow through the artery (Masuda *et al.* 1989). Changes in blood flow, brought upon by either increases or decreases in activity or other modifications, alter local wall shear stress levels which has been shown to stimulate vascular adaptations (Langille *et al.*, 1993). Chronic increases in wall shear stress are associated with an outward remodeling of the blood vessels while decreases cause the opposite (Masuda *et al.* 1989). Previous studies have shown that prolonged upper limb immobilization results in decreases in arterial compliance compared to a control limb (Giannattasio *et al.*, 1998), however studies have yet to determine the mechanistic relationship between shear rate, arterial compliance and endothelial function in humans. Studies have used inactive muscles from paraplegic individuals to demonstrate the model of extreme disuse and atrophy (Schmidt-Trucksass *et al.*, 2000); however, such a model

may not be physiologically relevant to study vascular adaptations to inactivity due to the altered vascular innervation and neurohumoral changes that accompany spinal cord injury. Thus, the present study will examine a muscle disuse model of inactivity to study the effects that such a condition has on the vascular system.

Although the effects of physical activity on the structure and function of the arterial vasculature has been extensively studied (Clarkson *et al.*, 1999; Dinenno *et al.*, 2001; O'Sullivan, 2003; Green *et al.*, 2004; Bleeker *et al.*, 2004), the effects of physical inactivity, in the form of deconditioning, on the vascular system, specifically endothelial function and arterial vascular compliance, have yet to be adequately examined in humans. While it has been demonstrated in healthy, young mice that physical inactivity causes endothelial dysfunction (Jasperse *et al.*, 1999), we are only aware of one study within the literature examining the effects of physical inactivity, or deconditioning, on vascular function in healthy young humans (Bleeker *et al.*, 2005a) . Such a study was able to conclude that within the human population, endothelial function does not show a decrease as animal models have previously shown (Bleeker *et al.*, 2005a)

It has been documented that muscle disuse leads to muscle atrophy and models such as limb un-weighting (Berg *et al.*, 1991; Dudley *et al.*, ; Adams *et al.*, 1994; Berg & Tesch, 1996), bed rest (LeBlanc *et al.*, 1992), microgravity (Hikida *et al.*, 1989) and immobilization (Hortobagyi *et al.*, 2000), have been shown to cause numerous functional adaptations within the affected muscle such as decreases in muscle strength and muscle atrophy (Hortobagyi *et al.*, 2000; Berg *et al.*, 1991). Loss of muscle mass or muscular atrophy is one of the most documented consequences of muscle disuse. Several studies

have concluded a loss of muscle strength resulting from muscle atrophy is very quick with rates of 1–6% per day during the first week (Vandenborne *et al.,* 1998). Muscle disuse has also been shown to cause significant metabolic and neurological alterations within the affected muscle groups such as muscle atrophy, orthostatic intolerance and decreased sympthatic control (Vandenborne *et al.,* 1998; Bonnin *et al.,* 2001; Shoemaker *et al.,* 1998a). It thus seems likely that the structural and functional changes that occur at the muscle level would be accompanied by changes in vascular structure and function.

Cross-sectional studies have confirmed decreases in vascular dimensions (Huonker *et al.*, 2003) and decreases in endothelial function (DeSouza *et al.*, 2000) in sedentary subjects compared to exercise-trained counterparts. Such findings may either indicate a down-regulation of vascular functioning accompanying physical inactivity or could represent an up-regulation of the vasculature through exercise training (Bleeker *et al.,* 2005a).

Longitudinal deconditioning intervention studies have shown disadvantageous effects of bed rest models of deconditioning on muscle function (Berg & Tesch, 1996; Bloomfield, 1997), and orthostatic tolerance (Takenaka *et al.*, 1994; Delp *et al.*, 2000). Vascular adaptations to physical deconditioning have utilized mainly measurements of blood flow and have focused primarily on the vascular bed of the upper extremities (Giannattasio *et al.,* 1998); however, the results that the majority of deconditioning studies show are confounded by the effects of microgravity and thus the effects on plasma volume become a confounding variable (Convertino *et al.*, 1989; Shoemaker *et al.*, 1998a; Shoemaker *et al.*, 1998b). Due to their functioning in standing and

locomotion, the legs are therefore more reflective of the effects of deconditioning interventions.

Unilateral Lower Limb Suspension (ULLS) is a model to elicit deconditioning and muscle adaptations to weight unloading of the lower extremities in human subjects (Berg *et al.*, 1991). The ULLS model allows for one limb of the lower leg to be free of weight-bearing while the subject uses crutches for locomotion. Previous studies have validated its effectiveness in inducing muscle atrophy and decreases in muscle strength (Berg *et al.*, 1991; Dudley *et al.*, 1992) and thus the ULLS model should be an effective model to study the potential effects of deconditioning on the vascular system without the potential confounding effects of microgravity and denervation.

The present study has specifically chosen Unilateral Lower-limb Immobilization (ULI) to study the arterial adaptations to physical inactivity. ULI corresponds to a ground-based protocol distinct from that of models of microgravity and dissimilar to injury-induced denervation such as in spinal cord injury (SCI) models. The model of ULI we specifically chose strongly resembles unilateral lower limb suspension (ULLS), which was initially developed in attempts to investigate effects of unloading on muscle and to imitate unloading in spaceflight. Both ULI and ULLS are based upon the avoidance of all weight-bearing in the limb being assessed while the participant relies on the support of crutches for unilateral support of the control leg (Berg *et al.*, 1991). The ULLS model has been proven to successfully induce deconditioning of the suspended leg with several studies indicating muscle atrophy and decreases in strength (Berg *et al.*, 1991; Dudley *et*

al., 1992). The ULI model was used in the present study to test the hypotheses that deconditioning would cause a decrease in arterial compliance, diameter and blood flow of both the popliteal and common femoral artery and a decrease in endothelial function throughout the 12-day time course. The ULI model is more suitable than the ULLS model since only a knee brace and crutches are required. Moreover, the concern of deep vein thrombosis is reduced with the ULI model compared to the ULLS model due to the fact that popliteal and femoral blood flows are not reduced. The preceding reasons justify the preference for using the ULI model over the ULLS model.

1.2 Summary and Hypotheses

The focus of the present study was to assess the impact of leg immobilization in the form of ULI on arterial endothelial function and compliance. We chose to include a group of immobilized subjects over a group of paraplegic subjects due to the fact that the paraplegic model may result in changes in vascular structure and function which are a result of both muscle disuse and altered vascular innervation and neurohumoral components (de Groot *et al.*, 2004). Thus, the ULI model is a more valid representation of a physically deconditioned lifestyle in a young, healthy and able bodied population.

The present research study addressed the impact of ULI on endothelial function and arterial compliance in both an immobilized (IMM) and NON-Immobilized (NIM) leg as well as arterial compliance in the common carotid artery as a representation of central cardiovascular structure. We hypothesized that 12 days of ULI would result in: a decrease in endothelial function, resting blood flow, and arterial compliance in the

immobilized leg compared to the control leg; no change in compliance or resting blood flow of the carotid artery.

Literature suggests that vascular dysfunction and thus the impairment of cardiovascular regulation, is an independent risk factor and predictor of cardiovascular disease (Moyna & Thompson, 2004). To our knowledge, whether arterial structural and functional properties are modified by decreases in activity, rather than becoming manifest only during exercise training has yet to be investigated. In the present study, we will address this issue by measuring endothelial function and arterial compliance in subjects in whom 1 leg will be immobilized in a brace for 12 days.

2.0 REVIEW OF LITERATURE

2.1 The Arterial Endothelium

The arterial vascular system has two main functions within the human body that are tightly correlated with each other: firstly the system is required to deliver an adequate supply of blood to all tissues of the body and secondly the system transforms the pulsatile ejection of blood from the left ventricle of the heart to a smooth and continuous peripheral flow (Izzo & Shykoff, 2001). The anatomy of the arterial vessel wall is divided into three main components that include the tunica intima, the tunica media and the tunica adventitia, (Hürlimann *et al.,* 2002). The tunica intima consists of a single layer of endothelial cells that line the lumen of the vessel and thus are direct contact with the blood. Next is the tunica media, which is composed of smooth muscular tissue and lastly

is the tunica adventitia which composes the external elastic lamina, terminal nerve fibers and also connective tissue that surrounds the blood vessel (Hürlimann *et al.*, 2002). The regulation of vasomotor tone depends on the adequate interaction of each of the three layers with each other. The function of all three components together is changed in pathologies such as atherosclerosis and other vascular diseases.

The endothelial layer of the blood is especially critical to the regulation of vasomotor tone in that it is an active organ that exerts its effects on the vasculature by secreting numerous mediators, either directly into the bloodstream (luminally) or to the surrounding smooth muscle cells of the tunica media (Hürlimann *et al.*, 2002). The endothelium is strategically placed within the vessel wall between the circulating blood and the vascular smooth muscle cells of the tunica media. The structure and function of the vasculature is mainly determined by the vascular smooth muscle cells and the endothelium. The endothelial cells have both direct and indirect means of exerting their effects on the vasculature. Not only does the endothelium regulate vascular tone and ultimately blood pressure, but it also plays very important roles with regards to its antithrombotic properties, its interactions with the circulating blood and, most importantly, its action as a paracrine organ, secreting vasoactive substances that regulate these varied functions (Cooke, 2000).

Endothelial cells are a critical source of important mediators that control the contractile state of the vascular smooth cells and also modulate platelet coagulation, function and monocyte adhesion to the vessel wall. A healthy and intact endothelium is able to produce and release both vasoconstricting and vasodilating substances (Furchgott

& Zawadzki, 1980). In the pathological condition of atherosclerosis and in the presence of cardiovascular disease risk factors, such as smoking (Zeiher *et al.,* 1995), diabetes (Johnstone *et al.,* 1993), hypercholesterolemia (Chowienczyk *et al.,* 1992) and hypertension (Linder *et al.,* 1990), there is a decreased functioning of the endothelial layer. Endothelial dysfunction may even contribute to vasoconstriction of the vessel, enhanced adhesion of platelets and monocytes and proliferation of vascular smooth muscle cells (Hürlimann *et al.,* 2002). Each of the preceding consequences of a dysfunctioning endothelium are known to be key early events in the development of atherosclerosis, (Hürlimann *et al.,* 2002).

Nitric oxide (NO), otherwise referred to as 'endothelium-derived relaxing factor', is released from the endothelium in response to shear stress brought about by the flow of blood in contact with the cells and also through the activation of surface receptors (Rubanyi *et al.,* 1996). The role of NO has been reported to be critical and perhaps the most important of the secretions from the endothelium within the vasculature as this substance plays a key role in the regulation of vasomotor tone (Ignarro *et al.,* 1987). Acetylcholine, bradykinin, and shear stress, act on the receptors on the endothelial cell surface and such an interaction causes intracellular calcium influx which in turn activates eNOS enzyme (Moncada *et al.,* 2002; Palmer *et al.,* 1988). NO is produced by the enzyme nitric oxide synthase (NOS) from the conversion of its precursor L-arginine and is in the free radical family and thus is able to cross biological membranes quickly and with ease (Palmer *et al.,* 1988). When NO diffuses from the endothelial cells into the smooth muscle cells of the vessel, its presence stimulates an increase in intracellular

cyclic-GMP through the increased activation of the enzyme guanylate cyclase, which ultimately leads to the relaxation of the smooth muscle cells and finally dilation of the vessel (Hürlimann *et al.*, 2002).

The conversion from L-arginine to NO can be inhibited by substances that act as artificial substrates for NOS, such as NG-monomethyl-L-Arginine (LNMMA). When inhibition of NO synthesis occurs, an increase in arterial blood pressure results due to the fact that the resting release of NO from the endothelium determines vasomotor arterial tone (Hürlimann *et al.*, 2002). Changes within the endothelium-derived L-Arginine/NO biological pathway may potentially be critical in pathologies such as cardiovascular disease due to the fact that NO is able to halt numerous processes within the atherogenic process leading to and causing the disease condition, (Hürlimann *et al.*, 2002). Either an increase in the breakdown of NO or a decrease in NO synthesis have the potential to lead to endothelial dysfunction and eventually atherosclerosis, and thus a process that decreases the ability of the endothelial cells to produce NO is a prospective accelerator of the process of atherosclerosis (Hürlimann *et al.*, 2002).

The functioning of the endothelium is most commonly measured as the ability of the artery to dilate its internal lumen diameter in response to physical or pharmacological stimuli. Physiological stimuli, such as shear stress, and pharmacological stimuli, such as acetylcholine, and serotonin, each exerts its effects on the endothelium through a cellular membrane receptor and transduces its signal through G-protein signal transduction, (Lüscher *et al.*, 1990). The ability of the artery to distend in response to the specific stimulus being applied is termed endothelial-dependent vasodilation which can be

measured directly in the coronary arteries, and also within the peripheral vasculature such as the brachial artery and other major arteries in the legs, (Lüscher *et al.,* 1990). High-resolution ultrasound presents a non-invasive method for measuring endothelial function and allows for insight into the pathogenesis of atherosclerosis, the earliest phase of which is a dysfunctioning endothelium, (Atkov *et al.,* 1998).

2.1.1 Endothelial Dysfunction as Pathology

The main outcome of a dysfunctioning endothelium is a decrease in the bioavailability of endothelium-derived NO and is directly associated with the vascular smooth muscle cells within the endothelium. The bioavailability of NO is able to be altered in three ways. These include: 1) a change in the availability of the NO precursor molecule L-arginine; 2) alterations in the rate of NO synthesis which is dependent on endothelial nitric oxide synthase (eNOS) expression, and (3) changes in the rate of NO breakdown in relation to the available reactive oxygen species (ROS) (Gielen & Hambrecht, 2001).

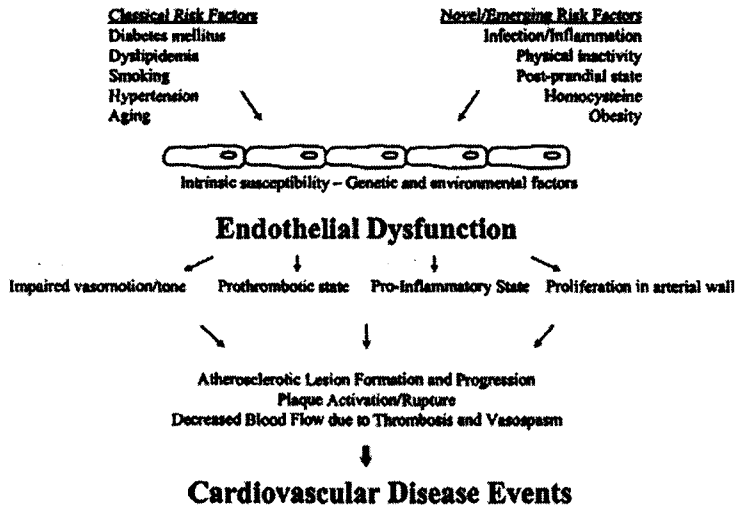
Interestingly, endothelial dysfunction has furthermore been correlated to an increase in oxidative stress (Gielen & Hambrecht, 2001) such that a disproportionate production of ROS is unable to be protected against by endogenous antioxidant defense mechanisms. Such an occurrence has been linked to the pathogenesis of many cardiovascular diseases. The pathogenesis of CVD has been shown to be mediated by NO bioavailability such that when NO bioavailability is reduced, endothelial dysfunction progressively becomes worse. In the state of excess free radical concentrations, interaction with NO molecules causes NO to become an inactive vasodilator. Such a

situation has the potential to lead to a reduced NO bioavailability and thus a decreased endothelial function (Cai & Harrison, 2000).

Endothelial dysfunction has been previously correlated to the pathogenesis of atherosclerotic vascular disease and acute cardiovascular events (Glasser *et al.,* 1996). In addition, it has been established that a reduction in the functioning of the endothelium occurs early in the process of atherogenesis prior to any histological or angiographic evidence of atherosclerosis (Mano *et al.,* 1996). Endothelial dysfunction also has prognostic implications and is associated with an increased risk of future cardiovascular events (Glasser *et al.,* 1996). It has been found that a decrease in endothelial function has negative functional costs and is also associated with harmful long-term physical properties associated with the vasculature including remodeling of the arterial vascular wall (Cameron *et al.,* 1994). It is generally accepted that a reduced bioavailability of NO is an important consequence of endothelial dysfunction and such an occurrence is thought to contribute to the development of atherosclerosis, (Ross, 1993).

Endothelial function is related to a modification in the phenotype of the endothelium and therefore it has the potential to contribute to the pathology of atherosclerosis and cardiovascular disease (Widlansky *et al.*, 2003). Endothelial dysfunction occurs as a result of a continuous loop that involves the inflammatory response, thus producing inflammatory factors that promote monocyte and T-Cell adhesion to the vessel wall, foam cell formation, extra-cellular matrix degradation, and smooth muscle cell migration and proliferation, all of which ultimately form atherosclerotic plaque buildup (Libby *et al.,* 2002). The relationship between endothelial

function and CVD and its associated risk factors is demonstrated in Figure 2.2 below. The risk factors associated with CVD promote a negative effect on endothelial homeostatic functions and contribute to the progression and expression of atherosclerosis and, ultimately, CVD (Widlansky *et al.*, 2003). The endothelium's response to CVD risk factors could potentially be connected to other factors such as genetics and dietary and exercise factors (Widlansky *et al.*, 2003).



(Widlansky *et al.*, 2003)

Figure 2.1 The role of endothelial dysfunction in the pathogenesis of cardiovascular disease events.

Since there is a direct association between endothelial dysfunction and atherosclerosis, there are many methods by which endothelial dysfunction can be evaluated in human research.

2.1.2 Methods of Measuring Arterial Endothelial Function

One common method of measuring endothelial function examines endothelial-dependant vasodilation. This method requires the stimulus that increases the production of endothelium-derived NO through the increase in blood flow and thus vascular wall shear stress, and has been known to be an effective and accessible indictor of endothelial functioning, (Atkov *et al.*, 1998). The basal release of NO can also be measured by using specific inhibitors of NOS, (Atkov *et al.*, 1998). Previous measures of endothelial dysfunction have also examined the vascular responses to infusions of acetylcholine (ACh) (Ludmer *et al.*, 1986) or increases in blood flow (Cox *et al.*, 1989).

When the endothelium is healthy, it responds to various stimuli by increasing the production of vasodilating factors, such as NO, which ultimately result in an increased lumen diameter (Widlansky *et al.*, 2003). In diseased states, such as CVD, patients show impaired coronary flow-mediated vasodilatory capacity and also a response to ACh that induces vasoconstriction as an alternative to the normal vasodilation. This vasoconstrictor response to ACh has been speculated to be due to a decrease in NO production and an impaired response of the vascular smooth muscle cells to ACh (Ludmer *et al.*, 1986).

Endothelial function may also be assessed using the non-invasive method of flow-mediated dilation (FMD) of conduit arteries in the human vascular system (Atkov *et al.*, 1998). This method of measuring endothelial function depends on the production of NO by the endothelial cells and also reflects the release of other vasodilators (Widlansky *et al.*, 2003). FMD is used frequently to assess endothelial function due to the fact that it is

non-invasive and can be applied to large and varied groups of individuals (Widlansky *et al.*, 2003).

The reproducibility of Doppler Ultrasound methods of evaluating vascular function throughout the literature has been known to vary between 1% to 1.5% for measures of diameter, from 9% to 18% for resting and hyperemic flow measurements and 15% to 16% for measurements of FMD (Demolis *et al.*, 1991; Hijmering *et al.*, 2001; Boot *et al.*, 2002).

2.1.3. Effects of Physical Activity on Endothelial Function

Proper function of the vascular endothelium is critical for the maintenance of the health of the vessel wall and vasomotor control at both the conduit and resistance vessel level (Blair *et al.*, 1995). Physical activity has been shown to reduce cardiovascular morbidity and mortality (Blair *et al.*, 1995) However, until recently, mechanisms of such a benefit have remained elusive. Physical activity has been shown to prevent cardiovascular disease (Blair *et al.*, 1995); moreover, physical training in the form of endurance training has proved to have beneficial effects on the cardiovascular system, increasing the functioning of the endothelium (Moyna & Thompson, 2004), inducing expansive arterial remodeling, and an increased resting blood flow (Dinenno *et al.*, 2001; Huonker *et al.*, 2003).

The improvement in endothelial function reported in studies examining the effects of exercise training on vascular function has been proposed to be the key beneficial effect that training has on the risk for developing cardiovascular disease (Moyna & Thompson,

2004). Healthy vessels are capable of accommodating to increases in blood flow by the act of dilating their internal vessel diameter. Studies using healthy young subjects have shown that exercise training increases endothelial-dependant vasodilation, as assessed with FMD (Clarkson *et al.*, 1999; Higashi *et al.*, 1999; Moyna & Thompson, 2004). On the contrary, endothelial function is suppressed in sedentary subjects compared to the values reported in their active counterparts (DeSouza *et al.*, 2000). Differences, however, have been proposed to be due to the up-regulation of FMD following exercise training and not due to down-regulation of FMD in sedentary individuals (Clarkson *et al.*, 1999). FMD has also been reported to be suppressed in patients exhibiting independent risk factors for cardiovascular diseases such as hypertension, diabetes, smoking and hypercholesterolemia (Laurent, 1990; Celermajer *et al.*, 1994; Steinberg *et al.*, 1997; Cosentino & Luscher, 1998).

Studies have shown that exercise training and an increased level of physical activity can improve endothelial function (Clarkson *et al.*, 1999). Such an improvement can be attributed to the changes in blood flow and shear stress levels and the resulting increases in both NO production and the NOS activity, (Clarkson *et al.*, 1999). Clarkson and colleagues in 1999 demonstrated that that endothelium-dependent response, endothelial functioning, in the brachial artery of healthy young men was improved following 10 weeks of regular physical exercise at an intensity that can be undertaken by the general public. This study was critical in that its results can be applied to an exercise regime beginning at a young age and to a population showing absence of clinical disease.

Clarkson and colleagues in 1999 proposed that elevated intermittent shear rates resulting from repeated bouts of exercise mediates the improved functioning of the endothelium (Clarkson *et al.*, 1999). Shear stress is not only responsible for mediating NO release from the endothelial cells thus promoting vasodilation, but also has effects on endothelial cell adhesion, monocyte binding and super-oxide production, (Tsao *et al.*, 1995). During periods of dynamic exercise, arterial blood flow is increased which leads to an acute increase in intra-luminal shear force against the walls of the arterial vasculature (Huonker *et al.*, 1996; Niebauer *et al.*, 1996). Such a force stimulates the vascular endothelium to release NO and causes reactive flow-dependent regional vasodilation. Chronic enhancements of shear forces induce endothelial cell-mediated alterations in gene expression as well as chronic structural adaptations of the vascular wall (Masuda *et al.*, 1989).

It has been proposed that the likely physiological mechanism behind the vascular adaptations to exercise training involve chronic increases in NO generation by increases in NOS gene expression (Clarkson *et al.*, 1999). The up-regulation in laminar shear stress that accompanies acute and chronic exercise training causes an increase in NOS mRNA expression, as has been previously shown in animal studies, (Sessa *et al.*, 1992; Sessa *et al.*, 1994; Koller *et al.*, 1995). A transcription factor binding site in the NOS promoter gene has been speculated to link the effects of shear stress on changes in vascular endothelial NO production, (Resnick *et al.*, 1995).

