

**Effects of Aging and Physical Activity on Cardiovascular Responses to Acute,
Induced Inflammation**

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THESIS

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TABLE OF CONTENTS

<u>CHAPTER</u>	<u>PAGE</u>
I. INTRODUCTION.....	1
A. INTRODUCTION.....	1
B. SIGNIFICANCE AND RELEVANCE OF THE RESEACH.....	3
II. LITERATURE REVIEW.....	5
A. AGING AND ARTERIAL STIFFNESS.....	5
B. AGING AND ENDOTHELIAL FUNCTION.....	9
C. AGING AND LEFT VENTRICULAR STRUCTURE AND FUNCTION.....	13
D. AGING AND VENTRICULAR-VASCULAR COUPLING.....	17
E. AGING AND THE IMMUNE SYSTEM.....	19
F. ACUTE INFLAMMATION AND THE CARDIOVASCULAR SYSTEM.....	24
G. SUMMARY.....	25
H. SPECIFIC AIMS.....	27
III. EFFECT OF AGING AND PHYSICAL ACTIVITY ON VENTRICULAR- VASCULAR COUPLING FOLLOWING ACUTE, INDUCED INFLAMMATION.....	28
A. INTRODUCTION.....	28
B. METHODS.....	31
C. RESULTS.....	37
D. DISCUSSION.....	42

TABLE OF CONTENTS (cont'd.)

<u>CHAPTER</u>	<u>PAGE</u>
IV. EFFECT OF AGING AND PHYSICAL ACTIVITY ON ENDOTHELIAL REACTIVITY AND BLOOD PRESSURE FOLLOWING ACUTE, INDUCED INFLAMMATION.....	49
A. INTRODUCTION.....	49
B. METHODS.....	51
C. RESULTS.....	57
D. DISCUSSION.....	60
V. SUMMARY AND RELEVENCE OF FINDINGS.....	85
LITERATURE CITED.....	87
VITA.....	96

LIST OF TABLES

<u>TABLE</u>	<u>PAGE</u>
I. SUBJECT CHARACTERISTICS.....	40
II. MEDICATION TYPES AND FREQUENCIES.....	41
III. CARDIAC PARAMETERS BEFORE AND AFTER VACCINATION IN OLDER AND YOUNGER ADULTS.....	49
IV. BLOOD PRESSURE RESPONSES TO VACCINATION IN OLDER AND YOUNGER ADULTS.....	74
V. ARTERIAL RESPONSES TO VACCINATION IN OLDER AND YOUNGER ADULTS.....	78

LIST OF FIGURES

<u>FIGURE</u>	<u>PAGE</u>
1. Age-related arterial stiffening and ventricular consequences.....	7
2. Role of NF- κ β and pro-inflammatory cytokines in inflammation-mediated vascular damage.....	12
3. Cytokine cascade and development of protection following an immune challenge.....	21
4. Arterial elastance response to acute, induced inflammation.....	43
5. Ventricular elastance response to acute, induced inflammation.....	44
6. Coupling response to acute, induced inflammation.....	45
7. IL-6 responses to acute, induced inflammation.....	52
8. CRP responses to acute, induced inflammation	53
9. Percent change in IL-6 at 24 and 48 hr post vaccination in older and younger adults.....	71
10. Percent change in CRP at 24 and 48 hr post vaccination in older and younger adults.....	72
11. Change in MAP post-vaccination in older and younger adults.....	75
12. Change in post-vaccination augmentation index in older and young adults.....	76
13. Flow-mediated dilation post-vaccination in older and younger adults	79