Kingwell and colleagues (1997) found that 4 weeks of cycle training increased resting NO production systemically by showing that an increase in basal NO production

occurs not only in the limbs of the exercising vasculature but also in the limbs that were not directly exposed to training. (Kingwell *et al.*, 1997). Such data suggests that some other factor not directly linked to local muscular adaptation to exercise training is responsible for increases in basal NO production or bioavailability between exercise bouts. This study suggests that exercise training, in the form of aerobic effort, increases the release of NO in the basal state by means of an increased laminar shear stress level caused by exercise-induced increases in blood flow. It also demonstrates that the increase in basal NO production occurs in vascular beds not directly associated with the mechanics of the exercise and thus the effects seem to be of a systemic nature, (Kingwell *et al.*, 1997).

The issue of the amount of training time becomes more important to consider when considering possible explanations for a failure of deconditioning to show the opposite effects of exercise training. Exercise training studies examining its long term effects (months or years) on the vasculature are scarce, however, the basal production of NO has been found to be similar in endurance trained individuals and control subjects (Kingwell *et al.*, 1997). Thus it was suggested that any alterations in endothelial function that result from short-term adaptations to exercise training are ultimately replaced by other vascular adaptations such as structural changes within the vessel wall (Green *et al.*, 2004). Such an observation was found in a study conducted by Green et al. (1996) in which arterial endothelial function of the brachial artery in tennis players was similar in the dominant and non-dominant upper limbs.

Thus, there is a substantial quantity of literature supporting the notion that exercise training increases the functioning of the endothelium by increasing the production of basal NO. The proposed mechanism within the literature seems to be consistent and relates to an elevation in arterial vascular wall shear stress via increases in blood flow brought upon by exercise training. Such findings have critical implications in disease states such as CVD, heart failure and hypertension, all of which show a decreases functioning of the endothelium.

2.1.4 Effects of Physical Inactivity on Endothelial Function

There have been many models used to study the effects of deconditioning on arterial vasculature. Such models included spaceflight (Vaziri *et al.,* 2000), simulated microgravity, bed rest (Takenaka *et al.*, 1994; Kamiya *et al.*, 2000; Bonnin *et al.*, 2001; Pawelczyk *et al.*, 2001), immobilization through casting (Giannattasio *et al.,* 1998), animal models (Jasperse *et al.*, 1999; Schrage *et al.*, 2000) and paraplegic models (De Groot *et al.*, 2003; de Groot *et al.*, 2004). While data on the effects of exercise and activity on endothelial vascular function seem to show consistent results from study to study, data on the effects of physical inactivity are conflicting. Studies using animal models to imitate physical inactivity by hindlimb unweighting are examples of such conflicting results. Various studies have shown a decrease in the NOS gene expression and a correlating decrease in endothelial function to unweighting (Jasperse *et al.*, 1999; Schrage *et al.*, 2000), while other studies report an upregulation NOS gene expression following models of inactivity (Vaziri *et al.*, 2000).

According to the theory of minimum cost, the arterial system should adjust its internal diameter in response to changes in blood flow in order to maintain a specific shear stress level (Langille, 1993). When vessel diameter decreases due to decreases in blood flow, such as in SCI individuals, the increase in basal shear stress may lead to an up-regulation of NOS gene expression due to the fact that shear stress is a potent physiological stimulus for the endothelial release of NO (Uematsu *et al.*, 1995). Uematsu and colleagues (1995) found that NOS gene expression in the endothelium is altered following increased shear stress while the same findings of an up-regulation of NOS gene expression were reported by Sessa et al (1994) using a dog model.

The paraplegic model of inactivity offers an extreme in the continuum of the variable of inactivity and offers a unique model to study the extreme effect of inactivity on vascular dynamics. A recent study conducted by De Groot et al (2004) evaluated vascular function in individuals with complete paraplegia. Contrary to their hypotheses, the study found that spinal cord injured individuals show a preserved or enhanced level of endothelial function in their inactive legs (de Groot *et al.*, 2004). The increased relative flow-mediated vasodilation response within this group of SCI individuals compared to a group of controls was an unanticipated observation given that exercise training studies demonstrate an increased FMD response and thus an increased endothelial function with exercise training (Hornig *et al.*, 1996; Clarkson *et al.*, 1999).

With regards to the failure to witness a decrease in endothelial functioning, both functional and structural changes in the vascular bed being innervated by the affected artery are proposed to cause such an occurrence, (de Groot *et al.*). The increase in arterial

endothelial function within the paraplegic model, although surprising, adds to the controversial findings of previous literature with some animal studies showing decreases in NOS activity and a concurrent decrease in endothelial function (Kamiya *et al.*, 2000; Schrage *et al.*, 2000), and others showing an up-regulated NOS system and thus an enhanced endothelial function (Vaziri *et al.*, 2000). Studies done in humans have also revealed inconsistent results. Humans exposed to 14 days of bed rest have revealed decreases in plasma nitrate and nitrite concentrations (an indicator of NO production) (Kamiya *et al.*, 2000) while Bonnin et al (2001) found enhanced endothelial function following 7 days of bed rest deconditioning (Bonnin *et al.*, 2001). Bonnin and colleagues failed to show any alteration in endothelium-independent function which suggests that the increased FMD is not due to changes in the sensitivity of the stimulus for NO release or to an increase in smooth muscle cell sensitivity to NO (Bonnin *et al.*, 2001).

In a recent study conducted by Bleeker et al (2005a), a human study utilizing the model of unilateral lower limb suspension (ULLS) to mimic deconditioning was employed for a period of 4 weeks. The primary findings of the study demonstrate that deconditioning causes no impairment of endothelial function of the femoral artery, contrary to their hypothesis. The authors concluded that the functional effects of deconditioning are not merely the inverse of those associated with exercise training (Bleeker *et al.*, 2005a). Such a study is critical in understanding the effects of inactivity in the form of deconditioning in humans since the ULLS model is not confounded by microgravity or denervation. Bleeker and colleagues (2005a) measured the functional changes in the artery in 7 able-bodied healthy humans by flow-mediated vasodilation

(FMD). The study found that absolute FMD values increased by 4 weeks of deconditioning; however, when FMD was corrected for the magnitude of the stimulus (shear stress) (Levenson *et al.*, 2001), no statistically significant increase was found. Endothelial independent dilation of the artery, however, did show an increase while the ratio of FMD to nitroglycerin- mediated dilation following the 4 weeks of ULLS did not show any change. The author suggested that this observation was due to altered sensitivity of the smooth muscle to NO or that the capacity of the smooth muscle to dilate the vessel was altered with deconditioning (Bleeker *et al.*, 2005a).

It has been proposed that the failure to show the opposite effects that exercise training has on the endothelium with deconditioning models is due to the absence of periods of high shear rate levels and decreases in diameter with deconditioning, (Bleeker *et al.*, 2005a) and thus the basal release of NO from the endothelium diminishes. Thus, the mechanism for such an increased FMD response and a concurrent increase in nitroglycerin-mediated dilation could potentially be an increased sensitivity of NO (Bleeker *et al.*, 2005a)). In a recent animal study, it was concluded that partial ligation of arterial flow resulted in increased sensitivity to NO (Rudic *et al.*, 2000) and thus it was proposed that a critical responsibility in the mechanism of arterial remodeling is endothelial function.

In the attempt to assess the effect of deconditioning on the contribution that NO has on vascular functioning in humans, a recent study by Bleeker and colleagues (2005b) utilized injections of an NOS blocker (Ngmonomethyl-L-arginine, L-NMMA) and an NO donor (Sodium nitroprusside, SNP) to evaluate vascular function in a group of SCI

individuals, a group of controls and a group of individuals deconditioned for 4 weeks through ULLS. The authors found that, contrary to their hypotheses, NO contribution to baseline vascular tone was not affected by deconditioning of human skeletal muscle. In both acute deconditioning of the legs in the ULLS group and within a chronic deconditioned state in the paraplegic group there was a preserved contribution of NO to baseline vascular tone. Such a conclusion was proven by showing a similar increase in leg vascular resistance in the paraplegic group, PRE versus POST ULLS and controls with an infusion of L-NMMA into the femoral artery. Moreover, it was shown that the response to the NO donor SNP was similar in the paraplegic group versus the control group and also in PRE versus POST ULLS, suggesting that there is similar smooth muscle cell responsiveness to NO. Since it has been well established that NO is potentially the most potent physiological stimulus for vasodilation and the critical role of NO is shown with the strong connection between its availability, thus endothelial function, and cardiovascular disease (Widlansky *et al.,* 2003), its capacity to function efficiently is important to consider. Defects in the NO pathway are visible in disease states such as CVD and also in CVD risk factors such as diabetes, smoking, hypertension and hypercholesterolemia (Widlansky *et al.,* 2003).

With deconditioning comes a decrease in leg blood flow and thus a potential decrease in the stimulus for the endothelial production of NO (Kamiya *et al.*, 2000; De Groot *et al.*, 2003). Such a mechanism has been proposed to cause arterial remodeling and, ultimately, atherosclerosis (Rudic *et al.*, 2000). It was thus hypothesized by Bleeker and colleagues (2005b) that a decrease in the contribution of NO to basal vascular

function and tone would occur in the models of deconditioning that were employed, SCI model and ULLS model. The SCI model offers a very unique state in that the muscle below the level of the lesion is extremely inactive and has been subjected to chronic, long-term deconditioning, and, more importantly exhibit neural denervation. Due to the confounding variables of the SCI model, the use of the ULLS model was incorporated in the attempt to distinguish between the SCI model's adaptation to deconditioning and a less extreme and more acute model. While the deconditioned legs of the ULLS group did not show decreases in leg blood flow which has proven to be a controversial issue within the literature, measures of muscle atrophy, arterial diameter, and muscle strength did show decreases. Such a finding by Bleeker and colleagues (2004) indicates that ULLS did evidently cause deconditioning of the leg, which others studies have also proven using the unloading protocol (Berg & Tesch, 1996; Schulze *et al.*, 2002). The authors concluded that both acute and chronic deconditioning of human skeletal muscle does not result in alterations of the contribution of NO to basal vascular tone. Thus, the increased levels of vascular resistance following a period of deconditioning cannot be considered to be due to an attenuated role of NO to basal vascular tone at the level of the conduit arteries (Bleeker *et al.*, 2005a).

Deconditioning by the use of forearm casting shows no alteration in the vasoconstrictor response to L-NMMA (Giannattasio *et al.*, 1998). However such a study could be confounded by the effects of physiological trauma and the response to injury that may increase resting blood flow through the forearm. Plasma nitrate and nitrite concentrations that were found to decrease following bed rest thus showing decreased

NO production and endothelial function may also not provide the most valid representation since these concentrations are representative of whole body NO metabolism (Kamiya *et al.*, 2000). The results of the study by Bleeker et al. (2005b) quantifies a more direct characterization of the specific role of NO in vascular tone (Bleeker *et al.*, 2005b). A reduction in blood flow or an increase in vascular tone in humans, as explained by Bleeker and colleagues in an attempt to justify their findings (Bleeker *et al.*, 2005b) cannot be attributed to a decreased production of NO during the basal state. The investigators found that, while baseline NO production was not changed through the 4 weeks, other stimuli responsible for the release of NO may still play a role in the mechanism of arterial remodeling and in the development of atherosclerosis (Bleeker *et al.*, 2005b).

Animal studies have also examined the impact of hindlimb unweighting in mice (Jasperse *et al.*, 1999; Delp *et al.*, 2000; Schrage *et al.*, 2000; Vaziri *et al.*, 2000). Conflicting results are also apparent with some studies (Jasperse *et al.*, 1999; Delp *et al.*, 2000; Schrage *et al.*, 2000) showing a decrease in endothelial function through observations of decreased eNOS expression while other studies show no changes in endothelial function following hindlimb unweighting (Vaziri *et al.*, 2000). Even when partial ligation of an artery in mice is utilized to mimic decreases in blood flow, it has been found that baseline NO production is markedly reduced (Rudic *et al.*, 1998; Rudic *et al.*, 2000). The authors correlated such a finding to arterial remodeling with an increased potential for the development of atherosclerosis (Rudic *et al.*, 1998; Rudic *et al.*, 2000).

2.2 ARTERIAL VASCULAR COMPLIANCE

Arterial compliance (AC) is characterized as the capability of an artery to accommodate an amplified blood volume (O'Rourke *et al.*, 2002). AC, or its inverse arterial stiffness, are both are used when describing alterations within the arterial vasculature to an intervention or therapy. Both, however, are often used interchangeably (O'Rourke *et al.*, 2002). AC is defined through two major elements, a passive factor that reflects the structural composition of the arterial vessel wall and also an active component that is associated with arterial vascular smooth muscle cells and their associated vascular tone (Kinlay *et al.*, 2001). Structurally the arteries of the vasculature consist of 3 layers, the tunica intima, which consists of a lining of endothelial cells that are in contact with the blood, a basement membrane, and a layer of elastic tissue referred to as the internal elastic lamina. The tunica media, the middle of the arterial wall, is considered the thickest layer and consists of elastic fibers and smooth muscle cells. Lastly, the external layer, the tunica adventitia consists mainly of elastic and collagen fibers (Faury, 2001).

AC is calculated as the change in arterial diameter cross sectional area as a consequence of a difference between systolic and diastolic blood pressures during a heart cycle. The denominator of the calculation of AC is a factor referred to as pulse pressure (PP). Pulse pressure is occasionally used as an independent determinate of arterial stiffness (Dart & Kingwell, 2001). An increased PP has been characterized as an independent risk factor for cardiovascular disease and it is thus a commonly utilized measure within clinical practice (Adji & O'Rourke, 2004; Dart & Kingwell, 2001). PP,

while able to provide crucial information regarding an individuals arterial function, cannot be used directly in the measurement of AC (Bulpitt *et al.*, 1999). Therefore, other techniques that are non-invasive in nature are used to provide a more comprehensive evaluation of arterial function. Such techniques include pulse wave velocity (PWV) and Doppler ultrasound (Woodman *et al.*, 2005).

A decrease in AC, or an increase in arterial stiffening, is associated with the aging process and is also associated with various disease states such as diabetes, atherosclerosis, myocardial infarction, and heart failure (Seals, 2003). Both structural and functional adaptations within the arterial vascular wall have been suggested to be involved in the mechanisms associated with an increase in arterial stiffness (Kingwell *et al.*, 2001). The vasculature must continuously remodel to meet the altering requirements for blood flow and also to accommodate changing in systemic blood pressures that are needed to drive such changes in blood flows, (Langille, 1993). The change in arterial diameter is highly dependant on changes in blood flow hemodynamics (Langille, 1993).

2.2.1 Mechanisms of Arterial Stiffening

It has been established that the composition of blood vessels influences the compliance of the arterial wall. Such a theory illustrates the connection between structural and functional properties of the vessel wall and relates them to the vascular smooth muscle, collagen, and elastic properties within (Benetos et al., 1997a). Elastic properties of an artery allow for distension when conditions of increased pressure are present while, at the same time, displaying the ability to recoil to its original shape once

the pressure load is removed. The collagen fibers of the vessel wall have a primary function of allowing for arterial distension over a physiologic range of pressures, however, they also protect the artery from injury. The compliance of an artery is dependant on the different ratios of elastin to collagen within its composition. If there exists a lower ratio of elastin to collagen, the compliance of the artery is lower and the stiffness relatively greater. Increases in smooth muscle tone and/or smooth muscle cell hypertrophy also increase stiffness and decrease compliance (Arnett *et al.*, 1994).

The arterial vessel wall undergoes structural and cellular adaptations in response to changes in hemodynamics of blood flow and pulse pressure and through such changes develops an increased stiffness (Zieman *et al.*, 2005), see Figure 2.2. Arterial stiffening can occur in differing ways, yet the mechanisms can also act synergistically. There are both stable and dynamic forces that act concurrently in the complex process of arterial stiffening and such forces involve both the structural and cellular components of the vessel wall (Figure 2.4) (Zieman *et al.*, 2005). Decreases in arterial compliance are not evenly dispersed throughout the vascular tree, nor do decreases in compliance have the same effect on all vessels. The process mainly occurs in central arteries as well as the large conduit arteries (Benetos *et al.*, 1993). The aorta and its major divisions are most susceptible to decreases in arterial compliance. It has been suggested that this is due to the ability of these vessels to accommodate large blood flows that are ejected from the heart and their responsibility for ensuring continuous blood flow throughout the remainder of the cardiac cycle (Dart & Kingwell, 2001).

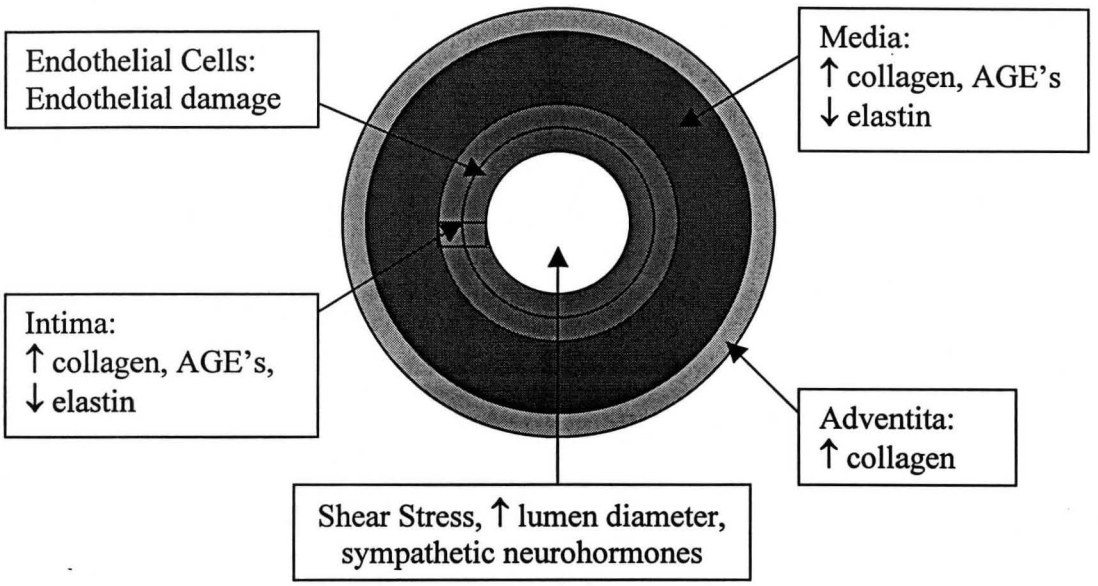


Figure 2.2 Causes and sites of arterial stiffening (Modified from Zieman et al., 2005)

Structural alterations have been suggested to contribute principally to age-correlated decreases in AC within large arteries (Lakatta, 2002). Elastin and collagen together provide structural integrity and elasticity within the extracellular matrix of the arterial vessel wall. Dysregulation of the normal balance of production and degradation of these proteins, as seen in the aging process, can lead to overproduction of abnormal collagen proteins and concurrently decreases the quantity of elastin (Zieman *et al.,* 2005).

The collagen molecules that supply the tensile strength of the arterial wall are enzymatically cross-linked soon after their formation which allows them to be insoluble to hydrolytic enzymes. Collagen has a low hydrolytic turnover rate, and therefore is quite susceptible to non-enzymatic glycation cross-linking. This process induces an increase in

collagen content and allows the artery to become increasingly rigid and less compliant (Zieman *et al.*, 2005). Ultimately, this less compliant state leads to a damaged endothelial layer that is less able to function normally.

The elastin molecules within the vessel wall are stabilized by a cross-linking mechanism and any kind of disturbance of this linking has been shown to lead to a weakening of the elastic properties within the vessel wall. Elastin has been shown to be fragmented and less dense in its less compliant form (Lakatta, 2003). The molecular alterations associated with the elastin protein which lead to a less compliant artery are observable as a visible thickening of the intima-media, as well as an increase in lumen diameter.

Arterial stiffness can also be associated with the actions of advanced glycation end products (AGE's), which result from non-enzymatic protein glycation to form irreversible cross links. Elastin and collagen are both susceptible to AGE crosslinking and, consequently, are vulnerable to an accretion of structurally inadequate molecules. AGE's are also able to affect endothelial cells through their destruction of bioavailable nitric oxide (Zieman *et al.*, 2005).

Not only does arterial stiffening occur structurally, but also through cellular mechanisms including endothelial cell signaling and vascular smooth muscle cell tone (Zieman *et al.*, 2005). The tone of the smooth muscle cells can be influenced through several factors such as mechano-stimulation, angiotensin II, endothelin, oxidative stress, and, in interest of the present discussion, nitric oxide (NO) (Zieman *et al.*, 2005). In relation to vascular stiffness, endothelial dysfunction is the result of an imbalance

between NO, endothelial-derived hyperpolarizing factor and constricting hormones (Zieman *et al.,* 2005). It has been shown that the condition of endothelial dysfunction and increased arterial stiffness may be the result of a decrease in NO production and a concurrent increase in the production of the NOS inhibitor, (Zieman *et al.,* 2005). A decrease in the bio-availability of NO and an increase in local endothelin-I and angiotensin-II are additional suggested mechanisms (Seals, 2003).

2.2.2 Consequences of Decreased Arterial Compliance

Elasticity of the arterial wall, or in other words compliance, facilitates normal, healthy behaviour of the cardiovascular system. Arterial Compliance reduces left ventricular wall pressure by dampening the rise in peak systolic pressure during systole and releasing the stored energy in diastole (Kinlay *et al.*). Arterial compliance has been shown to decrease with advancing age (Rowe *et al.,* 1987) and has also been shown to be decreased in pathological states such as hypertension, congestive heart failure, diabetes, and atherosclerosis (Scuteri *et al.,* 2004; 43:1388; Lakatta, 2003; 107:139; Safar *et al.,* 2003). Moreover, a decrease in arterial compliance, or distensibility, is now recognized as an independent risk factor for CVD (Arnett *et al.,* 1994).

The arterial system has two distinct, however, associated functions; first to deliver a sufficient amount of blood to the tissues of the body and second, to transform the pulsatile ejection of blood from the left ventricle of the heart to a smooth and continuous flow peripherally (Izzo, Jr. & Shykoff, 2001). Arteries primarily function as "cushions" and thus reduce the oscillations in blood pressure that result from the intermittent

ventricular ejection through their ability to distend and recoil (Safar *et al.*, 1987). Such a characteristic allows for the continuous and smooth blood perfusion to the organs and tissues. Through systole, the aorta distends to contain the stroke volume from the left ventricle of the heart and recoils during diastole to promote advancing flow (Somers *et al.*, 1992). Pressure increases during systole (SBP) due to the limited capacity of the aorta and this pressure is partially maintained during diastole (DBP) by the rebounding of the expanded arterial wall. Therefore when the arteries become less distensible, this cushioning function is impaired resulting in higher SBP and lower DBP (Arnett *et al.*, 1994).

There exist numerous unfavourable cardiovascular effects that accompany a reduction in the compliance of arteries within the central vasculature. First of all, the discharge of the stroke volume into a non-compliant aorta can potentially affect systolic blood pressure (SBP) and cause it to increase. Concurrently, a decrease in the associated loss of elastic recoil within the vessel may cause a reduction in diastolic blood pressure (DBP). Thus, through the preceding results of decreases in compliance, an increase pulse pressure occurs. If SBP and PP remain elevated over an extended period of time, damage to the vasculature may potentially occur and the risk of atherosclerosis could be intensified (Seals, 2003). As a result, the risk of myocardial infarction, thrombosis, and stroke are increased. Secondly, cardiac function and structure may be affected by a decrease in large artery compliance. Such effects have been speculated to be noted in the heart with left ventricular hypertrophy, an increase in left ventricular oxygen demand, delayed myocardial contraction, and, lastly, a reduction in early diastolic filling rate

(Seals, 2003). The penalty of the above modifications within the vasculature consist of an increased risk of congestive heart failure. Lastly, a decrease in the compliance of an artery may cause a reduction in the sensitivity of critical reflexes that are involved in the maintenance of vascular homeostasis. These reflexes include a decreased ability of arterial distension in response to an increased in intravascular pressure thus causing a decrease in the afferent signaling to the central nervous system by the arterial baroreceptors (Hunt *et al.,* 2001). As a result, there is a decrease in the efferent response which contributes to decreased blood pressure variability and potentially a decreased resistance to ventricular fibrillation and an increased vulnerability to sudden cardiac death (Hunt *et al.,* 2001).

A decrease in arterial compliance within the elastic arteries has been shown to be correlated with various detrimental adaptations within the cardiovascular system and such alterations may eventually lead to the pathology of cardiovascular disease or worse yet, mortality (Seals, 2003). An increase in systolic blood pressure (SBP) could potentially be a direct outcome of a decrease in arterial compliance. If the aorta is not capable of accommodating the ejected blood from the heart, the stroke volume, the pathology of systolic hypertension may develop. Moreover, a decreased compliant state reduces diastolic blood pressure (DBP) and, as a consequence, an increase in PP results. Such an elevation in SBP, if it occurs chronically, may lead to a damaged endothelium and, as a result, accelerate the development of cardiovascular disease (Seals, 2003).

2.2.3 Measurement of Arterial Compliance

Arterial compliance refers to the correlation between arterial diameter and the associated distending pressure, whereas distensibility index refers to the change in volume and the associated change in pressure multiplied by baseline volume. Increasing arterial compliance results in a more compliant, and thus less stiff, artery. Compliance changes in a non-linear manner with blood pressure and tends to be larger in states where there exists low blood pressures. Due to this fact, there exists the potential for incorrect conclusions if the distensibility index is shifted due to some type of intervention within the study design (Kinlay *et al,* 2001). Compliance curves are able to be utilized in order to provide investigators with a measure of elasticity that is independent of blood pressure alterations.

There are numerous methods to examine arterial compliance in vivo that are both invasive and non-invasive. The two most accepted and commonly use techniques involve the method of pulse wave velocity (PWV) and Doppler ultrasound imaging.

The method of PWV consists of an indirect evaluation of arterial compliance and has been shown to increase with advancing age and within the diseased state of hypertension (Izzo, Jr. *et al.,* 2001). This method is based on the theory that the velocity of a pressure waveform along an artery is inversely related to the distensibility, or compliance, of the arterial segment between measurement sites (Izzo, Jr. et al., 2001). Pressure waveforms within the artery are detected by transducers positioned above the artery of interest and by dividing the distance traveled between the transducers (meters)

by the time of travel of the pulse wave (the transmission time in seconds) the velocity is determined. Transmission time includes the elapsed time between the recognition of the peripheral pulse at two different transducers and is determined by approximating the delay between two successive waveforms (Arnett *et al.,* 1994). There are, however, limitations to such a technique. Firstly, the potential for significant measurement error exists when attaining transit times and distance traveled by the pulse wave. Secondly, difficulty in the determination of the predetermined pulse wave measured at the two points could result in timing error. Lastly, differences between individuals with regards to vascular anatomy can potentially lead to inaccuracies in distance determination between the two measuring sites (Izzo, Jr. *et al.,* 2001).

A second technique used in determining arterial compliance utilizes Doppler ultrasound imaging to measure arterial diameter, or cross-sectional area. This is combined with changes in pressure to allow for calculation of change in area over change in pressure. Through measure of the diameter change in one heart cycle and, concurrently, the measurement of a corresponding pressure change, arterial compliance can be assessed. Ultrasound imaging is the most commonly utilized method and allows for the beneficial visualization of the vessel diameter (Izzo, Jr. *et al.,* 2001). Mechanistically, the ultrasound transducer is positioned to direct ultrasound beams perpendicular to the artery in order to obtain the best possible sound reflection from the arterial walls of the vessel (Arnett *et al.,* 1994). There has recently been an increase in the use of ultrasound to assess arterial elasticity in humans. Such a protocol is mainly used in

the attempt to assess structural components affecting arterial compliance such as elastin, collagen and vascular smooth muscle cells (Bank *et al.,* 1996).

The brachial artery has been previously used to measure BP and is used in calculations to give compliance values. However, due to the fact that PP has been shown to be amplified in the peripheral vasculature, these pressure measurements may not represent a correct physiological central artery pulse pressure (Oliver & Webb, 2003). Thus there has been growing use of the procedure of applanation tonometry which is now used as means to obtain PP measures in the artery of interest. The theory behind applanation tonometry assumes that if a curved surface of a structure containing pressure can be flattened (or applanated) the circumferential forces within the wall of the structure maintain balance and the pressure obtained by the sensor is the same as the pressure intra-arterially (Kellyet al., 1989; Oliver *et al.,* 2003). Applanation tonometry is not commonly used to measure absolute systolic pressure; however, mean arterial pressure of the brachial artery and diastolic blood pressure can be assumed to be equal to that in the artery of interest and thus the absolute systolic pressure of the artery of interest can be calculated (Oliver *et al.,* 2003).

There are several advantages to utilizing ultrasound techniques in the attainment of calculating arterial compliance. This technique demonstrates extraordinary test reproducibility and also is a much more direct measure as the actual artery is being visually assessed compared to indirect measures involved with PWV measures. There also exist limitations to ultrasound measurements of arterial compliance. These include

the potential for measurement error from a number of sources including distortion of the vessel by the pressure of the transducer causing potential underestimation of vessel diameter change. In addition, the acquisition of accurate data may be restricted by participant effects that may include respiratory movements or persistent swallowing. Lastly, inaccurate recognition of the arterial walls during analysis may produce inaccurate diameter measurements (Arnett *et al.*, 1994).

2.2.4 Arterial Compliance and Levels of Physical Activity

Literature studying the effects of physical exercise on arterial compliance has mainly focused on results of cross-sectional studies and measures of central arterial compliance (Vaitkevicius *et al.*, 1993; Tanaka *et al.*, 1998). Research has demonstrated that endurance exercise training allows for the most potential in the reversal of age-related declines in arterial compliance (Vaitkevicius *et al.*, 1993; Tanaka *et al.*, 2000). Not only have the effects of endurance training on arterial compliance been studied, but also the effects of recreation and leisure time activities. It has been demonstrated through such studies, that even recreational types of activity are able to slow the progression of arterial stiffening, however to a lesser degree (Schmidt-Trucksass *et al.*, 2000). Several studies have shown that exercise training increases central arterial compliance in the able-bodied population (Wijnen *et al.*, 1991; Cameron *et al.*, 1994) and also in patients with cardiovascular disease (Fuchsjager-Mayrl *et al.*, 2002). Literature has also shown that complaince in peripheral arteries of endurance trained athletes is greater than their sedentary (Kool *et al.*, 1992) and spinal cord injured counterparts (Huonker *et al.*, 2003).

In study conducted by Tanaka et al. in 2000, it was shown that, compared to recreationally active and sedentary counterpart groups, an endurance trained group displayed a 20-35% greater arterial distensibility, while the recreationally active group, although not statistically significant, displayed a 10-17% greater central elastic arterial distensibility than the sedentary group (Tanaka *et al.*, 2000). It has thus been accepted throughout literature that aerobic exercise has the greatest influence on altering arterial compliance. Tanaka et al. (2000) was able to conclude that central arterial compliance can be increased following exercise training in healthy middle-aged and older men (Tanaka *et al.*, 2000). Thus, through such a longitudinal intervention study, it was found that even brisk walking, an activity in which the general population is able to include fairly easily in daily living, was able to partially reverse the age-correlated decrease in arterial distensibility. Although it was shown that this type of physical activity was associated with a greater distensibility, it must be noted that the findings were cross-sectional in nature and thus the differences between the trained and sedentary groups could be due to other lifestyle factors and genetic influences and not only on the effects of regular exercise.

The mechanisms through which regular aerobic exercise may alter central arterial stiffness are complex and have not received extensive study. It has been shown that compliance within the arteries is determined by the intrinsic elastic properties of the arteries, including the relative proportions of elastin and collagen that make up its composition, and also the activity of the smooth muscle cells within the vessel wall (Langille, 1993) and these are the potential sites in which alterations have been

speculated to take place that modify arterial compliance. One instance of such site-specific changes within the vasculature as a result of exercise training can be noted in the finding that collagen cross linking in the left ventricle of endurance trained rats was less than 50% of that observed in sedentary controls (Thomas *et al.*, 2000). Thus, although no definitive mechanisms have been identified in the alterations brought about by exercise training with regards to compliance, it is generally assumed that any influence that physical exercise has on arterial stiffness must involve a reduction or reversal of the mechanisms that have been anticipated to contribute to the age associated decrease in arterial compliance.

Although the effects of physical exercise has been proven to enhance central arterial compliance and distensibility (Vaitkevicius *et al.*, 1993; Cameron & Dart, 1994; Tanaka *et al.*, 1998; Schmidt-Trucksass *et al.*, 2000; Tanaka *et al.*, 2000), literature on the effects of exercise on the compliance of peripheral arteries is limited. Moreover, it has yet to be extensively studied if there occurs alterations in peripheral arterial compliance following deconditioning, or a decreased level of physical activity, in the human population. It has also not yet been universally determined whether adaptations within the vasculature are locally mediated, within the vessels directly involved in the intervention, or are more systemic in nature and occur throughout the entire vasculature.

In a study conducted by Wijnen et al in 1991, it was found that in severe paraplegia, compared to sedentary and endurance trained conditions, central arterial compliance was lower within the paraplegic model compared to both the able-bodied and athletes, while the athletes demonstrated the highest arterial compliance. Vessel wall

characteristics, including the distensibility coefficient and cross-sectional compliance, of the common carotid artery and the common femoral and brachial arteries were studied by use of ultrasound in 15 trained male cyclists and 15 healthy sedentary males matched for age, height, and weight. The investigators found that vessel wall properties and diameters of the common carotid artery showed no differences between the groups while diameter of the common femoral artery was significantly greater and cross-sectional compliance tended to be higher in cyclists. The diameter of the brachial artery was not different between the two groups; however, compliance of the brachial artery was found to be significantly higher in the cyclists. The results of this study suggest that this type of endurance training has the potential to cause structural adaptation of a peripheral artery, the common femoral artery, and a more general decrease in muscle tone of muscular arteries (Wijnen *et al.,* 1991).

Studies examining peripheral alterations in arterial stiffness have utilized animal models and have given insight into potential mechanisms that may be associated with the changes seen in compliance with increases or decreases in physical activity. Animal studies have shown that changes in arterial blood flow initiate structural and functional changes within the vasculature and such alterations have been associated with changes in local wall shear stress levels (Langille, 1993). An increase in blood flow and this shear stress leads to outward vascular remodeling while decreases cause inward structural remodeling (Masuda et al., 1989). Such a mechanism has been proposed to allow for a constant wall shear rate level within the vessel (Langille, 1993).

In their attempt to determine whether there exists any effects of an ordinary level of physical activity or a decreased level of physical activity on arterial peripheral compliance, Giannattasio et al. (1998) investigated the impact of prolonged immobilization on radial artery distensibility in humans and compared this to the recommencement of normal mobility. The study demonstrated that in an immobilized upper limb, a concurrent decrease in vessel diameter and compliance occurs (Giannattasio *et al.*, 1998). Seven normotensive subjects had one upper limb immobilized for 30 days due to a fracture with the contralateral limb used as a control. Immediately following removal of the plaster, radial artery distensibility was markedly less in the immobilized and fractured limb compared to the contralateral control limb and following 5 days of rehabilitation the distensibility of the radial artery was markedly increased in the previously fractured limb whereas no change was noted in the contralateral limb. These results suggest that a complete disruption of physical activity is linked to a clear decrease in arterial distensibility, indicating that even an ordinary level of activity plays a major role in modulation of arterial mechanical properties

An increase in the level of physical activity leads to changes in arterial blood flow to the working musculature and is speculated to cause increases in wall shear stress that leads to vascular outward remodeling and an increase in arterial compliance (Kingwell *et al.*, 1997; Giannattasio *et al.*, 1998). It was recently observed in a study conducted by Schmidt-Trucksass et al. (2000) that the common femoral artery showed a larger luminar diameter and a higher compliance in athletes compared to sedentary able-bodied individuals and paraplegic counterparts. However, the shear rates in the endurance

trained athletes and sedentary subjects were similar. Shear rates in the paraplegic subjects were significantly higher and diameter of the artery was significantly lower in this group compared to the trained and sedentary group. Such a finding indicates a disturbed response of the common femoral artery to alterations in blood flow within the paraplegic condition. Within the study, only local changes were found, as there were no differences in the central common carotid artery between any of the groups (Schmidt-Trucksass *et al.*, 2000).

Such a study confirms previous literature findings of chronic increases or decreases in blood flow inducing an increase or reduction in arterial diameter within animal populations (Masuda *et al.*, 1989; Langille, 1993). Within the trained group, the chronic increases in blood flow brought about by exercise training bouts are accompanied by a larger arterial diameter, On the contrary, within a paraplegic model, there is evidence showing a reduced blood flow and a smaller arterial diameter of the common femoral artery (Schmidt-Trucksass *et al.*, 2000; Hopman et al., 1996; Huonker et al., 1998).

The differences in compliance found by Schmidt-Trucksass et al. (2000) were also found to be correlated to vessel dimension adaptations with a correlation value of 0.62. Compliance was found to be lowest in the paraplegic model that also demonstrated the smallest arterial diameter. The trained group showed a higher compliance and also the largest arterial diameter of the three groups. This study also there existed a difference in compliance between the groups with trained subjects showing higher arterial compliance and the paraplegic subjects showing the lowest. Mechanisms that could potentially be

responsible for the greater arterial compliance in trained versus the sedentary and paraplegic groups include the following; a decreased sensitivity to the vasoconstrictive effects of norepinepherine (an alpha-2-adrenergic-receptor mechanism) or a decreased basal smooth muscle sympathetic tone accompanying exercise training (Schmidt-Trucksass *et al.,* 2000).

The arterial system, in its attempt to maintain constant shear stress levels has shown evidence of being able to adjust its internal diameter to changes in blood flow and thus allows for such a balance to be maintained (Masuda *et al.,* 1989). Thus there exists a minimum work theory that allows for constant average wall shear stress that is independent of the diameter of the vessel (Schmidt-Trucksass *et al.,* 2000). The increase in blood flow that occurs with exercise training to the active muscles causes an endothelium-mediated dilation of the vessel and through the actions of NO and other prostacyclins, and thus counteracts the vasoconstrictive effects of norepinepherine on the alpha-2-adrenergic receptors of the endothelial cells (Rubanyi *et al.*, 1986; Delp, 1995). A potential mechanism that has been speculated to play a role in arterial adaptation to physical activity includes the increases or decreases in shear stress which is speculated to lead to outward and inward vascular remodeling, respectively. Knowledge into such a mechanism has recently been extended with the findings that NO is invloved in the regulation of the mechanical properties of peripheral arteries in humans and suggests that the inhibition of NO synthesis is associated at this level with the expansion of balancing vasodilating mechanisms.

More recently, the involvement of NO has been receiving more attention as a potential mediator of vascular tone and thus its effect on arterial compliance has been recently examined (Kinlay *et al.,* 2001). In a study conducted by Kinlay et al. (2001), the investigators attempted to identify the endogenous vasoactive substances that regulate arterial compliance which had not previously been accomplished in human populations. The investigators hypothesized that NO would modulate arterial compliance and such a theory proved correct when they found that NO, an endothelium-mediated vasodilator, enhanced arterial elasticity in the human brachial artery and, therefore, does in fact contribute to compliance of the vessel in healthy humans. To determine this, the investigators used L-NMMA, an inhibitor of NOS, and found that when infused with this inhibitor, measures of arterial elasticity were attenuated. This technique, although invasive, allows for very accurate measures of elasticity and relations of changes in arterial caliber to changes in pressure over a wide range of transmural pressures. The findings confirmed the investigators' hypotheses that a reduced bioavailability of endothelium-derived NO would decrease arterial compliance, whereas the administration of nitroglycerin, an exogenous NO donor, would reverse the loss of compliance induced by L-NMMA. Thus, the researchers concluded that a loss of endothelial-derived NO that has been shown to be associated with cardiovascular risk factors may adversely affect arterial compliance in humans (Kinlay *et al.,* 2001).

The most probable mechanism for functional adaptations in arterial compliance is related to alterations in vascular smooth muscle tone influenced by vascular-endothelium-dependent vasodilation, thus NO bioavailability (Seals, 2003). It has been shown that

moderate-intensity aerobic exercise restores the age-related decreases in endothelium-dependent function within a population of previously sedentary middle aged men (DeSouza *et al.*, 2000). The increase in NO availability associated with aerobic exercise training has been suggested to restrain smooth muscle tone within the arterial wall and as a result increase compliance.

In conclusion there are numerous adverse effects of a decreased compliance of the central and peripheral arteries in the human population (Zieman *et al.*). A decrease in central arterial compliance has been shown to be associated with the incidence and progression of cardiovascular disease (Van Merode *et al.*, 1988). Moreover it has been shown that a physically inactive lifestyle is a well established risk factor for CVD (Blair *et al.*, 1995). Due to the connection between arterial compliance, CVD and physical activity, it thus seems important to study the effects of activity on the functioning of the vasculature and attempt to determine mechanisms involved. The growing occurrence and associated risk of arterial stiffness provides motivation to better comprehend the underlying mechanisms and the resultant physiological impact of such a condition.

2.3 Models of Inactivity

Within the literature there are various models of physical inactivity and cardiovascular deconditioning that have been used to mimic a physically deconditioned state. Models which have been proven to induce muscle atrophy and decreases muscle strength such include spaceflight (Vaziri *et al.*, 2000), simulated microgravity (Hikida *et al.*, 1989), bed rest (Takenaka *et al.*, 1994; Kamiya *et al.*, 2000; Bonnin *et al.*, 2001; Pawelczyk *et al.*, 2001), immobilization through casting (Giannattasio *et al.*, 1998),

animal models (Jasperse *et al.*, 1999; Schrage *et al.*, 2000) and paraplegic models (De Groot *et al.*, 2003; de Groot *et al.*, 2004). Models of cardiovascular deconditioning have several advantages and disadvantage associated with their use.

The bed rest model has served as a primary means of studying deconditioning and its effects on the cardiovascular and musculoskeletal system (Adams *et al.*, 1994). Bed rest studies have shown detrimental effects of this type of deconditioning on the cardiovascular system and muscle function such as muscle atrophy and decreases in muscle strength (Berg & Tesch, 1996; Bloomfield, 1997) and orthostatic intolerance (Bonnin *et al.*, 2001). Orthostatic intolerance has been shown to occur with models of bed rest deconditioning, thus simulated microgravity, as well as spaceflight models and such models are associated with changes throughout the cardiovascular system such as hypovolemia (Convertino *et al.*, 1989; Eckberg & Fritsch, 1992), decreases in baroreflex responsiveness (Convertino *et al.*, 1989; Eckberg & Fritsch, 1992) and increases in venous pooling (Convertino *et al.*, 1989). Studies have also mainly focused on the upper arm vascular beds and in conditions of head-down tilt (Shoemaker *et al.*, 1998a). In such studies in which bed rest is associated with head down tilt, confounding variables such as shifts in plasma volume are affected and such a model does not seem to be representative physiologically of a state of inactive or deconditioned sedentary lifestyle. Within a head down tilt bed rest state, blood flow changes to the legs during the first 24-48 hours are largely due to pronounced decreases in plasma volume and thus are not valid representations of an upright lifestyle in which most humans partake (Convertino, 1996). Due to the legs being responsible for the actions of standing and locomotion, it is best to

represent a physically inactive lifestyle, or cardiovascular deconditioned state, with the lower limbs in a normal physiological upright position. Such a model more accurately reflects changes in vasculature to cardiovascular deconditioning (Bleeker *et al.*, 2005b). Spaceflight models of deconditioning are hindered by limited number of human spaceflights and expenses and logistical problems associated with studying humans in space.

The model of ULLS was originally developed to study muscle adaptation to unloading (Berg *et al.*, 1991) and is based on the prevention of single lower limb weight bearing while the participant utilizes crutches for locomotion. The ULLS model has been proven to induce significant factors of deconditioning, which included muscle atrophy and a decrease in muscle strength (Berg *et al.*, 1991; Dudley *et al.*, 1992; Bleeker *et al.*, 2005a). The model of ULLS seems to be a very valid representation of a physically inactive, or physically deconditioned, lifestyle in able-bodies humans, as it is not confounded by the influences of denervation, such as in the SCI, or microgravity, such as in the models of bed rest and spaceflight. Thus, ULLS provides an accurate representation of a sedentary lifestyle within the able-bodied population. ULLS is also a very logical approach to study deconditioning within the human population as it allows subjects to conduct normal everyday activities and also the procedure reduces expenses and logistical issues that are associated with bed rest.

Another model that has been used to study the effects of inactivity or deconditioning is the model of spinal cord injury (SCI). SCI provides a unique model to study peripheral vascular adaptations to an extremely inactive paralyzed state (De Groot

et al., 2003). Although extremely valuable, the results of studies using paraplegic models should be interpreted with caution and application of results of such studies should be used with vigilance due to the state of paraplegia being associated with other unique pathologies such as a disturbed sympathetic innervation. However, it has been shown that patients that lack vascular sympathetic innervation but engage in normal physical activity show no signs of vascular adaptations (Eisenach *et al.*, 2002). Moreover, it has been shown that there exists similar shear stress levels in an SCI group with lower motor neuron lesion, thus with nerve degeneration, and in an SCI group with upper motor neuron lesion, thus without nerve degeneration (Boot *et al.*, 2002). Thus in their attempt to distinguish between the effect of nerve degeneration and inactivity the authors found that inactivity plays a more significant part in the increased shear stress levels found within the femoral artery in SCI individuals than denervation. Thus, it was concluded that the vascular adaptations in the SCI population result principally from the effects of deconditioning.

ULLS is a model to elicit deconditioning and muscle adaptations to weight unloading of the lower extremities in human subjects (Berg *et al.*, 1991). Such a model allows for one limb of the lower leg to be free of weight-bearing while the subject uses crutches for locomotion. Previous studies have validated its effectiveness in inducing muscle atrophy and decreases in muscle strength (Berg *et al.*, 1991; Dudley *et al.*, 1992) and thus such a model was proposed to be an effective model to study the potential effects of deconditioning on the vascular system. The ULLS model has been shown to elicit muscular adaptations in healthy young humans such as muscle atrophy and

decreases in muscle strength (Dudley *et al.*, 1992; Berg & Tesch, 1996; Bleeker *et al.*, 2005a). Bleeker and colleagues, in the attempt to justify ULLS as an effective form of deconditioning, found that ULLS caused a significant decrease in calf circumference, skin temperature, and strength of the quadriceps muscle within the suspended leg (Bleeker *et al.*, 2005a). Such findings are in close agreement with earlier studies reporting a decrease of 13-21% in maximum voluntary contraction of the quadriceps muscle after 10 days to 6 weeks of unloading (Dudley *et al.*, 1992; Berg & Tesch, 1996; Schulze *et al.*, 2002).

Studies utilizing animal models most commonly rely on the model of hindlimb unweighting to mimic physical deconditioning (Jasperse *et al.*, 1999; Schrage *et al.*, 2000; Vaziri *et al.*, 2000). HLU, most commonly in rats, is a model that has been widely used to simulate human bed rest or exposure to microgravity (Morey *et al.*, 1979). Within such a model, investigators are able to use more invasive measurement techniques to study more cellular adaptations to deconditioning such as protein and gene expression and enzyme levels and activity (Jasperse *et al.*, 1999; Schrage *et al.*, 2000). Animal models should be interpreted with caution as there are a vast amount of species differences between upper and lower mammals and humans (Berg & Tesch, 1996). Such differences include growth rates, anatomy, fiber type characteristics and distributions and anatomical functioning (Berg & Tesch, 1996). Animal muscles are mainly homogenous with regards to fiber type distribution and thus animal vascular and muscular adaptations could differ between human adaptations in which fiber type is much more heterogamous in nature (Berg & Tesch, 1996).

To our knowledge there is limited amount of literature examining the effects of lower limb immobilization on alterations within the arterial vascular system in healthy, young humans with only few known studies characterizing such adaptations (Bleeker *et al.*, 2005a/b). Thus it is in the interest of the present study to extend previous studies conducted on animals to determine the time course of potential alterations within the vasculature of healthy young humans.

3.0 MATERIALS AND METHODS

3.1 Subjects

15 healthy participants (8 female, 7 male; male: 20.85 ± 0.7 years, Female: 20.37± 0.75 years) were recruited from McMaster University to participate in the study. Participants were recruited from McMaster University under volunteer conditions. All subjects were screened with a medical history questionnaire and none exhibited any medical problems that would interfere with their safety in the study. Exclusion criteria were smoking, recent bone fractures of the limbs, cardiovascular disease, diabetes, oral contraceptive medication and certain medications known to interfere with safety of the subjects and of the measures being examined. None of the subjects were endurance-trained and subjects were excluded if they exercised more than five hours per week. If inclusion criteria were met, informed, written consent was obtained from each participant. All testing procedures utilized in the study were approved by the McMaster University Medical Ethics Board and were performed in the Exercise and Metabolism Research Laboratory in the Ivor Wynne Center at McMaster University. Subject characteristics are summarized in Table 1.

Table 1. Subject Characteristics

SUBJECT	AGE	LEG	HEIGHT (cm)	WEIGHT (Kg)	PULSE PRESSURE (mmHg)	PULSE PRESSURE (mmHg)	BMI (Kg/m^2)
MALE							
MM	20	R	175	61.7	64.8	60.6	20.1
JW	20	R	177	87.4	47.2	44.7	27.9
SB	20	L	177	78.4	56.8	36.4	25.0
BC	21	L	173	72.2	54.1	51.6	24.1
CL	25	L	174	91.2	49.9	51.4	30.1
MC	20	R	189	84.9	58.4	54.6	23.8
JD	20	L	191	111.1	69.8	65.0	30.5
MEAN	**20.85**		**179.4**	**83.8**	**57.3**	**52.0**	**25.9**
SEM	**0.70**		**2.79**	**5.9**	**7.97**	**9.6**	**1.4**
FEMALE							
NZ	19	R	167	61.3	46.4	47.6	22.0
JB	19	R	170	63.7	48.1	45.0	22.0
SS	19	R	159	50	46.1	35.5	19.8
AT	23	R	157	52.1	52.4	49.1	21.1
NB	21	L	165	59.9	52.0	42.5	22.0
RA	20	L	160	66.1	55.2	47.2	25.8
HV	18	R	163	61.7	45.6	42.5	23.2
KB	24	L	170	70.1	32.6	52.4	24.3
MEAN	**20.37**		**164**	**60.6125**	**47.3**	**45.2**	**22.5**
SEM	**0.75**		**1.7572**	**2.3794**	**6.4**	**4.8**	**0.6647**

3.2 Study Design

Participants in the study were single-leg immobilized for 12 days. Testing sessions occurred prior to immobilization and following 12 days of immobilization. Testing sessions consisted of measurements to determine potential changes in arterial compliance, arterial distensibilty, and endothelial function through the immobilization protocol.

The investigation employed a two-way repeated measures analysis of variance (ANOVA) to assess the effects of single leg immobilization on all parameters over the two time points. Immobilization (IMM and NIM) and time (PRE and 12-DAY) were within subjects factors. For the analysis of the effects of gender on the responses of the deconditioning, a three-way ANOVA was used with gender, time and immobilization as between subject variables.

3.3. Unilateral Lower Limb Immobilization (ULI) Protocol

One leg of the participant was suspended from weight bearing activity by the application of a knee brace that was held in place over the knee joint to prohibit knee flexion and extension. The knee was immobilized in a slightly flexed position of approximately 130 degrees to reduce the risk of development of deep vein thrombosis in the suspended limb; however the hip and ankle joints were fully mobile and were able to move about in a full range of motion. Each brace was wrapped with tape and signed by the investigators to ensure participant protocol compliance. Each subject utilized crutches for locomotion. Subjects were introduced to the crutches and the mechanics of

locomotion in order to minimize any compensatory activity of the contra-lateral leg. Participants reported to the lab once per day throughout the 12 day protocol to take off their knee braces and stockinette and take a shower in the laboratory without bearing weight on the treatment leg. The knee brace was then reapplied, and new tape was applied. Subjects spent an overall time of 10–15 min with the brace removed.

During testing protocol, subjects were informed to lie still comfortably and relax in a supine position on a bed with a pillow elevating their head slightly for comfort purposes. Vascular function measurements were performed after a brief acclimatization period.

3.4 Testing Procedures

All 15 subjects were measured at two testing time points: PRE and POST (12-Days) in the Exercise and Metabolism Research Laboratory at McMaster University, Hamilton, Ontario. POST testing was scheduled following 12 days of immobilization and not 14 days due to POST scheduling conflicts and biopsy priority testing of a concurrent study. We felt that the testing may be uncomfortable to the subjects if done on the same day as biopsy samples and thus we chose to test at 12 days into immobilization. Prior to the initial testing session, all participants visited the lab and were familiarized with the testing procedures to which they would be subjected.. An information brochure was given to each participant to ensure their full comprehension of the study's procedures, its rationale and its objectives.

All attempts were made to perform measurements at the same time of day for each individual subject and only under a few circumstances did times slightly vary between testing times for individual subjects. Subjects were advised to refrain from caffeine, nicotine, alcohol and heavy exercise for 24 hours prior testing sessions. Subjects were furthermore instructed to refrain from eating for 4 hours prior to the commencement of the testing procedures and were advised to consume a similar dietary regime prior to the testing times. The preceding instructions were applied to negate any effects a different diet may have on vascular dynamics and allow for consistent measuring conditions during each time point throughout the 12 day intervention protocol. Testing room temperature was controlled between approximately 22-24°C. Subjects were informed to go about their normal daily activities and not increase or decrease the level of activity in which they were engaging in prior to the commencement of the study.

3.4.1 Heart Rate

Upon entering the laboratory for a testing session, 2 sets of 3 electrocardiogram (ECG) electrodes were placed in the CM5 configuration on each participant's chest in order to obtain continuous heart rate (HR) monitoring throughout the testing session with two separate heart rate monitoring systems. Electrode placement consisted of adhering 2 electrodes inferior to the right clavicle, two inferior to the right clavicle and two on the left side of the torso inferior to the subjects' nipple. The ECG signal from one set of 3 electrodes was obtained with the Cardiomatic (Model MSC 71233, Medical Systems Corp., Miami, FL,USA). This analogue ECG signal was sampled at 200Hz for analogue

to digital conversion (Powerlab 16sp, AD Instruments) and recorded on Powerlab Chart 5 Software (Ad Instruments, Colorado Springs, CO, USA). Data was stored on a personal computer (IBM NeVista x86 compatible processor, White Plains, NY, USA) for subsequent analysis. The second set of 3 ECG electrodes was used to acquire an ECG tracing on the Doppler Ultrasound unit for gating of vessel images with the heart cycle (Model System Five, GE Medical Systems, USA).

3.4.2 Resting Blood Pressure

A measure of resting blood pressure was taken at the commencement of each testing session using an automated oscillometric BP measurement device (Model CBM-7000, Colin Medical Instruments, San Antonio, TX, USA). A standard blood pressure cuff was placed over the right brachial artery distal to the elbow joint and participants were instructed to remain still in a supine position. Measures were obtained in triplicate and average at each testing time point.

The non-invasive assessment of pulse pressure was also acquired via applanation tonometry on a beat by beat basis concurrently with the acquisition of arterial images. A pencil-type probe enclosing a high fidelity strain-gauge transducer (Model SPT-301, Millar Instruments Inc., Texas, USA) was held on each subject's skin over the site where the greatest pulsation of the common carotid artery occurred. Arterial pressure waveforms were represented by the analogue signal produced by the strain gauge transducer. When a consistent and regular blood pressure pulse waveform was acquired for several beats of the cardiac cycle, the pressure waves were sampled at 200Hz and

stored for later analysis (Chart 5, Powerlab Software, ADI Instruments, Colorado Springs, CO, USA). The pencil-like tonometer was calibrated to continuous pressure waveforms obtained by use of automated radial artery applanation tonometry (Model CBM-7000, Colin Medical Instruments, San Antonio, TX, USA). A sensor was placed over radial artery at the point of maximal pulsation and beat-by-beat blood pressure waveforms were acquired. The radial artery waveforms were automatically calibrated to brachial oscillometric cuff pressure with the use of an automated arm pressure cuff (Model CBM-7000, Colin Medical Instruments, San Antonio, TX, USA). The time of image acquisition was marked on the Powerlab Chart 4 software with an external trigger for future analysis (Figure 3.1).

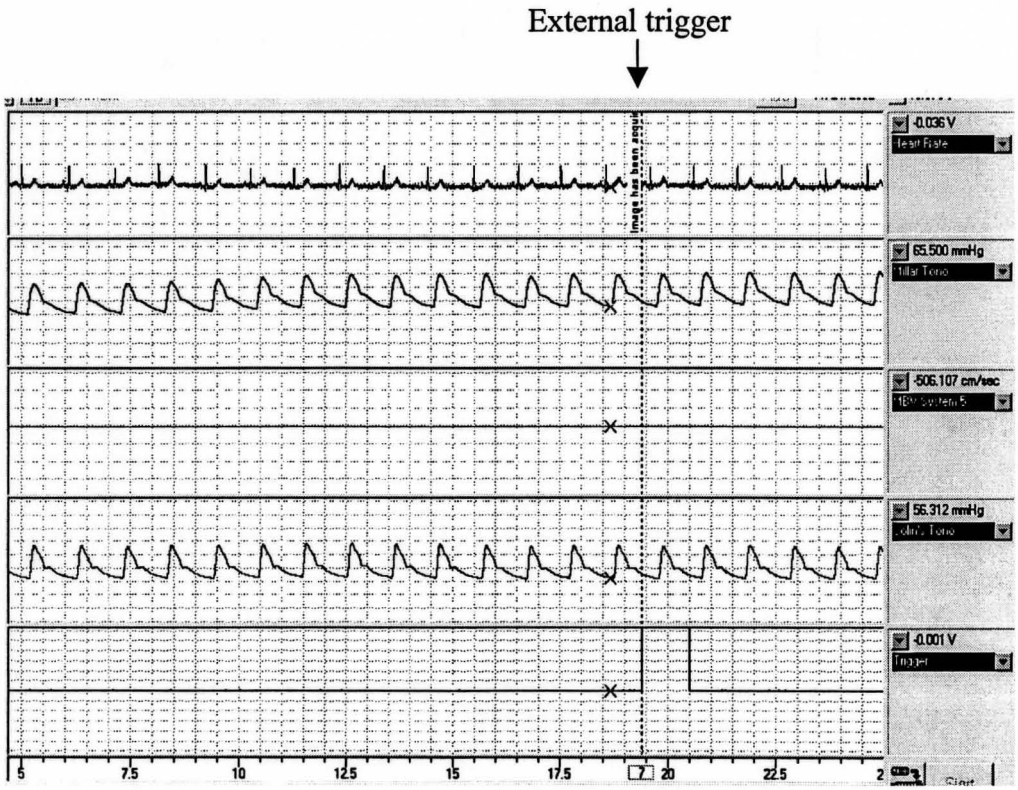


Figure 3.1: Powerlab data displaying the heart rate, Millar BP (carotid tonometry) and Colin BP (radial tonometry) channels. Ultrasound image recorded is marked with the external trigger.

The carotid pressure waveform was converted to mmHg units using the radial artery pulse pressure as a reference. The pressure signal obtained by the process of applanation tonometry in the carotid artery was calibrated by assigning the diastolic pressure acquired from radial artery tonometry to the value corresponding to minimum pressure, and the mean pressure (calculated by the formula: [(1/3(SBP-DBP) + DBP)] as assessed by radial tonometry to its average value. This procedure is based upon the assumptions that mean blood pressure does not change within large conduit arteries and, moreover, that diastolic blood pressure does not significantly differ among carotid, popliteal and femoral arteries (Kelly *et al.,* 1989). Systolic blood pressure, however, does not remain similar between central (carotid) arteries and peripheral (popliteal and femoral) arteries due to the amplification of pulse waves as the arterial pressure wave is transmitted down the arterial tree (Izzo & Shykoff, 2001). Therefore, to determine SBP in the carotid artery, the diastolic and mean arterial pressures obtained by radial tonometry were equated with the corresponding signal in the carotid pressure waveform. The SBP in the carotid artery was then extrapolated from this relationship (Figure 3.2).

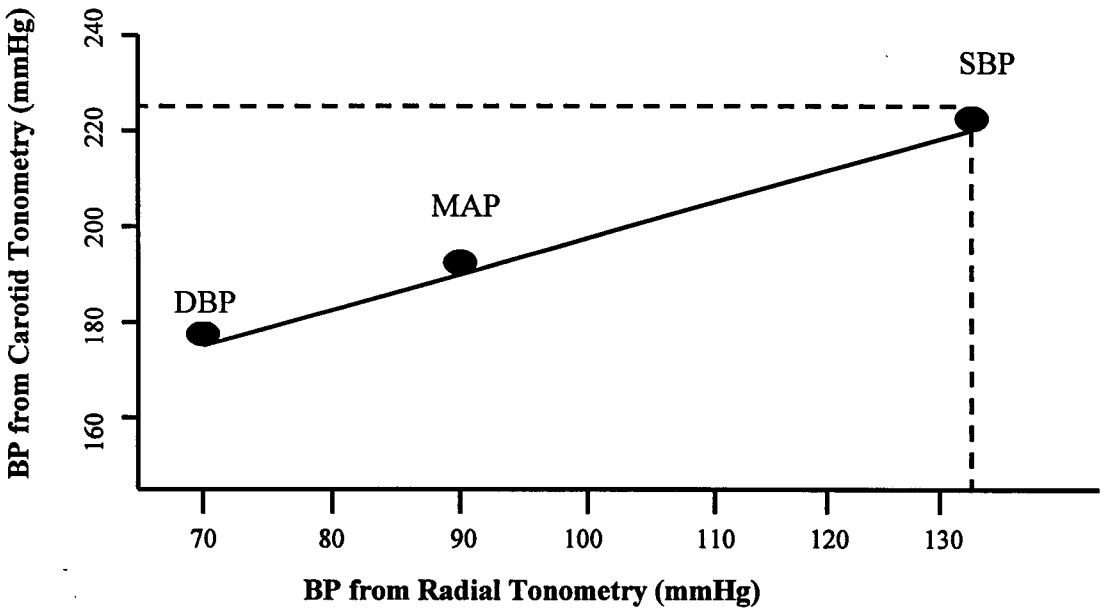


Figure 3.2 Systolic blood pressure (SBP) in the carotid artery in mmHg is calculated by extrapolating from the above relationship between diastolic blood pressure (DBP) and mean arterial pressure (MAP), whose values are known in both mmHg and mV.

3.4.3 Arterial Imaging

Common carotid, common femoral and popliteal artery distensibilty and compliance was assessed through concurrent imaging of the respective artery by use of Doppler ultrasound and simultaneous applanation tonometry. The diameter of the artery was determined from images using B-mode ultrasound techniques (Model System Five, GE Medical Systems, Horten, Norway). A 10 MHz high resolution linear array ultrasound probe transducer was positioned on the skin over the artery. When an image, viewed on the monitor, was obtained where the near and far walls of the artery were visible and clear, 3 images were digitally stored for later off-line analysis. Each

individual image corresponded to one R-R interval of the subjects ECG heart cycle. Images were saved digitally and then transferred to a DICOM (digital imaging and communication in medicine) JPEG file for future analysis and also saved on videocassette. The same investigator analyzed all images using an automated edge-detecting software program (AMS II, Chalmers University, Gotenberg, Sweden). The interface between the adventitia layer of the vessel wall and the media layer of the vessel wall was measured and this represented the distance between the artery's near-wall boundary and the far wall boundary, respectively. Maximum diameter of the artery, or systolic expansion, and minimum diameter, or diastolic relaxation, was measured to establish the diameter change through one heart cycle.

3.5 Measurements

3.5.1 Blood Flow and Diameter Analysis

Arterial blood flow and arterial diameter were assessed by the utilization of Echo Doppler (Model System Five, GE Medical Systems, Horten, Norway) of the Left Common Carotid Artery (CCA), Suspended and Contra-lateral Leg Common Femoral Artery (ULLS-CFA and C-CFA, respectively), Suspended and Contra-lateral leg Popliteal Artery (ULLS-PA and C-PA, respectively), and Suspended and Contra-lateral leg Popliteal Vein (ULLS-PV and C-PV, respectively). The average of 10-12 Doppler

spectra waveforms was used to calculate mean blood velocity. Mean blood flow (ml/min) was calculated as

$$\frac{1}{4} \cdot \pi \cdot (\text{mean diameter})^2 \cdot \text{mean velocity (cm/s)} \cdot 60 \quad \textbf{(Equation 5)}$$

The calculations of peak blood flow (ml/min) was obtained by using the following equation,

$$\frac{1}{4} \cdot \pi \cdot (\text{systolic diameter})^2 \cdot \text{peak velocity (cm/s)} \cdot 60 \quad \textbf{(Equation 6)}$$

For resting arterial diameter measurements, two successive longitudinal vessel images were analyzed at the peak systolic and end-diastolic phase of the heart cycle. Mean diameter was calculated as ⅓· systolic diameter + ⅔· diastolic diameter. The average of at least 10-12 Doppler spectra waveforms was used to calculate mean blood velocity in order to obtain accurate and steady data values.

3.5.2 Arterial Compliance Measurements

Cross sectional compliance of the common carotid artery, common femoral artery and the popliteal artery was assessed in all participants at each of the three testing sessions. A combination of ultrasound imaging and simultaneous applanation tonometry was used for the non-invasive determination of arterial distensibility. Cross sectional

compliance was calculated using an equation containing values of PP and changes in diameter. Cross-sectional compliance was calculated as follows:

$$CSC = \frac{\Delta \text{Area}}{PP} = \frac{\pi r^2_{max} - \pi r^2_{min}}{PP} = \frac{\pi(d_{max}/2)^2 - \pi(d_{min}/2)^2}{PP} \quad \textbf{(Equation 9)}$$

where, CSC = cross-sectional compliance
PP = pulse pressure
r = radius of the artery
d = diameter of the artery
max = maximal value
min = minimum value

In order to calculate the compliance and distensibilty of the artery, the diameter changes (Δ D) as well as the corresponding PP values are required. Diameter change was calculated by subtracting of the minimum diameter from the maximum diameter in each of the two arterial images.

Δ D = maximum diameter – minimum diameter (Equation 1)

Pulse Pressure (Δ P) was obtained by subtracting the DBP from the SBP. The blood pressure waveform that was analyzed included one heart cycle prior to the trigger in the Powerlab Chart 5 Software (Ad Instruments) file. The single heart cycle corresponded to the image that was obtained by Doppler Ultrasound.

$$\Delta P = SBP - DBP \quad \textbf{(Equation 2)}$$

Arterial compliance was defined as the change in cross sectional area relative to the pulse pressure according to the formula

$$(\Delta D/D_{mean})/(2\cdot\Delta P)\cdot\pi\cdot D_{mean}^{2} \quad (mm^2/mmHg) \quad \textbf{(Eqation 3)}$$

Δ D is the difference between systolic and diastolic diameter, D mean is 1/3 · systolic diameter + 2/3 · diastolic diameter, Δ P is the difference between systolic and diastolic pressure.

Arterial distensibility was defined as the relative diameter change in an artery for a given change in pressure.

$$\textbf{Arterial Distensibility} = \Delta D / (\Delta P \times D) \quad \textbf{(Equation 4)}$$

where D is resting diameter. The value used was minimum arterial diameter. Arterial distensibility was consequently calculated for the two images and their corresponding pulse pressures. These two values were then averaged to give a measure of arterial distensibility at all three testing time points.

3.5.3 Flow Mediated Vasodilatation (FMD) Measurement

Reactive hyperemia and flow mediated vasodilatation (FMD) was assessed by placing a pressure cuff around the calf at its maximum girth and was then inflated to a suprasystolic pressure of approximately 200-220 mmHg for 9 minutes. Following the cuff deflation at 9 minutes, hyperemic blood flow velocity in the popliteal artery was assessed and recorded on videotape for 15 seconds following release followed by continuous acquisition of popliteal artery diameter for 2 minutes to establish FMD data. Regional peak wall shear rate (PWSR) was calculated as,

$$4 \cdot \text{peak velocity/ systolic diameter } (s^{-1}) \quad \textbf{(Equation 7)}$$

Mean wall shear rate (MWSR) was calculated as

$$4 \cdot \text{mean velocity / mean diameter } (s^{-1}) \quad \textbf{(Equation 8)}$$

Delta flow, delta PWSR and delta MWSR were defined as the differences between resting responses and hyperemic responses. Vessel diameters following reactive hyperemia were measured off-line from 30, 60, 90, 120 and 180 seconds following cuff release. FMD was expressed as the maximal relative diameter change from baseline of the end-diastolic diameter. Since the FMD response is directly proportional to the magnitude of the stimulus (Levenson *et al.*, 2001), the FMD response was also expressed

relative to the delta shear rate. The following ratios were calculated: FMD / delta MWSR and FMD / delta PWSR.

3.6 Statistical Analysis

Data were analyzed by using a 2 way repeated measures ANOVA (condition by time) design. Differences between experimental condition (immobilized limb and control limb), or time points, expressed as PRE and 12-DAY time points, were analyzed for each dependent variable. A p-value of < 0.05 was used to evaluate statistical significance.

4.0 RESULTS

4.1 Subject Characteristics

Subject descriptive characteristics are displayed in Table 1. Significant differences between the Male and Female groups were noted for height ($p=0.0003$), weight ($p = 0.002$), Pulse Pressure ($p= 0.02$) and BMI ($p= 0.041$).

Table 1. Subject Characteristics

SUBJECT	AGE	LEG	HEIGHT (cm)	WEIGHT (Kg)	PULSE PRESSURE (mmHg)	PULSE PRESSURE (mmHg)	BMI (Kg/m^2)
MALE							
MM	20	R	175	61.7	64.8	60.6	20.1
JW	20	R	177	87.4	47.2	44.7	27.9
SB	20	L	177	78.4	56.8	36.4	25.0
BC	21	L	173	72.2	54.1	51.6	24.1
CL	25	L	174	91.2	49.9	51.4	30.1
MC	20	R	189	84.9	58.4	54.6	23.8
JD	20	L	191	111.1	69.8	65.0	30.5
MEAN	**20.85**		**179.4**	**83.8**	**57.3**	**52.0**	**25.9**
SEM	**0.70**		**2.79**	**5.9**	**7.97**	**9.6**	**1.4**
FEMALE							
NZ	19	R	167	61.3	46.4	47.6	22.0
JB	19	R	170	63.7	48.1	45.0	22.0
SS	19	R	159	50	46.1	35.5	19.8
AT	23	R	157	52.1	52.4	49.1	21.1
NB	21	L	165	59.9	52.0	42.5	22.0
RA	20	L	160	66.1	55.2	47.2	25.8
HV	18	R	163	61.7	45.6	42.5	23.2
KB	24	L	170	70.1	32.6	52.4	24.3
MEAN	**20.37**		**164**	**60.6125**	47.3	45.2	**22.5**
SEM	**0.75**		**1.7572**	**2.3794**	6.4	4.8	**0.6647**

Initial three-way ANOVA statistical analysis of all cardiovascular variables with SEX (Male and Female), as a between factor and LEG (Immobilized and NON-Immobilized) and TIME (Pre and Post) as the within factors, revealed only main effects for SEX and TIME (Appendix A: Section B). These results indicate that there are obvious differences between some of the baseline vascular characteristics of males and

females, (i.e. Males have larger popliteal arterial diameters, larger popliteal arterial blood flows and greater pulse pressures), while other variables such as compliance and FMD showed only main effects for time. These findings do not impact on the responses of the 2 groups to immobilization. Due to the similar responses of males and females to immobilization, the data for both groups was pooled to create a single data set for all further analysis.

4.2 Popliteal Artery Characteristics at Rest

Vascular characteristics of the popliteal artery are shown in Table 2.

Table 2: Popliteal Artery Characteristics

Variable	Immobilized		Non-immobilized	
	PRE	12-DAY	PRE	12-DAY
Systolic Diameter (cm)	0.60±0.02	0.52±0.02[a,*]	0.62±0.02	0.58±0.02[a]
Diastolic Diameter (cm)	0.56±0.02[*]	0.49±0.02[a]	0.58±0.02	0.55±0.02[a]
Mean Diameter (cm)	0.57±0.02	0.50±0.02[a,*]	0.59±0.02	0.55±0.02[a]
Mean Blood Velocity (cm/sec)	1.5±0.1	2.2±0.1[a]	1.7±0.1	2.0±.2[a]
Mean Blood Flow (mL/min)	24.2±2.1	26.2±2.7	28.1±2.7	27.1±1.9
Pulse Pressure (mmHg)	59.1±2.5	63.4±2.2	58.0±2.8	61.8±1.7
Cross Sectional Compliance ($10^{-4}mm^2/mmHg$)	5.7±0.4	3.8±0.4[a]	6.7±0.9	5.5±0.6[a]

All data were obtained with the participant at rest and are presented as means ±SEM. [a] indicates differences within a leg over time, * indicates differences from the NON-immobilized leg at the same time point.

Popliteal artery systolic diameter at rest decreased from PRE to 12-DAY in both the Immobilized and NON-Immobilized legs; however, the decrease was greater in the Immobilized leg and resulted in differences between the groups at the 12-DAY time point (Table 2). Resting popliteal artery diastolic diameter decreased from PRE to 12-DAY in both the Immobilized and NON-Immobilized legs. Analysis of popliteal artery mean resting diameter for the Immobilized (I) and NON-Immobilized (N) legs at the two testing time points showed a significant interaction effect of leg by time ($p=0.00001$). Mean arterial diameter of both the I and N decreased from PRE to 12-DAY; however, the

Immobilized leg showed a greater decrease such that the mean popliteal artery diameter was smaller than in the NON-Immobilized leg after 12 days (Table 2 and Fig. 4.1).

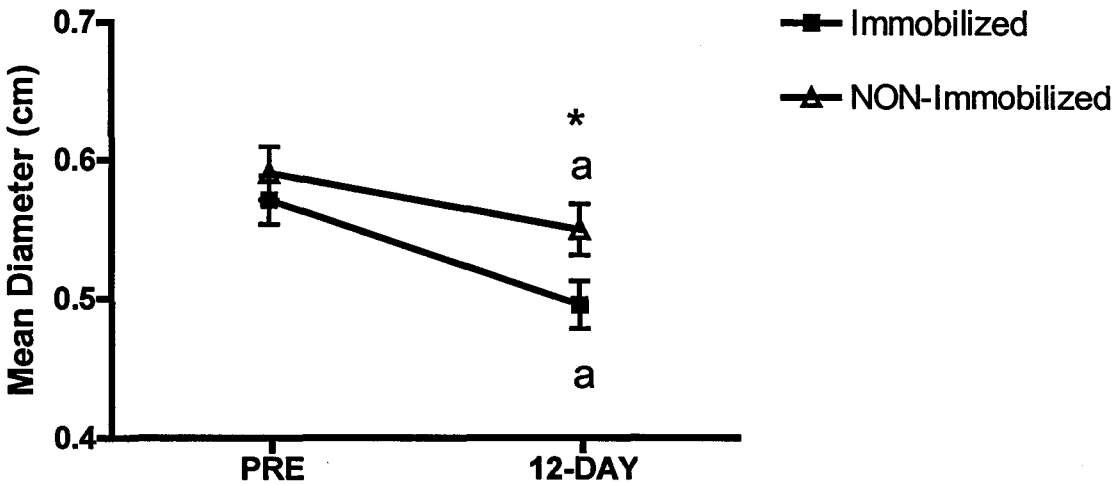


Figure 4.1 Popliteal Artery Mean Resting Diameter in Immobilized leg (I) (n=15), and NON-Immobilized (N) (n=15). Values are plotted as mean ± SEM. Means with different letters refer to values that are different over time. * indicates differences between conditions.

Resting popliteal arterial mean blood velocity increased over time in both legs; however, the increase was greater in the Immobilized leg (Table 2). As a result of decreases in diameter and increases in velocity over time, popliteal artery blood flows did not change throughout the immobilization period and did not differ between the legs at any time point (Table 2). Popliteal arterial cross-sectional compliance decreased from PRE to 12-DAY in both the Immobilized and the NON-Immobilized legs (Table 2, Figure 4.2)

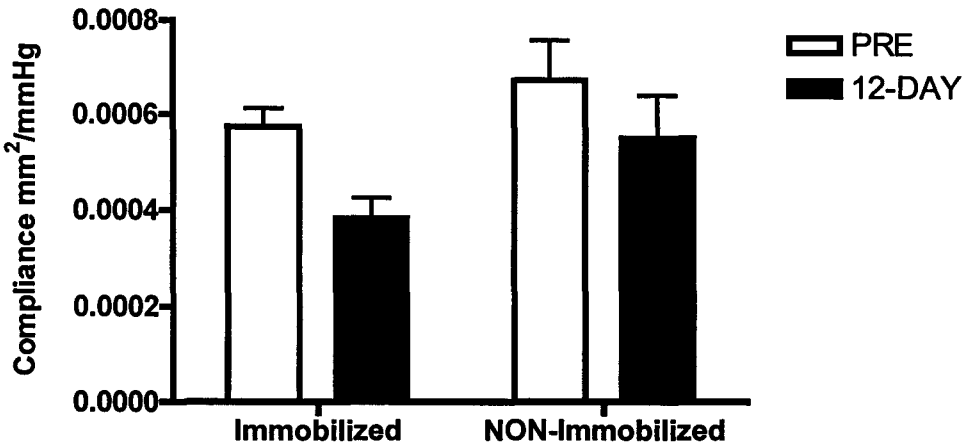


Figure 4.2 Popliteal Artery Cross Sectional Compliance in Immobilized leg (I) (n=15), and NON-Immobilized (N) (n=15). Values are plotted as mean ± SEM. Significant main effect of TIME (p=0.000).

4.3 Common Femoral Artery Characteristics at Rest

Vascular characteristics of the Common Femoral artery are shown in Table 3.

Table 3: Femoral Artery Characteristics

Variable	Immobilized		Non-immobilized	
	PRE	12-DAY	PRE	12-DAY
Systolic Diameter (cm)	0.86±0.04	0.79±0.03[a]	0.83±0.04	0.81±0.03[a]
Diastolic Diameter (cm)	0.81± 0.04	0.75±0.03[a]	0.78±0.03	0.76±0.03[a]
Mean Diameter (cm)	0.83± 0.04	0.77±0.03[a]	0.81±0.03	0.77±0.03[a]
Mean Blood Velocity (cm/sec)	3.6±0.20*	4.6±0.24[a]*	4.8±0.40	5.1±0.4
Mean Blood Flow (mL/min)	122.7±12.5	135.6±15.3	139.6±13.3	141.1±12.6
Cross Sectional Compliance ($10^{-4}mm^2/mmHg$)	1.2 ±0.1	.79 ±0.1[a]*	1.15 ±0.1	1.0±0.1[a]

All data are means ±SEM. [a] indicates differences within a leg over time, * indicates differences from the NON-immobilized leg at the same time point.

Common femoral systolic and diastolic and mean resting diameters decreased from PRE to 12-DAY in both the Immobilized and NON-Immobilized legs (Table 3) . Resting femoral artery mean blood velocity were lower in the immobilized versus the NON-immobilized leg at the PRE testing time point and increased over time in the immobilized leg but did not change in the control leg (Table 3).

There were no differences in resting mean arterial blood flow between the Immobilized and NON-Immobilized legs at any time point and no changes were observed within any leg over time (Table 3). Common femoral artery compliance changed

differently over time between the two legs. In the Immobilized leg, cross-sectional compliance decreased from PRE to 12-DAY while compliance of the NON-Immobilized leg was not altered over time (Table 3 and Figure 4.3).

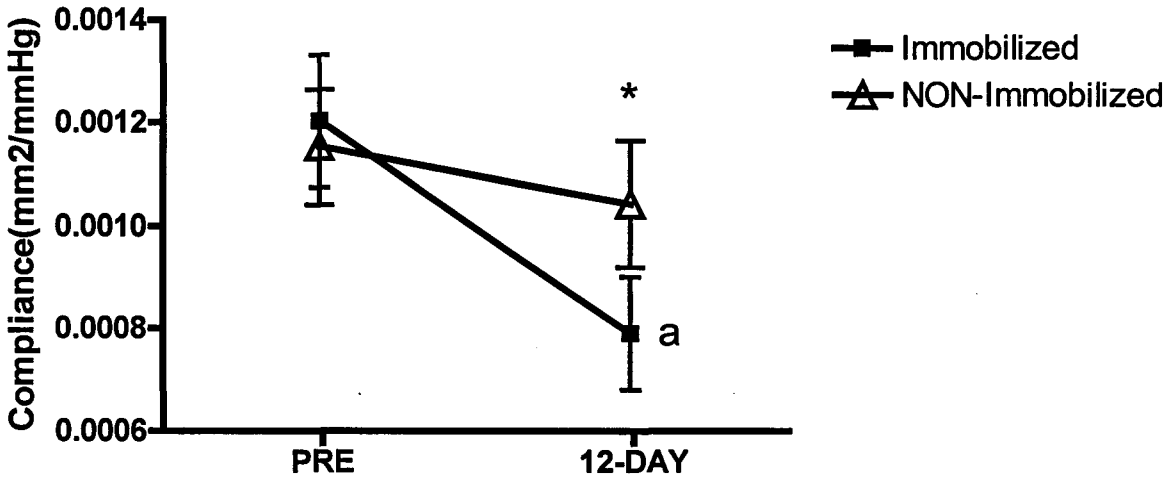


Figure 4.3 Common Femoral Artery Cross Sectional Compliance in Immobilized leg (I) (n=15), and NON-Immobilized (N) (n=15). Values are plotted as mean ± SEM. Different letters indicate differences over time within a condition. * indicates differences between conditions at a single time point.

4.4 Popliteal Artery Flow Mediated Vasodilation

Analysis of Popliteal artery relative FMD for the Immobilized (I) and NON-Immobilized (N) legs at the two testing time points (PRE and 12-DAY) showed a significant interaction effect of LEG x TIME ($p=0.0148$). Although there were no differences in the response of the popliteal artery to an occlusive challenge prior to immobilization, after 12 days relative FMD was only found to increase in the

immobilized leg (Figure 4.4). Popliteal Artery Relative FMD values for the Immobilized legs are as follows; 6.43 ± 1.38 % and 13.57 ± 2.73 % for PRE and 12-DAY time points respectively. Values associated with the NON-Immobilized are as follows; 6.29 ± 1.41 and 8.94 ± 1.65 % for PRE and 12-DAY time points respectively.

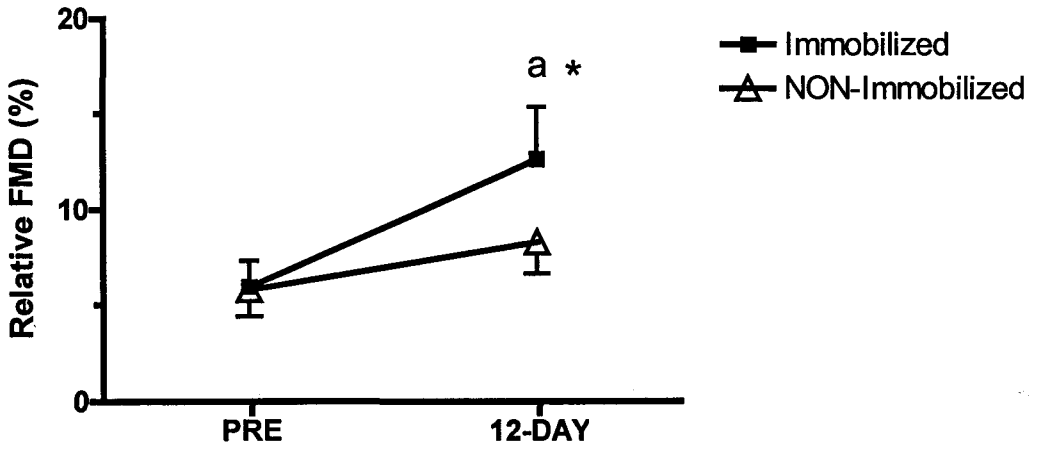


Figure 4.4 Popliteal Artery Relative FMD in Immobilized leg (I) (n=15), and NON-Immobilized (N) (n=15). Values are plotted as mean ± SEM. [a] indicates differences over time, within a condition. * indicates differences between conditions at a single time point.

Popliteal Artery Peak Wall Shear Rate (PWSR) measured after release of the cuff in the FMD protocol was not different between the legs at any time point and did not change within any leg over time (Immobilized PRE: 389.1 ± 23.83 sec^{-1}, 12-DAY 412.7 ± 23.14sec^{-1} ;NON-Immobilized PRE: 407.0 ± 29.18sec^{-1}, 12-DAY: 437.5 ± 34.56sec^{-1})

When the relative FMD response in the Popliteal artery was normalized to the PWSR resulting in Normalized FMD, a significant INTERACTION EFFECT (p=0.011)

was observed. Post hoc testing revealed that Normalized FMD increased in the immobilized leg over time (PRE: 0.0167 ± 0.007%/$^{sec-1}$, 12-DAY: 0.0316 ± 0.008 %/$^{sec-1}$) while remaining unchanged in the non-immobilized leg (PRE: 0.0163 ± 0.003%/$^{sec-1}$, 12-DAY: 0.0223 ± 0.004 %/$^{sec-1}$) (Figure 4.5).

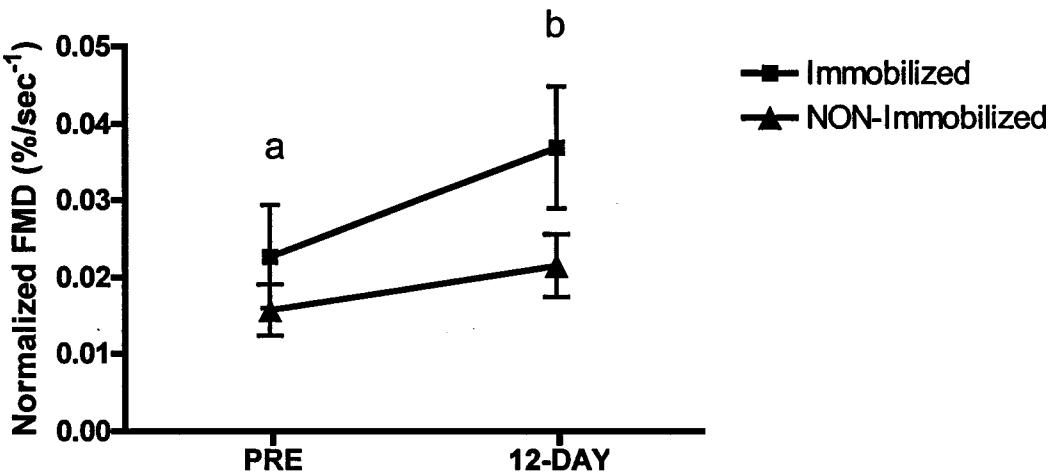


Figure 4.5 Popliteal Artery Normalized FMD in Immobilized leg (I) (n=15), and NON-Immobilized (N) (n=15). Values are plotted as mean ± SEM. Different letters indicate differences between conditions at a single time point.

4.5 Carotid Artery Resting Characteristics

Carotid artery compliance was not different over time (p=0.7657). Carotid artery compliance values are as follows; 0.001209 ± 0.000067 mm^2/mmHg and 0.001230 ± 0.00085 mm^2/mmHg for PRE and 12-DAY time points respectively (Figure 4.6).

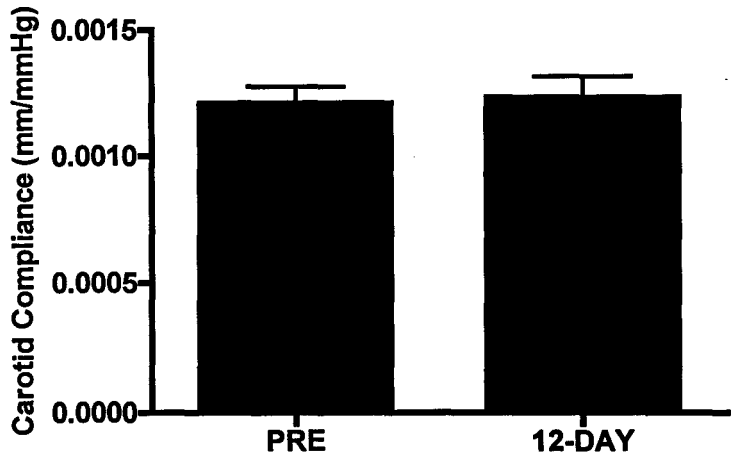


Figure 4.6 Carotid Artery Cross Sectional Compliance (n=15). Values are plotted as mean ± SEM

Similarly, carotid artery blood flow was not different at the two testing time points (PRE: 242.8±14.2 ml/min; 12-DAY: 226.0±14.27 ml/min) (Figure 4.7).

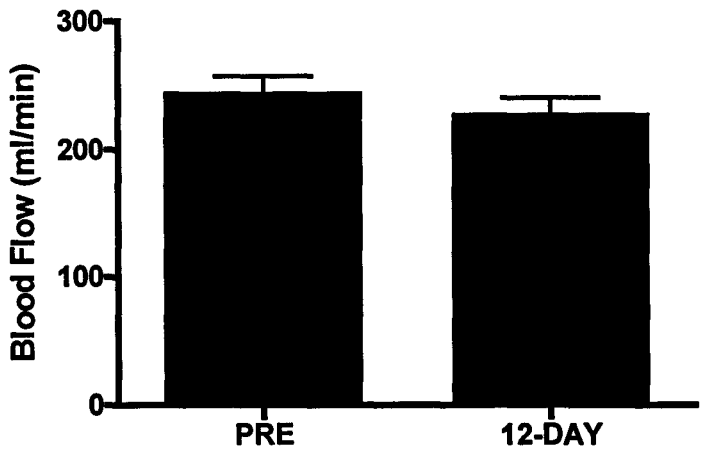


Figure 4.7 Carotid Artery Blood Flow (n=15). Values are plotted as mean ± SEM.

4.6 Control Group Analysis

In a separate subset of 5 healthy male individuals, identical testing procedures were conducted at a 12 day interval to assess the repeatability of our measures over time (Table 4). No differences were found for any variable over time.

Table 4. Control Data

	PRE	12-DAY	P-Value
Popliteal Artery			
Systolic Diameter (cm)	0.60±0.06	0.60±0.06	0.65
Diastolic Diameter (cm)	0.56±0.05	0.55±0.06	0.18
Mean Diameter (cm)	0.57±0.06	0.56±0.06	0.29
Blood flow (ml/min)	31.1±8.5	29.9±10.1	0.73
Blood Velocity (cm/sec)	2.0±0.6	2.0±0.8	0.90
Compliance (mm^2/mmHg)	0.068±0.02	0.069±0.02	0.85
Relative FMD (%)	11.0±4.4	10.9±6.2	0.92
Normalized FMD ($\%/sec^{-1}$)	0.024±0.01	0.026±0.01	0.60
Common Femoral Artery			
Systolic Diameter (cm)	0.85±0.09	0.88±0.1	0.40
Diastolic Diameter (cm)	0.78±0.1	0.82±0.12	0.34
Mean Diameter (cm)	0.81±0.09	0.84±0.11	0.36

Blood flow (ml/min)	87.9±31.6	90.9±28.5	0.61
Blood Velocity (cm/sec)	2.8±0.52	2.7±0.58	0.54
Compliance (mm^2/mmHg)	0.15±0.05	0.13±0.031	0.13

5.0 DISCUSSION

The purpose of the present study was three-fold: 1) to examine the effects of a unilateral lower-limb immobilization (ULI) protocol on vascular arterial compliance, resting blood flow, and endothelial function in young, healthy humans; 2) to examine any sex-based differences associated with such a protocol on vascular parameters of arterial compliance, resting blood flow, and endothelial function; 3) to determine if any potential vascular alterations that may occur with inactivity are locally mediated, specific to the immobilized limb, or occur systemically with changes occurring in central arteries.

The present study utilized ULI to study the arterial adaptations to physical inactivity. As such ULI represents a ground-based protocol unlike that of microgravity and dissimilar to injury-induced denervation. The model of ULI we utilized closely resembles unilateral lower limb suspension (ULLS), which was originally developed to examine the effects of unloading on muscle and to mimic unloading in spaceflight. Both models are based upon the avoidance of all weight-bearing in the limb being assessed while the participant relies on the support of crutches for unilateral support of the control leg (Berg *et al.,* 1991). The ULLS model has been proven to elicit successful deconditioning of the suspended leg with several studies demonstrating muscle atrophy and decreases in strength (Berg *et al.,* 1991; Dudley *et al.,* 1992). The ULI model was used in the present study to test the hypotheses that deconditioning would cause a decrease in arterial compliance, diameter and blood flow of both the popliteal and common femoral artery and a decrease in endothelial function throughout the 12 day time course. The ULI model is more convenient than the ULLS model since all this is required

is a knee brace and crutches and the issue of deep vein thrombosis is reduced with the ULI model versus the ULLS model since popliteal and femoral blood flows are not reduced. Hence, for these reasons we chose to use the ULI and not the ULLS model.

In agreement with our hypotheses, we found the following to occur through 12 days of ULI; a decrease in popliteal arterial resting systolic and mean arterial lumen diameter in the IMM and NIM leg with the IMM leg showing greater decreases compared to the NIM leg; a decrease in popliteal artery diastolic diameter in both the IMM and NIM legs over time; a decrease in common femoral artery systolic, diastolic and mean diameters in both the IMM and NIM legs; an increase in both popliteal and common femoral arterial resting mean blood velocity in both the IMM and NIM legs over time with the IMM leg increasing at a greater rate; a decrease in common femoral arterial cross sectional compliance in the Immobilized (IMM) compared to the NON-Immobilized (NIM) leg over time; and a decrease in popliteal arterial cross sectional compliance in both the IMM and NIM leg over time. Contrary to our hypotheses, however, our results showed no change in resting arterial blood flow in either the IMM or NIM leg in either the popliteal or common femoral artery and also showed a surprising increase in both relative and normalized flow-mediated vasodilation (FMD) of the popliteal artery in IMM leg compared to the NIM leg. Central arterial vascular results showed no change in carotid artery cross sectional compliance or blood flow through the 12 day of ULI.

There are several epidemiological studies that have provided evidence that a sedentary lifestyle is an independent risk factor for atherosclerosis and cardiovascular

disease (Paffenbarger *et al.*, 1993; Blair *et al.*, 1995). Physical exercise has been proven to prevent cardiovascular disease (Blair *et al.*, 1995). Moreover, the effect of exercise training on vascular dimensions (Dinenno *et al.*, 2001; Huonker *et al.*, 2003) and its effects on the functioning of the endothelium (Moyna & Thompson, 2004) has been studied extensively. Nevertheless, potential alterations that occur within the vasculature, such as arterial dimension, blood flow and endothelial function as a result of physical inactivity or cardiovascular deconditioning have yet to be clearly distinguished.

5.1 Popliteal Artery Endothelial Function Following ULI

In the present study we found that relative flow mediated vasodilation increased significantly ($p<0.05$) in the IMM leg while no significant changes were observed in the NIM leg throughout the 12 day ULI period. Moreover, when relative FMD values were corrected for the magnitude of the shear stress stimulus, a significant increase was still observed ($p<0.05$), with the IMM leg showing significant increases in normalized FMD and the NIM leg showing no changes. If, as we had hypothesized, one considers the evidence that inactivity reduces arterial reactivity then our findings require some explanation. Improvement in the functioning of the endothelium that has been demonstrated within the literature could be the key advantageous effect that physical activity has on such a risk factor for cardiovascular disease (CVD) (Bleeker *et al.*, 2005a). Investigations utilizing healthy subjects have shown that physical exercise training increases the function of the endothelium in humans by enhancing endothelium-dependent vasodilation (Clarkson *et al.*, 1999; Higashi *et al.*, 1999). If inactivity is

considered on a continuum then humans with a spinal cord injury would have local (i.e., below the level of the lesion) deconditioning of their musculature and presumably their vasculature also. With regards to the functioning of the endothelium in paraplegic populations endothelial function, as assessed using flow-mediated vasodilation (FMD), is reduced compared to able-bodied sedentary individuals and active controls (DeSouza *et al.*, 2000). Such differences have been suggested to be due to a relative up-regulation of endothelial function through physical exercise rather than a down-regulation through physical inactivity (Bleeker *et al.*, 2005b). Furthermore, it has been shown in other studies, just as it has presently, that deconditioning may not cause a decrease in endothelial function and may actually be preserved or show an increase (Bonnin *et al.*, 2001; de Groot *et al.*, 2004).

The measure of FMD was used in the present study to examine the functional alterations that potentially occur in the arteries of the leg following ULI deconditioning. FMD is an indicator of endothelial function and NO bioavailability which is brought upon through the stimulatory action of wall shear stress (Bleeker *et al.*, 2005b). The present study found that FMD, both relative and normalized to shear stress stimulus (Levenson *et al.*, 2001), increased significantly over the 12 days of ULI in the IMM leg. This finding is in contrast with the findings of Bleeker et al. (2005) who noted that when the significantly increased relative FMD values were corrected for the shear stress stimulus, no significant increases occurred (Bleeker *et al.*, 2005b). The authors suggested that such a finding may be due to insufficient power within the study for the type of correction made to the FMD values. Since Bleeker et al had no control group or a control

(i.e., non-immobilized) leg their pre- to post-immobilization findings showing a 'lack' of change are difficult to interpret. An additional parameter that Bleeker and colleagues examined was endothelial-independent vasodilation which they found to increase following their deconditioning period of 4 weeks. This finding, although unexpected, is in accordance with the results of a training study conducted by Rywik et al. (1999) who demonstrated an endothelial-independent dilation increase following exercise training (Rywik *et al.*, 1999). Although such a study utilized the opposite intervention to deconditioning, exercise training, their results suggest that endothelium-independent dilation, and thus the smooth muscle sensitivity to other vasoactive factors and NO donors, is modifiable over a range of levels of physical activity. The investigators, however, did not find any change with training in the ratio of FMD and nitroglycerin-mediated vasodilation suggesting that it is primarily a measure of sensitivity to NO within the smooth muscle cells of the vessel wall or the capacity of the smooth muscle cells to vasodilate that is responsible for the changes in endothelial function (Rywik *et al.*, 1999). It may be that our model of ULI does not truly mimic that of long-term inactivity (i.e., a sedentary lifestyle). It may also be that the changes we observed in FMD are short-term in nature and are not the same as those seen in the long-term. We favour this explanation and propose that a reduction in arterial function may take much longer than 12 days to be fully expressed since endothelial dysfunction is likely a long-term process. Further, actual physical changes in arterial structure, such as those due to remodeling of the collagen lattice that supports the vessel, would likely also take longer to occur.

With regards to the effects of deconditioning in other models, it has been shown that within a group of paraplegic subjects, relative FMD is increased in the legs (de Groot *et al.*, 2004). Although when normalized to the shear stress stimulus this increase was no longer statistically significant, the investigators did witness an increased FMD response per change in shear rate which suggests that the sensitivity of the response of FMD might be increased, but that the magnitude is either unchanged or reduced. Moreover, a bed rest study by Bonnin and colleagues (2001) also demonstrated increases in FMD in the brachial artery following only 7 days of bed rest deconditioning (Bonnin *et al.*, 2001). Taken together, the preceding literature provides no verification of a true impairment in FMD following chronic deconditioning in models of paraplegia or acute deconditioning following periods of ULLS or bed rest (Bonnin *et al.*, 2001; Bleeker *et al.*, 2005a). In fact, if deconditioning causes any alterations in the functioning of the endothelium, the present results as well as the results of Bleeker et al (2004) and Bonnin et al (2001) suggest that an increase in FMD is more likely to occur than a decrease.

Considering literature has proven that endothelial function is enhanced following exercise training (Clarkson *et al.*, 1999) it is somewhat counterintuitive that the changes in endothelial function following deconditioning are not simply the opposite of those with exercise. One possible rationale for such a finding could be related to NO and a possible decrease in basal production and/or bioavailability within the endothelium, which would occur when periods of high shear rates are absent and diameter is decreased. Such a situation would occur in a deconditioned state compared to a state of chronic exercise training (Rush *et al.*, 2005). In this situation, the stimulus to produce NO, shear stress, is

less frequent and thus NO sensitivity could increase and explain the increase in FMD response in the present study and the increased NTG-mediated dilation found by Bleeker et al. in 2005. In accordance with such a rationale, Rudic et al. in 1999 found a hypersensitivity of the endothelium to NO following partial arterial ligation, and thus decreased blood flow and shear rate levels, in mice (Rudic *et al.*, 2000). Thus, it appears that the functioning of the endothelium plays a critical role in the remodeling of the arterial vessel wall (Rudic *et al.*, 2000).

The bioavailability of NO depends on processes controlling both NO synthesis and NO degradation and also on the sensitivity of the target vasculature (Rush *et al.*, 2005). Prospective mechanisms that may be involved in the adaptations of the endothelium to either physical exercise training or deconditioning could result from one or more of the following 3 major determinants of NO bioavailability; *1*) the rate of NO production in the endothelium by endothelial NOS (eNOS); *2*) the rate of NO destruction from other biological molecules such as reactive oxygen species (ROS); and/or *3*) the sensitivity of vascular smooth muscle to NO. Increased oxidative stress in the vasculature has been shown to be a significant preceding event in many cardiovascular disease states and has the potential to influence one or more aspects of NO bioavailability (Rush *et al.*, 2005).

Recently there has been evidence to support a significant contribution of oxidative stress-induced destruction of NO to endothelial dysfunction that is found within numerous cardiovascular disease states including hypertension, diabetes, chronic heart failure, and atherosclerosis (Rudic *et al.*, 2000). There is also proof that there exists a role

for an improvement in NO bioavailability accompanying exercise training due to enhanced synthesis and reduced oxidative stress mediated destruction (Rush *et al.*, 2005). Targets that may potentially be sensitive to the exercise training effect include the endothelial nitric oxide synthase (eNOS) and the antioxidant enzyme superoxide dismutase (SOD), an antioxidant enzyme that if upregulated would reduce the level of oxidative stress. Recently Rush and colleagues proposed the theory that the various mechanisms contributing to endothelial dysfunction in CVD can be targeted and reversed by the signals related with regular physical activity. Rush and colleagues (2005) have attributed the mechanisms responsible for exercise -associated improvements in arterial endothelial function to enhancements in the mediation of vascular oxidative stress. Such a mechanism may provide critical contributions to the increased NO bioavailability and thus an increased endothelial function (Rush *et al.*, 2005). An adequate NO bioavailability has been thought to be maintained by a delicate balance between NO and ROS (Rush *et al.*, 2005). A major role of oxidative stress in mediating NO bioavailability within the vessel has been proposed due to several lines of evidence including: exposure to endogenous or exogenous superoxide radical (O_2^-) decreases endothelium-dependent vasodilation to acetylcholine (Rush *et al.*, 2005) inhibition of SOD, a scavenger of ROS that can quench NO, impairs agonist-evoked endothelium-dependent NO-mediated vasodilation (Carneado *et al.*, 2002); addition of SOD or chemical antioxidants in vitro protects NO availability against O_2^- (Zalba *et al.*, 2000); and improvement of vascular wall SOD by gene or protein transfer in vivo re- establishes the NO action weakened by ROS overproduction (Chu *et al.*, 2003; Fennell *et al.*, 2002). It is thus suggested that

oxidative stress-induced destruction of NO contributes to endothelial dysfunction. Any decrease in the ability of antioxidant factors to buffer O_2^- results in a destruction of NO and therefore an impaired NO bioavailability. In addition, supplemental SOD and antioxidant treatments contribute to the buffering of excess O_2^- production and thus allows for the prospective restoration of endothelium-dependent dilation (Taddei *et al.*, 1995; Carneado *et al.*, 2002; Gokce *et al.*, 2002). Thus, oxidative stress-induced decreases in NO bioavailability can be potentially studied as a pathophysiological mechanism in CVD.

It has been shown that acute exercise increases oxidative stress due to an increase in ROS generation and also can result in an increased availability of antioxidant defenses within the vasculature (Rush *et al.*, 2000; Rush *et al.*, 2003). Rush and colleagues (2000, 2003) have shown that there is an increased mRNA, protein, and enzymatic activity of SOD-1 in coronary arterioles and aortic endothelium in exercised pigs compared to a group of sedentary controls suggesting that exercise has encouraging effects on vascular antioxidant pathways (Rush *et al.*, 2000; Rush *et al.*, 2003). It has also been shown recently that there occurs a possible reduction in aortic NADPH oxidase expression associated with improved NO-dependent aortic vasodilation in exercise-trained compared to sedentary hypertensive rats (Graham & Rush, 2004). Such a finding suggests a possible means for improvements NO-mediated endothelial function resulting from regular aerobic exercise may include an improved balance of O_2^- and NO (Graham & Rush, 2004). It thus may be possible that deconditioning has an altering effect on

oxidative stress, specifically a decrease that may increase bioavailable NO and contribute to an increase in endothelial function.

5.2 *Arterial diameter changes of the leg caused by ULI*

In agreement with other studies utilizing models of inactivity (De Groot *et al.*, 2003; Bleeker *et al.*, 2005a), resting arterial diameter (popliteal) was significantly reduced ($p<0.05$) following 12 days of ULI in the IMM versus the NIM leg and baseline mean wall shear rate levels were significantly elevated ($p<0.05$) in both the immobilized leg and non-immobilized leg. Previously, studies have utilized various models of inactivity to determine vascular adaptations to inactivity in humans such as spinal cord injury (SCI) (De Groot *et al.*, 2003), limb suspension (Bleeker *et al.*, 2004; Bleeker *et al.*, 2005a), and bed rest (Takenaka *et al.*, 1994; Bonnin *et al.*, 2001) as well as hind-limb suspension in rodents (Rudic *et al.*, 2000). In the present study a decrease in the diameter of both the common femoral and popliteal artery may potentially have resulted from either structural remodeling of the artery and/or an increase in vasomotor tone of the smooth muscle cells of the artery in response to the ULI protocol. Previous literature studying the structural alterations within the vessel wall in response to study interventions have mainly focused on animal studies and have involved the use of ex-vivo measurements (Rudic *et al.*, 2000). Bleeker and colleagues (2004) utilized nitroglycerine (NTG) to initiate a maximal hyperemic response of the artery and investigated the change in diameter of the common femoral artery. The maximal diameter of the hyperemic response to FMD and also the hyperemic response to NTG decreased significantly over

the 4 week deconditioning protocol. Collectively, these results suggest that a greater part of the diameter change from deconditioning is due to structural arterial changes within the vessel (Bleeker *et al.*, 2005a).

The present study demonstrated that the deconditioning model of ULI caused arterial diameter to decrease significantly through the 12 days of deconditioning in conduit arteries in the IMM leg while showing no changes in the NIM leg. Literature on the effects of arterial diameter changes following periods of deconditioning or decreased levels of physical activity are limited; however, the recent study conducted by Bleeker and colleagues (2005a) examined 4 weeks of ULLS deconditioning in the legs of healthy young humans and such a study provided novel insight into vascular changes that occur with cardiovascular deconditioning (Bleeker *et al.*, 2005a). This was the first study to demonstrate the vascular effects of ULLS protocol and deconditioning on arterial diameter in healthy, young humans. In accordance with the present study's findings, Bleeker and colleagues found a decrease in common and superficial femoral artery diameter of 12% following 4 weeks of ULLS.

Other studies have also examined the condition of physical inactivity on vascular changes in the lower legs by studying the vascular properties in states of paraplegia (De Groot *et al.*, 2003). It was found that in extreme states of inactivity, the state of paraplegia, common femoral artery diameter is 30% less than in a group of healthy control individuals and is virtually completed within 6 weeks following spinal cord injury (De Groot *et al.*, 2003). The spinal cord injured (SCI) individuals offer a very unique

human model of inactivity that allows researchers to examine peripheral vascular changes that occur with extreme inactivity. A study conducted by De Groot et al (2003) demonstrated very valuable information regarding the time course of vascular adaptations to inactivity by finding that within 6 weeks post injury, the diameter of the common femoral artery in individuals with complete SCI decreased by 30% and mean wall shear rate (MWSR) increased by 100% or more, (De Groot *et al.*, 2003). Such adaptations showed no further changes after 6 weeks. Such a finding suggests that vascular adaptations to inactivity occur within weeks, not months, as previously thought (De Groot *et al.*, 2003).

Despite limited data on the effects of deconditioning on arterial diameter, literature on the arterial diameter changes following exercise training are available. Huonker and colleagues (2003) demonstrated a 21% larger diameter of the common femoral artery in competitive road cyclists compared to a group of sedentary controls (Huonker *et al.*, 2003). This cross-sectional finding is of questionable significance from an adaptation prospective, however, since these differences may be pre-existing. Three months of leg training increased the diameter of the common femoral artery by 9% and kept shear stress levels and blood flow unaltered (Dinenno *et al.*, 2001). Such findings suggest that the arterial diameters adaptations that occur with exercise training are the results of structural expansive arterial remodeling and changes to correct for the peak shear stress levels experienced during exercise training (Dinenno *et al.*, 2001). Along the same line of reasoning, the decrease in conduit arterial diameter found in the present

study may potentially by due to structural remodeling of the artery as a result of a decreased exposure to periods of high peak shear rates (Bleeker *et al.*, 2005c).

5.3 Blood flow alterations in the leg caused by ULI

Blood flow was unchanged in both the popliteal artery and common femoral artery following the deconditioning period of 12 days in both the IMM and NIM limb. It is has been reported that exercise training increases arterial diameter but does not alter baseline blood flow and it is suggested that such an occurrence results from a physiological adaptive mechanism of arterial diameter to peak flow rates that occur during exercise bouts rather to resting blood flow values (Dinenno *et al.*, 2001; Miyachi *et al.*, 2001). Moreover, even in the extreme chronic deconditioned state of paraplegia there is evidence of remarkable decreases in arterial diameter with no changes in resting blood flow (Oliver & Webb, 2003; de Groot *et al.*, 2004). Although literature on the changes in blood flow following deconditioned states is inconsistent with some studies showing decreases in resting blood flow and others demonstrating no change in resting blood flow values, technical limitations should be considered. It is known, for example, that the reproducibility of blood flow measures is not as high-quality as measures of arterial diameter (de Groot *et al.*, 2004).

Bleeker and colleagues (2005a) found that, following 4 weeks of ULLS, resting MWSR and peak wall shear rate (PWSR) were elevated and such a finding is suggestive of a failure of the legs to maintain an equal wall shear rate during an acute deconditioned state (Bleeker *et al.*, 2005b). Other studies have also shown similar results. De Groot et al

(2003) and Schmidt-Trucksass (2000) found an increase in MWSR and PWSR in paraplegic subjects which suggests, just as the present study and the results of Bleeker et al (2005a), an impaired ability of the artery to maintain a constant wall shear stress. In contrast to the preceding findings found in deconditioned states, exercise training studies have demonstrated no change in resting shear stress levels in healthy individuals (Dinenno *et al.,* 2001). Overall, it seems as if resting wall shear stress levels are maintained during exercise training, but are increased following acute physical deconditioning and chronic deconditioning such as that in spinal cord injury. Such results are suggestive of a dissimilar or disturbed vascular diameter response to deconditioning compared to the response witnessed with exercise training.

As shown in animal experiments, alterations in arterial blood flow instigate structural and functional adaptations (Langille, 1993); S11) of which the structural adaptations have been postulated to be the result of alterations in local wall shear stress (Langille, 1993). An increase in blood flow and shear rate has been suggested to cause vascular remodeling resulting in an increased diameter of the vascular wall while a decrease causes a decreased diameter (Masuda *et al.,* 1989). Such a process of outward and inward remodeling of the vascular wall has been thought of a mechanism adapted by the body to maintain constant wall shear stress levels, (Masuda *et al.,* 1989). It has been generally accepted that arteries adapt to changes in blood flow by going through compensatory adjustments with regards to their internal diameter (Langille, 1993).

5.4 Changes in Arterial Cross Sectional Compliance Following ULI

In the present study it was found that popliteal arterial cross-sectional compliance decreased from PRE to 12-DAY in both the IMM and the NIM legs whereas the common femoral artery compliance decreased from PRE to 12-DAY in the IMM leg and showed no change in the NIM leg.

Animal experiments have shown that alterations in arterial blood flow induce both structural and functional adaptations within the arterial vasculature (Langille, 1993). Such alterations have been attributed to changes in local wall shear stress which has been shown to be a primary stimulus for alterations in the arterial vasculature (Langille, 1993). Therefore, an increase in arterial blood flow causes an outward vascular remodeling (20), whereas a decrease causes an inward remodeling of the arterial wall (Masuda *et al.*, 1989). Several studies have investigated the alterations in the arterial system to both exercise and deconditioning models and have also provided some potential insight into mechanisms that may be responsible for such adaptations.

With regards to the effects of exercise on arterial compliance it has been shown that exercise training increases central arterial compliance in healthy subjects (Cameron & Dart, 1994) and also in patients with congestive heart failure (Parnell *et al.*, 2002). In addition, previous cross-sectional studies have shown that common femoral artery compliance was significantly greater in endurance trained subjects compared to sedentary counterparts (Kool *et al.*, 1992). Another study conducted by Huonker and colleagues

also found a higher compliance in highly trained athletes compared to spinal cord injured individuals (Huonker *et al.*, 2003). Such findings imply that physical activity has the potential to improve central and peripheral arterial compliance.

While the effects of exercise training are to increase central arterial compliance and distensibility (Vaitkevicius *et al.*, 1993; Cameron & Dart, 1994; Tanaka *et al.*, 1998; Schmidt-Trucksass *et al.*, 2000; Tanaka *et al.*, 2000), the effects of exercise on the compliance of peripheral arteries are not well understood. Alterations in peripheral arterial compliance following physical deconditioning in humans have not, to our knowledge, been extensively characterized.

Wijnen et al (1991) found that in paraplegic models of deconditioning compared to sedentary and endurance trained conditions, central arterial compliance was lower in the paraplegic model compared to both the able-bodied and athletes, while the athletes demonstrated the highest arterial compliance. This suggests that exercise training has the potential to initiate structural adaptations within peripheral arteries (Wijnen *et al.*, 1991). Studies investigating peripheral alterations in arterial stiffness have also used animal models and have given insight into potential mechanisms involved in changes in compliance with increases or decreases in physical activity. Such studies demonstrate that changes in arterial blood flow instigate structural and functional changes within the vasculature that are related to changes in local wall shear stress levels (Langille, 1993). An increase in blood flow and this shear stress contributes to an outward vascular remodeling while decreases cause inward structural remodeling (Masuda *et al.*, 1989).

Such a mechanism has been proposed to allow for a constant wall shear rate level within the vessel (Langille, 1993).

In the paraplegic model of deconditioning, it is both the extreme level of deconditioning in addition to the decreased sympathetic vascular control that may be responsible for the decrease in arterial compliance found within this population. However, one may expect a positive effect in arterial compliance when considering a loss of sympathetic tone, which would likely result in a reactive vasodilation and a more compliant artery. Studies have yielded results of a decreased compliance found within a paraplegic population (Kooijman *et al.,* 2003). Kooijman and colleagues (2003) found the the α-adrenergic involvement to basal vascular tone was actually conserved below the lesion level in paraplegic subjects compared to a group of control subjects. In the paraplegic model, it has been suggested that there is both a structural and a functional component to the observed adaptation of a decreased arterial compliance (De Groot et al., 2003). In a study conducted by Giannattasio et al (1998) it was found that with prolonged upper arm casting over a period of 30 days, radial artery distensibility was significantly decreased following removal of the cast (Giannattasio *et al.,* 1998).

Mechanistically it is an increase in the level of physical activity that leads to alterations in arterial blood flow to the working musculature and is assumed to cause increases in wall shear stress leading to vascular outward remodeling and an increase in arterial compliance (Kingwell *et al.*, 1997; Giannattasio *et al.*, 1998). Schmidt-Trucksass et al. (2000) showed that the common femoral artery showed a larger arterial diameter

and a higher compliance compared with sedentary and paraplegic counterparts. Nonetheless, the shear rates in the endurance trained athletes and sedentary subjects were similar. Shear rates in the paraplegic subjects were significantly higher and diameter of the artery was significantly lower compared to the trained and sedentary groups. Such a finding indicates a disturbed response of the common femoral artery to changes in blood flow within the paraplegic condition. Within the study, as was also shown in the present study, only peripheral changes were found, as there were no differences in the compliance within the carotid artery between any of the groups (Schmidt-Trucksass *et al.*, 2000).

With regards to failure of the present study and previous other studies (Huonker *et al.*, 2003) to note significant changes within the diameter of the systemic vasculature in response to training or chronic deconditioning (Schmidt-Trucksass *et al.,* 2000) it has been shown that the change in blood flow volume to the head compared to that going to the working musculature is much less, with only a 30-40% increase going to the head and a 20-30 fold increase going to the working muscles during exercise bouts (Hellstrom *et al.*, 1996). In contrast to the acute deconditioning period of 12 days, present study, and 4 weeks (Bleeker *et al.*, 2005a), Cameron et al. (1991) demonstrated that a 4 week exercise training protocol induces short-term adaptations in central arterial compliance and accredited such alterations to alterations in arterial smooth muscle tone, which in turn alters the relative loading of collagen and elastin fibers within the vessel wall (Cameron & Dart, 1994).

Mechanisms involved in the decreased arterial compliance found through 12 days of ULI could involve any of the elements of the arterial wall that primarily determine arterial compliance such as the relative amounts of collagen and elastin in the tunica media, and thus structural characteristics, and also the smooth muscle vascular tone. It could potentially be that a decrease in peak blood flows and periods of relatively greater shear rates experienced during deconditioning (Bleeker *et al.*, 2005c) caused an inward structural remodeling of the elastin and collagen within the vessel wall. It may also be possible that functional changes within the vascular wall may also influence smooth muscle tone and contribute to a decrease in arterial compliance. Such a speculation revolves around literature showing that an upregulation of bioavailable NO may directly relax the vascular smooth muscle and also that NO may have the potential to directly inhibit smooth muscle cell proliferation (De Groot *et al.*, 2003).

Shear rate has been proposed as the key factor in altering arterial compliance and it has been speculated that it is this stimulus that induces structural adaptations within the vascular wall (Langille, 1993; Masuda *et al.*, 1989). In an attempt to maintain constant shear stress levels the arterial system has shown evidence of being able to regulate its internal diameter to alterations in blood flow (Masuda *et al.*, 1989). The increase in blood flow to the active musculature that occurs with exercise training results in an endothelium-mediated dilation of the vessel and through the actions of NO and other metabolites the vasoconstrictive effects of norepinepherine on the endothelial cells are counteracted (Vanhoutte & Miller, 1989; Delp, 1995).

There is a more recent explanation speculated by Kinlay et al (2001) involving the contribution of NO to baseline vascular tone that may explain alterations in arterial compliance found throughout the literature and may explain the decrease in compliance of both arteries examined in the present study. Kinlay and colleagues found that NO increased arterial elasticity in the human brachial artery and thus it was concluded that NO contributes to compliance of arterial vessel in healthy humans (Kinlay *et al.*, 2001). When an artery was infused with an inhibitor of NO synthase (L-NMMA) measures of arterial elasticity were attenuated and suggested that there is a reduced bioavailability of endothelium-derived NO that would decrease arterial compliance. When infused with NTG, an exogenous NO donor, there occurred a reverse of the loss of compliance induced by L-NMMA. Thus, it was concluded that a loss of endothelial-derived NO that has been shown to be associated with cardiovascular risk factors may adversely affect arterial compliance in humans (Kinlay *et al.*, 2001).

5.5 Limitations

The present study addresses questions regarding the impact that physical deconditioning has on the vascular functioning in healthy young humans and the quick time course to which such adaptations can occur. However, although we provide insight into the vascular adaptations to a deconditioned and thus sedentary lifestyle, limitations within the study do exist.

Measurement techniques used within the present investigation have the potential to be vulnerable to investigator variability and error. While every precaution was taken

to obtain the most accurate and reliable measures possible, there still exists a chance of human technical error. Doppler Ultrasound use to obtain visual images of vessels of interest has been shown to be a reliable and reproducible method; however, there exists the possibility for investigator error. It is critical that there is a consistent image of the vessel of interest throughout the study testing time points. Thus, it is of extreme importance that relative positions and anatomic landmarks are distinguished for every vessel measurement acquired.

The method of flow-mediated vasodilation (FMD) when assessing endothelial function is also susceptible to error that may affect the reliability of the results. For example, the use of Ultrasonographic assessment of a small artery, such as the popliteal artery, is a challenging task and thus should be conducted by a well-trained ultrasound technician. There are various technical aspects that have the potential to affect the accuracy of the FMD technique. Such aspects include confidence of the examiner, transducer movement, and, as mentioned above, utilizing anatomical landmarks. A second aspect that could affect the reliability of the FMD protocol is the lack of control that there is over shear stress exerted on the endothelium on a recurring daily basis. It was established recently that improvement of a technique should be implemented into the assessment of endothelial function that allows a constant shear stimulus (Pyke *et al.*, 2004). Moreover, the assessment of post-ischemia blood flow should be represented relative to the tissue volume being affected which would provide an indication of the extent of muscle atrophy that occurs as a result of deconditioning and address the issue of

whether the changes in blood flow response following ischemia are in fact the consequence of an increase in resistance vessel dilation.

5.6 Summary

In closing, the present study demonstrated that a short deconditioning period of unilateral lower limb immobilization (ULI) was able to induce structural and functional changes within the arterial vasculature of healthy, young humans. In agreement with our hypotheses, we found that femoral arterial cross sectional compliance decreased in the immobilized limb with no changes occurring in the non-immobilized limb. Both the measure of blood flow and flow mediated vasodilation (both relative values and values normalized to shear stress stimulus) were in disagreement with our hypotheses with blood flow showing no change in either limb through the 12 day immobilization period and FMD values increasing significantly in the immobilized limb while showing no change in the non-immobilized limb. Thus it can be concluded that even an acute period of deconditioning can lead to arterial vascular adaptations that have previously been found to be detrimental to cardiovascular health, such as decreases in compliance. The increase in popliteal FMD in the present study was an unanticipated finding, As suggested above, such a phenomenon could be the result of many factors that may accompany a deconditioned state such as a decrease in the bioavailability of NO (through either a decrease in its production or an increase in its destruction by other biological molecules) or an increase in the sensitivity of the smooth muscle cells within the vessel to a lower NO concentration (Rudic *et al.*, 2000; Bleeker *et al.*, 2005a).

The present study was one of the first to examine the impact of deconditioning in a healthy, young population. While Bleeker and colleagues (2005a) did demonstrate a trend towards an increase in FMD following 4 weeks of ULLS, the investigators attributed the failure to see a significant increase in FMD to a small sample size of only 8 participants. Thus the present study increased the power of the measure and increases in FMD were observed in only 12 days compared to 4 weeks.

Previous literature supports the notion that a sedentary lifestyle could potentially increase the risk of developing CVD (Paffenbarger *et al.*, 1993 ; Blair *et al.*, 1995)and that physically active populations show adaptations within the cardiovascular system that deem to be advantageous to cardiovascular health (DeSouza *et al.*, 2000; Kingwell *et al.*, 1997). It is surprising that the effects of deconditioning are not simply the inverse of the effects of exercise training with regards to endothelial function and such an occurrence deserves additional research, potentially invasive in nature, to determine specific cellular mechanisms controlling NO bioavailability in humans in a deconditioned state.

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APPENDIX A

STATISTICAL TABLES

SECTION A:

SUBJECT CHARACTERISTICS STATISTICS TABLES

HEIGHT (cm)

Summary of all Effects
1-GENDER

Effect	Df Effect	MS Effect	Df Error	MS Error	F-Ratio	p-Level
1	1	903.1440	13	38.50687	23.45410	.000321

Tukey's HSD
Probabilities for Post Hoc Tests
Main Effect: GENDER

	MALE	FEMALE
MEANS	179.4286	163.8750
M {1}		.000482
F {2}	.000482	

* Significant differences between MALE and FEMALE

WEIGHT (Kg)

Summary of all Effects
1-GENDER

Effect	Df Effect	MS Effect	Df Error	MS Error	F-Ratio	p-Level
1	1	2014.691	13	137.1728	14.68726	.002076

Tukey's HSD
Probabilities for Post Hoc Tests
Main Effect: GENDER

	MALE	FEMALE
MEANS	83.84286	60.61250
M {1}		.002242
F {2}	.002242	

* Significant differences between MALE and FEMALE

BODY MASS INDEX (Kg)

Summary of all Effects
1-GENDER

Effect	Df Effect	MS Effect	Df Error	MS Error	F-Ratio	p-Level
1	1	43.24805	13	8.423791	5.134036	.041184

Tukey's HSD
Probabilities for Post Hoc Tests
Main Effect: GENDER

	MALE	FEMALE
MEANS	25.92857	22.52500
M {1}		.041325
F {2}	.041325	

* Significant differences between MALE and FEMALE

PULSE PRESSURE (mmHg)

Summary of all Effects
1-GENDER 2-TIME
MAIN EFFECT: GENDER

Effect	Df Effect	MS Effect	Df Error	MS Error	F-Ratio	p-Level
1	1	527.0720	13	74.69651	7.056180	.019772
2	1	99.9619	13	36.51431	2.737609	.121940
12	1	18.7326	13	36.51431	.513021	.486502

Tukey's HSD
Probabilities for Post Hoc Tests
Main Effect: GENDER

	MALE	FEMALE
MEANS	54.66429	46.26250
M {1}		.019932
F {2}	.019932	

SECTION B:

SEX-BASED ANOVA TABLES

POPLITEAL ARTERY COMPLIANCE

Summary of all Effects

1-GROUP, 2-GENDER, 3-TIME
Mixed 2-Way ANOVA
MAIN EFFECT: TIME

Effect	Df Effect	MS Effect	Df Error	MS Error	F-Ratio	p-Level
1	1	.000000	26	.000000	2.88989	.101072
2	1	.000000	26	.000000	.46491	.501367
3	**1**	**.000000**	**26**	**.000000**	**43.08349**	**.000001**
12	1	.000000	26	.000000	.01336	.908865
13	1	.000000	26	.000000	1.08677	.306785
23	1	.000000	26	.000000	.16729	.685881
123	1	.000000	26	.000000	1.63515	.212289

COMMON FEMORAL ARTERY COMPLIANCE

Summary of all Effects

1-GROUP, 2-GENDER, 3-TIME
Mixed 2-Way ANOVA
MAIN EFFECT: TIME

Effect	Df Effect	MS Effect	Df Error	MS Error	F-Ratio	p-Level
1	1	.000000	24	.000000	.43677	.514982
2	1	.000000	24	.000000	.00454	.946843
3	**1**	**.000001**	**24**	**.000000**	**12.49332**	**.001691**
12	1	.000000	24	.000000	.84839	.366174
13	1	.000000	24	.000000	2.78605	.108083
23	1	.000000	24	.000000	.22260	.641324
123	1	.000000	24	.000000	.17806	.676801

POPLITEAL RELATIVE FLOW-MEDIATED VASODILATION

Summary of all Effects

1-GROUP, 2-GENDER, 3-TIME
Mixed 2-Way ANOVA

Effects	df Effect	MS Effect	df Error	MS Error	F-ratio	p-level
1	1	194.8925	24	109.6937	1.77670	.195072
2	1	4.7309	24	109.6937	.04313	.837237
3	**1**	**232.1428**	**24**	**21.2496**	**10.92459**	**.002974**
12	1	1.1525	24	109.6937	.01051	.919212
13	1	31.5414	24	21.2496	1.48433	.234940
23	1	26.5143	24	21.2496	1.24776	.275040
123	1	42.5335	24	21.2496	2.00162	.169975

SECTION C:

STUDY ANOVA TABLES

POPLITEAL ARTERY SYSTOLIC DIAMETER

Summary of all Effects

1-GROUP, 2-TIME
Mixed 2-Way ANOVA
INTERACTION EFFECT

Effect	Df Effect	MS Effect	Df Error	MS Error	F-Ratio	p-Level
1	1	.028992	28	.008025	3.61279	.067677
2	**1**	**.050454**	**28**	**.002333**	**21.62670**	**.000072**
12	1	.007643	28	.002333	3.27624	.081036

POPLITEAL ARTERY DIASTOLIC DIAMETER

Summary of all Effects

1-GROUP, 2-TIME
Mixed 2-Way ANOVA
INTERACTION EFFECT

Effect	Df Effect	MS Effect	Df Error	MS Error	F-Ratio	p-Level
1	1	.025592	28	.008915	2.87065	.101305
2	**1**	**.036204**	**28**	**.000622**	**58.18242**	**.000000**
12	**1**	**.008304**	**28**	**.000622**	**13.34474**	**.001056**

Tukey's HSD
Probabilities for Post Hoc Tests
INTERACTION EFFECT

		PRE	12-DAY	PRE	12-DAY
MEANS		.5590733	.4864167	.5768500	.5512500
I	1 {1}		.000164	.230408	.825781
I	2 {2}	.000164		.000164	.000164
N	1 {3}	.230408	.000164		.041939
N	2 {4}	.825781	.000164	.041939	

*Significant differences between:
Immobilized PRE and Immobilized 12-DAY;
Immobilized 12-DAY and NON-Immobilized 12-DAY;
Immobilized 12-DAY and NON-Immobilized PRE;
NON-Immobilized PRE and Immobilized 12-DAY

POPLITEAL ARTERY MEAN DIAMETER

Summary of all Effects

1-GROUP, 2-TIME
Mixed 2-Way ANOVA
INTERACTION EFFECT

Effect	Df Effect	MS Effect	Df Error	MS Error	F-Ratio	p-Level
1	1	.020325	28	.009220	2.2045	.148784
2	**1**	**.050800**	**28**	**.000481**	**105.5559**	**.000000***
12	**1**	**.004572**	**28**	**.000481**	**9.5011**	**.004577***

Tukey's HSD
Probabilities for Post Hoc Tests
INTERACTION EFFECT

	PRE	12-DAY	PRE	12-DAY
MEANS	**.5713044**	**.4956500**	**.5906556**	**.5499200**
I 1 {1}		**.000164***	.097280	.057206
I 2 {2}	**.000164***		**.000164***	**.000165***
N 1 {3}	.097280	**.000164***		**.000269***
N 2 {4}	.057206	**.000165***	**.000269***	

*Significant differences between; Immobilized PRE and Immobilized 12-DAY;
Immobilized 12-DAY and NON-Immobilized PRE;
Immobilized 12-DAY and NON-Immobilized 12-DAY;
NON-Immobilized PRE and NON-Immobilized 12-DAY

POPLITEAL ARTERY MEAN BLOOD VELOCITY

Summary of all Effects

1-GROUP, 2-TIME
Mixed 2-Way ANOVA
INTERACTION EFFECT

Effect	Df Effect	MS Effect	Df Error	MS Error	F-Ratio	p-Level
1	1	.000882	28	.432363	.00204	.964302
2	**1**	**3.512291**	**28**	**.096312**	**36.46768**	**.000002**
12	**1**	**.481116**	**28**	**.096312**	**4.99537**	**.033573**

Tukey's HSD
Probabilities for Post Hoc Tests
INTERACTION EFFECT

		PRE	12-DAY	PRE	12-DAY
MEANS		**1.538813**	**2.201800**	**1.710240**	**2.015040**
I	**1 {1}**		**.000175**	.443698	**.001433**
I	**2 {2}**	**.000175**		**.001042**	.369391
N	**1 {3}**	.443698	**.001042**		.054763
N	**2 {4}**	**.001433**	.369391	.054763	

*Significant differences between; Immobilized PRE and Immobilized 12-DAY;
Immobilized 12-DAY and NON-Immobilized PRE;
Immobilized PRE and NON-Immobilized 12-DAY
NON-Immobilized PRE and NON-Immobilized 12-DAY

POPLITEAL ARTERY MEAN BLOOD FLOW

Summary of all Effects

1-GROUP, 2-TIME
Mixed 2-Way ANOVA
NO EFFECTS
OR INTERACTIONS

Effect	Df Effect	MS Effect	Df Error	MS Error	F-Ratio	p-Level
1	1	95.67676	28	172.0214	.556191	.462014
2	1	6.64255	28	16.4028	.404965	.529704
12	1	6.87255	28	16.4028	.418987	.522715

POPLITEAL ARTERIAL COMPLIANCE

Summary of all Effects

1-GROUP, 2-TIME
Mixed 1-Way ANOVA
MIAN EFFECT: TIME

Effects	df Effect	MS Effect	df Error	MS Error	F-ratio	p-level
1	1	.000000	28	.000000	2.46128	.127917
2	**1**	**.000000**	**28**	**.000000**	**45.00452**	**.000000***
12	1	.000000	28	.000000	2.21950	.147456

COMMON FEMORAL SYSTOLIC DIAMETER

Summary of all Effects

1-GROUP, 2-TIME
Mixed 2-Way ANOVA
MAIN EFFECT: TIME

Effect	Df Effect	MS Effect	Df Error	MS Error	F-Ratio	p-Level
1	1	.000849	24	.029686	.028609	.867103
2	**1**	**.026183**	**24**	**.002828**	**9.258266**	**.005605**
12	1	.007913	24	.002828	2.797855	.107378

COMMON FEMORAL DIASTOLIC DIAMETER

Summary of all Effects

1-GROUP, 2-TIME
Mixed 2-Way ANOVA
MAIN EFFECT: TIME

Effect	Df Effect	MS Effect	Df Error	MS Error	F-Ratio	p-Level
1	1	.002277	24	.030658	.074269	.787551
2	**1**	**.017152**	**24**	**.002810**	**6.103625**	**.020982**
12	1	.004192	24	.002810	1.491892	.233786

COMMON FEMORAL ARTERY MEAN DIAMETER

Summary of all Effects

1-GROUP, 2-TIME
Mixed 2-Way ANOVA
MAIN EFFECT: TIME

Effect	Df Effect	MS Effect	Df Error	MS Error	F-Ratio	p-Level
1	1	.000937	24	.029698	.03155	.860507
2	**1**	**.030112**	**24**	**.001599**	**18.83308**	**.000223***
12	1	.001270	24	.001599	.79443	.381613

COMMON FEMORAL ARTERY MEAN BLOOD VELOCITY

Summary of all Effects

1-GROUP, 2-TIME
Mixed 2-Way ANOVA
INTERACTION EFFECT

	Effect	Effect	Error	Error	F	p-level
1	1	10.04022	26	2.921119	3.43711	.075126
2	**1**	**6.02703**	**26**	**.188725**	**31.93543**	**.000006**
12	**1**	**1.76447**	**26**	**.188725**	**9.34941**	**.005114**

Tukey's HSD
Probabilities for Post Hoc Tests
INTERACTION EFFECT

			Pre	**12-DAY**	**PRE**	**12-DAY**
MEANS			**3.592136**	**4.603274**	**4.794000**	**5.095114**
I	1	{1}		**.000174**	**.000169**	**.000169**
I	2	{2}	**.000174**		.655775	**.028605**
N	1	{3}	**.000169**	.655775		.280931
N	2	{4}	**.000169**	**.028605**	.280931	

*Significant differences between;
Immobilized PRE and Immobilized 12-DAY;
NON-Immobilized PRE and Immobilized PRE;
Immobilized PRE and NON-Immobilized 12-DAY;
Immobilized 12-DAY and NON-Immobilized 12-DAY

* TWO SUBJECTS DISCLUDED DUE TO IMPROPER IMAGING

COMMON FEMORAL ARTERY BLOOD FLOW

Summary of all Effects

1-GROUP, 2-TIME
Mixed 2-Way ANOVA
NO MAIN EFFECTS OR INTERACTIONS

Effect	Df Effect	MS Effect	Df Error	MS Error	F-Ratio	p-Level
1	1	1498.369	22	3532.452	.424172	.521608
2	1	620.914	22	820.374	.756866	.393705
12	1	388.929	22	820.374	.474087	.498310

COMMON FEMORAL ARTERY COMPLIANCE

Summary of all Effects

1-GROUP, 2-TIME
Mixed 2-Way ANOVA
INTERACTION EFFECT

Effect	Df Effect	MS Effect	Df Error	MS Error	F-Ratio	p-Level
1	1	.000000	24	.000000	.39572	.535249
2	**1**	**.000001**	**24**	**.000000**	**25.16265**	**.000040***
12	**1**	**.000000**	**24**	**.000000**	**8.43614**	**.007777***

Tukey's HSD
Probabilities for Post Hoc Tests
INTERACTION EFFECT: GROUPX TIME

			PRE	12-DAY	PRE	12-DAY
MEANS			**.0012026**	**.0007905**	**.0011517**	**.0010418**
I	**1**	**{1}**		**.000208***	.899270	.156189
I	**2**	**{2}**	**.000208***		**.000431***	**.011448***
N	**1**	**{3}**	.899270	**.000431***		.457028
N	**2**	**{4}**	.156189	**.011448***	.457028*	

* Significant differences between;
Immobilized PRE and Immobilized 12-DAY;
Immobilized 12-DAY and NON-Immobilized PRE;
Immobilized 12-DAY and NON-Immobilized 12-DAY

POPLITEAL ARTERY RELATIVE FLOW-MEDIATED VASODILATION

Summary of all Effects

1-GROUP, 2-TIME
Mixed 2-Way ANOVA
INTERACTION EFFECT

Effect	Df Effect	MS Effect	Df Error	MS Error	F-Ratio	p-Level
1	1	74.1231	24	84.45121	.87770	.358164
2	**1**	**312.2116**	**24**	**9.49021**	**32.89827**	**.000007***
12	**1**	**65.4999**	**24**	**9.49021**	**6.90184**	**.014768***

Tukey's HSD
Probabilities for Post Hoc Tests
INTERACTION EFFECT

		PRE	12-DAY	PRE	12-DAY
MEANS		**6.431358**	**13.57665**	**6.288169**	**8.944159**
I	**1 {1}**		**.000185***	.999450	.188480
I	**2 {2}**	**.000185***		**.000181***	**.004267***
N	**1 {3}**	.999450	**.000181***		.152535
N	**2 {4}**	.188480	**.004267***	.152535	

*Significant differences between;
Immobilized PRE and Immobilized 12-DAY;
Immobilized 12-DAY and NON-Immobilized PRE;
Immobilized 12-DAY and NON-Immobilized 12-DAY

* TWO SUBJECTS DISCLUDED DUE TO IMPROPER IMAGING

POPLITEAL ARTERY PEAK WALL SHEAR RATE (DURING OCCLUSIVE CHALLENGE)

Summary of all Effects

1-GROUP, 2-TIME
Mixed 2-Way ANOVA
NO MAIN EFFECTS
OR INTERACTIONS

Effect	Df Effect	MS Effect	Df Error	MS Error	F-Ratio	p-Level
1	1	6837.69	28	18901.24	.361759	.552370
2	1	11042.27	28	4717.56	2.340676	.137255
12	1	178.15	28	4717.56	.037762	.847324

POPLITEAL ARTERY NORMALIZED FLOW-MEDIATED VASODILATION

Summary of all Effects

1-GROUP, 2-TIME
Mixed 2-Way ANOVA
INTERACTION EFFECT

Effect	Df Effect	MS Effect	Df Error	MS Error	F-Ratio	p-Level
1	1	.000305	24	.000431	.70821	.408346
2	**1**	**.001434**	**24**	**.000034**	**42.10271**	**.000001***
12	**1**	**.000255**	**24**	**.000034**	**7.49127**	**.011487***

Tukey's HSD
Probabilities for Post Hoc Tests
INTERACTION EFFECT

		PRE	12-DAY	PRE	12-DAY
MEANS		**.0167080**	**.0316398**	**.0162946**	**.0223666**
I	**1 {1}**		**.000172**	.997919	.090408
I	**2 {2}**	**.000172**		**.000171**	**.002550**
N	**1 {3}**	.997919	**.000171**		.062604
N	**2 {4}**	.090408	**.002550**	.062604	

*Significant differences between;
Immobilized PRE and Immobilized 12-DAY;
Immobilized 12-DAY and NON-Immobilized PRE;
Immobilized PRE and NON-Immobilized 12-DAY

* TWO SUBJECTS DISCLUDED DUE TO IMPROPER IMAGING

CAROTID ARTERY COMPLIANCE

Summary of all Effects

1-TIME
1 Way ANOVA
NO MAIN EFFECTS

Effect	Df Effect	MS Effect	Df Error	MS Error	F-Ratio	p-Level
1	1	.000000	14	.000000	.092374	.765651

CAROTID ARTERY BLOOD FLOW

Summary of all Effects

1-TIME
1 Way ANOVA
NO MAIN EFFECTS

Effect	Df Effect	MS Effect	Df Error	MS Error	F-Ratio	p-Level
1	1	2115.683	14	2142.680	.987400	.337244

APPENDIX B

Study Data

HYPOTHESES #1

Popliteal Artery Cross-Sectional Compliance (mm^2/mmHg)

Immobilized Subject	Group	PRE	12-DAY
MM	I	0.000464	0.000262
JW	I	0.000516	0.000295
SB	I	0.000505	0.000411
BC	I	0.000558	0.000309
CL	I	0.000555	0.000239
MC	I	0.000488	0.000347
JD	I	0.000758	0.000473
NZ	I	0.000627	0.00053
JB	I	0.000385	0.00028
SS	I	0.000404	0.000264
AT	I	0.000376	0.000206
NB	I	0.000795	0.000864
RA	I	0.000846	0.000438
HV	I	0.000655	0.000461
KB	I	0.000667	0.000345

NON-Immobilized Subject	Group	PRE	12-DAY
MM	N	0.000291	0.000328
JW	N	0.00031	0.000179
SB	N	0.000604	0.000591
BC	N	0.00078	0.00062
CL	N	0.000574	0.000503
MC	N	0.00097	0.00072
JD	N	0.000977	0.000882
NZ	N	0.001295	0.001021
JB	N	0.000788	0.000538
SS	N	0.000312	0.000217
AT	N	0.000356	0.000284
NB	N	0.001316	0.000862
RA	N	0.000319	0.000369
HV	N	0.000582	0.000619
KB	N	0.000592	0.000502

Popliteal Artery Mean Blood Flow (ml/min)

Immobilized Subject	Group	PRE	12-DAY
MM	I	29.49337	34.83039

JW	I	35.77095	42.74505
SB	I	34.38167	34.02693
BC	I	17.52819	17.75427
CL	I	22.67228	26.29622
MC	I	35.13401	30.42778
JD	I	24.66209	43.37122
NZ	I	17.48272	18.26524
JB	I	12.72323	14.29488
SS	I	15.60628	14.65969
AT	I	23.193	24.14559
NB	I	35.89977	33.84928
RA	I	25.31027	28.77862
HV	I	19.84399	19.97588
KB	I	13.66166	10.33647

NON-Immobilized Subject	Group	PRE	12-DAY
MM	N	34.83006	35.797
JW	N	32.98095	37.85458
SB	N	28.82553	28.81099
BC	N	21.80618	26.05552
CL	N	23.17797	26.60106
MC	N	20.93009	22.7009
JD	N	58.24204	39.36811
NZ	N	24.05431	27.80341
JB	N	30.01259	16.07989
SS	N	33.35542	25.75427
AT	N	20.68899	22.60652
NB	N	35.59161	37.19127
RA	N	17.58642	18.58277
HV	N	16.45854	35.24691
KB	N	22.36403	21.03446

Popliteal Artery Mean Lumen Diameter (cm)

Immobilized Subject	Group	PRE	12-DAY
MM	I	0.61475	0.0224
JW	I	0.7215	0.0187
SB	I	0.630283	0.5364
BC	I	0.5269	0.4342

CL	I	0.48835	0.4646
MC	I	0.626917	0.0299
JD	I	0.542033	0.506217
NZ	I	0.576467	0.03765
JB	I	0.5083	0.01445
SS	I	0.51805	0.02
AT	I	0.525833	0.01595
NB	I	0.643033	0.552867
RA	I	0.61575	0.52675
HV	I	0.522833	0.02665
KB	I	0.508567	0.442367

NON-Immobilized Subject	Group	PRE	12-DAY
MM	N	0.6158	0.6074
JW	N	0.71545	0.648433
SB	N	0.683333	0.036
BC	N	0.533617	0.0335
CL	N	0.606733	0.0351
MC	N	0.643467	0.598467
JD	N	0.629633	0.04595
NZ	N	0.598233	0.529033
JB	N	0.609467	0.575883
SS	N	0.510033	0.472583
AT	N	0.4823	0.445133
NB	N	0.6715	0.0506
RA	N	0.473067	0.0309
HV	N	0.5408	0.5117
KB	N	0.5464	0.02825

Popliteal Artery Mean Wall Shear Rate (sec^{-1})

Immobilized Subject	Group	PRE	12-DAY
MM	I	10.77576	0.143492593
JW	I	8.084269	12.31378049
SB	I	11.65571	18.71439224
BC	I	10.17119	18.40994933
CL	I	16.52421	22.25742574
MC	I	12.10368	21.71761676
JD	I	13.14532	28.37994271
NZ	I	7.746502	14.60443506

JB	I	8.22349	12.72004377
SS	I	9.528038	18.42753653
AT	I	13.54041	0.116087037
NB	I	11.46068	17.00229109
RA	I	9.202436	16.71381111
HV	I	11.78578	19.5520747
KB	I	8.81615	10.13548338

NON-Immobilized Subject	Group	PRE	12-DAY
MM	N	12.6606	13.55943365
JW	N	7.64442	11.78532874
SB	N	7.668293	7.813830179
BC	N	12.18178	13.80128874
CL	N	8.808483	12.05242922
MC	N	6.668255	8.989640192
JD	N	19.80581	25.76051098
NZ	N	9.536747	15.93926029
JB	N	11.25312	7.146586403
SS	N	21.33978	20.71253747
AT	N	15.65333	21.75617792
NB	N	9.977662	13.63369884
RA	N	14.10034	17.82764811
HV	N	8.83284	22.33027164
KB	N	11.6369	14.28655244

Common Femoral Artery Compliance (mm^2/mmHg)

Immobilized Subject	Group	PRE	12-DAY
MM	I	0.001293	0.000892
JW	I	0.000422	0.000278
BC	I	0.001552	0.000888
CL	I	0.000614	0.000229
MC	I	0.001882	0.001123
JD	I	0.001357	0.000668
NZ	I	0.00163	0.000832
JB	I	0.001841	0.001744
AT	I	0.001029	0.000608
NB	I	0.001347	0.00105

RA	I	0.000793	0.000493
HV	I	0.000753	0.000551
KB	I	0.00112	0.00092

NON-Immobilized Subject	Group	PRE	12-DAY
MM	N	0.001731	0.00189
JW	N	0.000638	0.000464
BC	N	0.001414	0.001381
CL	N	0.001118	0.000665
MC	N	0.00091	0.000727
JD	N	0.001862	0.001556
NZ	N	0.001105	0.000852
JB	N	0.001119	0.001404
AT	N	0.000801	0.000805
NB	N	0.001659	0.001115
RA	N	0.00064	0.000566
HV	N	0.00105	0.000733
KB	N	0.000924	0.001386

Common Femoral Artery Mean Diameter (cm)

Immobilized Subject	Group	PRE	12-DAY
MM	I	0.925900	0.870750
JW	I	0.786583	0.763467
BC	I	0.984900	0.950100
CL	I	0.666333	0.654983
MC	I	0.927017	0.820000
JD	I	0.747000	0.708233
NZ	I	0.706450	0.704667
JB	I	0.771033	0.706183
AT	I	0.674083	0.616750
NB	I	1.157667	0.988133
RA	I	0.833717	0.750450
HV	I	0.912100	0.840950
KB	I	0.635550	0.599500

NON-Immobilized

Subject	Group	PRE	12-DAY
MM	N	0.785150	0.828450
JW	N	0.998567	0.763333
BC	N	0.957817	0.944150
CL	N	0.891033	0.902567
MC	N	0.643497	0.598467
JD	N	0.850583	0.780650
NZ	N	0.776000	0.746533
JB	N	0.661967	0.609617
AT	N	0.647167	0.612817
NB	N	0.867500	0.823683
RA	N	0.769333	0.749250
HV	N	0.871517	0.883533
KB	N	0.769333	0.749250

Common Femoral Artery Mean Blood Flow (ml/min)

Immobilized Subject	Group	PRE	12-DAY
MM	I	132.0355	183.7679
JW	I	122.001	113.4781
BC	I	117.6339	134.8334
CL	I	78.69557	98.73834
MC	I	120.7075	110.6668
JD	I	80.95606	99.89495
NZ	I	103.7551	119.4034
SS	I	168.6405	128.0847
AT	I	60.65313	61.93445
NB	I	210.1357	261.4452
RA	I	104.4623	123.1409
HV	I	173.3033	192.2268

NON-Immobilized Subject	Group	PRE	12-DAY
MM	N	114.7794	141.3593

JW	N	189.4497	118.6406
BC	N	195.9837	182.8653
CL	N	180.4454	184.241
MC	N	46.35022	44.5562
JD	N	161.8429	154.6462
NZ	N	118.2293	116.7534
SS	N	68.41853	215.4978
AT	N	148.9997	138.7824
NB	N	140.0589	108.9808
RA	N	160.5901	144.8155
HV	N	150.2391	142.2511

Common Femoral Artery Mean Wall Shear Rate (sec^{-1})

Immobilized Subject	Group	PRE	12-DAY
MM	I	14.11945	23.62699
JW	I	21.27886	21.64518
BC	I	10.45142	13.3447
CL	I	22.57849	29.82732
MC	I	12.86147	17.03707
JD	I	16.48568	23.86897
NZ	I	24.97955	28.96575
SS	I	25.30687	33.84284
AT	I	16.8086	22.40908
NB	I	11.49657	23.00135
RA	I	15.30112	24.73183
HV	I	19.38647	27.43611

NON-Immobilized Subject	Group	PRE	12-DAY
MM	N	20.12915	21.10302
JW	N	16.15035	22.64175
BC	N	18.93181	18.44283

CL	N	21.65127	21.27001
MC	N	14.76496	17.64442
JD	N	22.3235	27.59239
NZ	N	21.47629	23.81997
SS	N	20.02095	41.07783
AT	N	46.66124	51.18725
NB	N	18.21049	16.55345
RA	N	29.93605	29.22496
HV	N	19.26527	28.19679

Popliteal Artery Relative Flow Mediated Vasodilation

Immobilized Subject	Group	PRE	12-DAY
MM	I	4.929394	9.746672
JW	I	2.982456	3.14017
BC	I	17.75847	40.38669
CL	I	8.11097	16.36158
MC	I	3.616212	12.53632
JD	I	5.035832	12.9558
NZ	I	6.507824	19.27541
JB	I	2.664797	6.591462
SS	I	2.680412	5.205343
NB	I	4.487588	9.518039
RA	I	2.235196	5.418623
HV	I	5.982073	11.63112
KB	I	16.61644	23.72921

NON-Immobilized Subject	Group	PRE	12-DAY
MM	N	3.157122	2.822107
JW	N	1.337087	2.836277
BC	N	20.66228	21.55442
CL	N	5.806126	12.86351
MC	N	3.704296	6.831658

JD	N	6.553037	7.674419
NZ	N	3.79681	7.224635
JB	N	2.812868	2.093085
SS	N	2.75063	3.257608
NB	N	6.883835	9.561286
RA	N	11.30229	16.75393
HV	N	3.092784	8.684316
KB	N	9.887032	14.11681

Popliteal Artery Normalized FMD (%/sec^{-1})

Immobilized Subject	Group	PRE	12-DAY
MM	I	0.016059	0.029873
JW	I	0.009376	0.010314
BC	I	0.034385	0.069901
CL	I	0.026564	0.047017
MC	I	0.011932	0.032553
JD	I	0.008862	0.032093
NZ	I	0.016595	0.038532
JB	I	0.005556	0.012917
SS	I	0.006568	0.009342
NB	I	0.018059	0.031904
RA	I	0.006366	0.01088
HV	I	0.011755	0.02301
KB	I	0.045127	0.062975

NON-Immobilized Subject	Group	PRE	12-DAY
MM	N	0.005279	0.007004
JW	N	0.003901	0.009585
BC	N	0.049985	0.049776
CL	N	0.023418	0.043919
MC	N	0.014376	0.021064
JD	N	0.011933	0.013888
NZ	N	0.010155	0.01604
JB	N	0.007699	0.005396
SS	N	0.005695	0.006413
NB	N	0.025036	0.036432

RA	N	0.026208	0.031814
HV	N	0.006257	0.012286
KB	N	0.021887	0.037149

Popliteal Artery Peak Wall Shear Rate (Occlusive Challenge)

Immobilized Subject	Group	PRE	12-DAY
MM	I	306.964333	326.268545
JW	I	318.079727	304.454184
SB	I	379.844385	503.305821
BC	I	516.453656	325.280411
CL	I	305.339079	347.990678
MC	I	303.065584	385.108078
JD	I	568.262585	403.692696
NZ	I	392.164274	500.247363
JB	I	479.585480	510.305873
SS	I	408.072672	557.179877
NB	I	248.493451	298.331631
RA	I	351.129385	498.040453
HV	I	508.906784	505.484511
KB	I	368.217092	376.804482

NON-Immobilized Subject	Group	PRE	12-DAY
MM	N	598.102491	402.911942
JW	N	342.776667	295.921387
SB	N	280.000591	366.329335
BC	N	413.366073	433.029473
CL	N	247.933195	292.890926
MC	N	257.673816	324.327542
JD	N	549.159186	552.610923
NZ	N	373.876065	450.418999
JB	N	365.349934	387.890657
SS	N	482.975485	507.983396
NB	N	274.952846	262.439859

RA	N	431.257198	526.620394
HV	N	494.307523	706.838900
KB	N	451.721325	380.001429

HYPOTHESES #2

Carotid Artery Compliance (mm^2/mmHg)

	Subject	PRE	12-DAY
1	MM	.0014772	.0011574
2	JW	.0013352	.0014804
3	SB	.0013586	.0018722
4	BC	.0014079	.0014880
5	CL	.0010764	.0011269
6	MC	.0008667	.0009861
7	JD	.0015216	.0014239
8	NZ	.0008719	.0007410
9	JB	.0007701	.0007009
10	SS	.0010897	.0015085
11	AT	.0009936	.0008688
12	NB	.0012217	.0014581
13	RA	.0011914	.0011395
14	HV	.0013138	.0014399
15	KB	.0016385	.0010624

Carotid Artery Blood Flow (ml/min)

	Subject	PRE	12-DAY
1	MM	188.5371	223.7008
2	JW	289.1863	162.1214
3	SB	261.5001	197.6037
4	BC	268.7832	222.6713
5	CL	287.7170	173.1553
6	MC	322.6028	236.7709
7	JD	290.9099	285.4963
8	NZ	160.3284	186.8978
9	JB	173.8873	214.0814
10	SS	234.4245	238.6302
11	AT	211.6914	260.0335
12	NB	146.8470	139.1716

13	RA	235.4456	281.5431
14	HV	277.9988	358.4258
15	KB	291.6031	209.2258

HYPOTHESES #3

Arterial Pulse Pressure (Main effect GENDER)

ARTERIAL PULSE PRESSURE

Summary of all Effects

1-GROUP, 2-GENDER, TIME, 3-TIME
Mixed 2-Way ANOVA
MAIN EFFECT: TIME
MAIN EFFECT: GENDER

Effect	Df Effect	MS Effect	Df Error	MS Error	F-Ratio	p-Level
1	1	24.674	24	67.05927	.36794	.549824
2	**1**	**1751.363**	**24**	**67.05927**	**26.11664**	**.000031**
3	**1**	**233.922**	**24**	**21.70383**	**10.77791**	**.003140**
12	1	12.943	24	67.05927	.19300	.664358
13	1	1.165	24	21.70383	.05369	.818720
23	1	15.327	24	21.70383	.70618	.409010
123	1	28.952	24	21.70383	1.33396	.259473

Tukey's HSD
Probabilities for Post Hoc Tests
MAIN EFFECT: GENDER

	MALE	FEMALE
MEANS	**66.13895**	**54.95425**
M {1}		**.000178**
F {2}	**.000178**	

*MALE means are significantly different FEMALE means ($p<0.05$)

Tukey's HSD

Probabilities for Post Hoc Tests
MAIN EFFECT: TIME

	PRE	12-DAY
MEANS	**58.50279**	**62.59042**
1 {1}		**.003284**
2 {2}	**.003284**	

* TIME means significantly different from PRE TO 12-DAY ($p<0.05$)

Popliteal Artery Mean Diameter (Main effect GENDER)

POPLITEAL ARTERY MEAN DIAMETER

Summary of all Effects

1-GROUP, 2-GENDER, 3-TIME
Mixed 2-Way ANOVA
INTERACTION EFFECT: GENDER x TIME

Effect	Df Effect	MS Effect	Df Error	MS Error	F-Ratio	p-Level
1	1	.021485	26	.007458	2.88066	.101590
2	**1**	**.060032**	**26**	**.007458**	**8.04897**	**.008708**
3	**1**	**.050135**	**26**	**.000507**	**98.84339**	**.000000**
12	1	.004214	26	.007458	.56499	.459009
13	**1**	**.004476**	**26**	**.000507**	**8.82476**	**.006319**
23	1	.000216	26	.000507	.42489	.520228
123	1	.000072	26	.000507	.14232	.709052

Tukey's HSD
Probabilities for Post Hoc Tests
MAIN EFFECT: GENDER

	MALE	FEMALE
MEANS	**.5856976**	**.5222943**
M {1}		**.008853**
F {2}	**.008853**	

* MALE means significantly different from FEMALE means (p=0.05)

Tukey's HSD
Probabilities for Post Hoc Tests
INTERACTION EFFECT
GROUP X TIME

	PRE	12-DAY	PRE	12-DAY
MEANS	**.5726581**	**.4974034**	**.5932756**	**.5526467**
I 1 {1}		**.000169**	.082366	.095599
I 2 {2}	**.000169**		**.000169**	**.000170**
N 1 {3}	.082366	**.000169**		**.000361**
N 2 {4}	.095599	**.000170**	**.000361**	

- Immobilized 12-DAY different from Immobilized PRE
- NON-Immobilized PRE different from Immobilized 12-DAY
- Immobilized 12-DAY different from NON-Immobilized 12-DAY
- NON-Immobilized 12-DAY different from NON-Immobilized PRE

Popliteal Artery Mean Blood Flow

(Main Effect GENDER)

POPLITEAL ARTERY MEAN BLOOD FLOW

Summary of all Effects

1-GROUP, 2-GENDER, 3-TIME
Mixed 2-Way ANOVA
MAIN EFFECT: GENDER

Effect	Df Effect	MS Effect	Df Error	MS Error	F-Ratio	p-Level
1	1	81.945	26	140.7075	.582375	.452252
2	**1**	**1045.622**	**26**	**140.7075**	**7.431179**	**.011320**
3	1	7.288	26	17.2946	.421422	.521922
12	1	112.582	26	140.7075	.800115	.379265
13	1	7.717	26	17.2946	.446222	.510021
23	1	3.693	26	17.2946	.213525	.647862
123	1	5.925	26	17.2946	.342572	.563394

Tukey's HSD
Probabilities for Post Hoc Tests
MAIN EFFECT: GENDER

	MALE	FEMALE
MEANS	**31.30491**	**22.93715**
M {1}		**.011458**
F {2}	**.011458**	

* MALE means significantly different from FEMALE means ($p<0.05$)

Common Femoral Artery Mean Wall Shear Rate (Main effect GENDER)

Summary of all Effects

1-GROUP, 2-GENDER, 3-TIME
Mixed 2-Way ANOVA
MAIN EFFECT: GENDER
MAIN EFFECT: TIME

Effect	Df Effect	MS Effect	Df Error	MS Error	F-Ratio	p-Level
1	1	238.9010	20	115.9890	2.05969	.166696
2	**1**	**690.2375**	**20**	**115.9890**	**5.95089**	**.024151***
3	**2**	**171.8246**	**40**	**10.8322**	**15.86244**	**.000008***
12	1	110.7410	20	115.9890	.95475	.340184
13	2	9.0340	40	10.8322	.83400	.441719
23	2	12.9907	40	10.8322	1.19927	.312019
123	2	.4823	40	10.8322	.04452	.956501

Tukey's HSD
Probabilities for Post Hoc Tests
Main Effect: GENDER

	MALE	FEMALE
MEANS	**19.73122**	**25.92368**
.... M .... {1}		**.024288***
.... F .... {2}	**.024288***	

* MALE means significantly different from FEMALE means ($p<0.05$)

* THREE SUBJECTS DISCLUDED DUE TO IMPROPER IMAGING

Tukey's HSD
Probabilities for Post Hoc Tests
Main Effect: TIME

	PRE	6-DAY	12-DAY
MEANS	**20.02399**	**23.10452**	**25.35383**
1 {1}		**.006718**	**.000125***
2 {2}	**.006718**		.058139
3 {3}	**.000125***	.058139	

*TIME means are significantly different from PRE to 6-DAY and PRE to 12-DAY ($p<0.05$)

* THREE SUBJECTS DISCLUDED DUE TO IMPROPER IMAGING