LIST OF ABBREVIATIONS

AGE Advanced Glycation End-product

Aix Augmentation Index

BMI Body Mass Index

BSA Body Surface Area

CO Cardiac Output

COX Cyclooxygenase

cPWV Central Pulse Wave Velocity

CRP C-Reactive Protein

CVD Cardiovascular Disease

E' Early Diastolic Tissue Velocity

Ea Arterial Elastance

EDV End-diastolic Volume

EF Ejection Fraction

Elv Left Ventricular Elastance

eNOS Endothelial Nitric Oxide Synthase

ESP End-systolic Pressure

ESV End-systolic Volume

ET-1 Endothelin-1

FMD Flow Mediated Dillation

FS Fractional Shortening

ICAM Intracellular Adhesion Molecule

IL-6 Interleukin-6

IMT Intima-media Thickness

iNOS Inducible Nitric Oxide Synthase

LV Left Ventricle

LVM Left Ventricular Mass

MAP Mean Arterial Pressure

NO Nitric Oxide

ONOO- Peroxynitrate

S' Systolic Tissue Velocity

SS Shear Stress

SV Stroke Volume

TNF- α Tumor Necrosis Factor Alpha

VCAM Vascular Cellular Adhesion Molecule

VVC Ventricular-vascular Coupling Ratio

SUMMARY

Aging is characterized by increased arterial and ventricular stiffness, at least partly due to chronic, low-grade systemic inflammation. This leads to a concomitant rise in both arterial elastance (Ea: total arterial load) and ventricular elastance (Elv: left ventricular contractility or stiffness). Their ratio (Ea/Elv) determines ejection fraction and exercise capacity. Comorbidities common in aged individuals transiently cause further up-regulation of inflammation, which may predispose older adults to cardiac events. Physically active older adults, however, retain a more compliant cardiovascular system, partially mediated by reduced systemic inflammation.

Thus, we investigated the effects of acute inflammation on cardiovascular (Ea and Elv), and endothelial function and blood pressure, in older versus younger adults and sought to determine the relationship between physical activity (intensity and volume) and any inflammation-induced changes. Applanation tonometry and ultrasonography were used to measure pressures, cardiac volumes and other indices of performance before and at 24 and 48 hr after induction of inflammation using an influenza vaccination. Endothelial function was assessed using flow-mediated dilation, and physical activity was measured with accelerometry.

Following inflammation, there was no change in Ea or Ea/Elv ($p > 0.05$), but a reduction in Elv ($p < 0.05$) in both older and younger adults. Systolic performance was only reduced in older adults, but diastolic performance was attenuated in both groups at 24 hr post-inflammation ($p < 0.05$ for all). Younger adults attenuated flow-mediated dilation at 24 and 48 hr post-inflammation and older adults did not, ($p < 0.05$). Older adults decreased peripheral and central

systolic pressure after inflammation, and younger adults did not, ($p < 0.05$). Physical activity intensity was inversely related to the change in Elv at 24 hr post-vaccination, $p < 0.05$.

Our data indicate that aging does not affect the elastance response but does affect the blood pressure and endothelial responses to acute, induced inflammation. Physical activity was inversely related to the change in Elv following acute inflammation.

Chapter I: Introduction

Arterial stiffness increases and endothelial function decreases with aging [1, 2]. The left ventricle (LV) also undergoes alterations such as stiffening and attenuated systolic or diastolic function in older adults [3-5]. Consequently, arterial elastance (Ea) and ventricular elastance (ELV) both increase with aging, demonstrating a greater arterial load and resultant compensation by the LV [6]. However, the ratio of Ea to ELV, or ventricular-vascular coupling (VVC), does not change with age due to homogenous augmentation of both components [6]. Maintaining optimal VVC is important, as it mediates myocardial perfusion and cardiac energetics as well as enabling increases in cardiac output without drastically increasing cardiac wall stress [4]. VVC is also associated with mortality in patients with a prior myocardial infarction (MI) [7].

Aging is also associated with impaired immune function, such as changes in innate immunity and less regulation of inflammatory responses [8, 9]. Indeed, increased levels of inflammatory markers are associated with decreased ventricular and vascular function in cross sectional studies [10-12]. Acute systemic inflammation also temporarily increases risk of cardiovascular events [13-15], although the underlying mechanisms are not fully understood. Furthermore, acute inflammation decreases arterial function in young adults [16, 17], but it is not known if aging affects this response. Importantly, the direct effect of acute inflammation on arterial and ventricular function and their interaction in older (and younger) adults is unknown as studies to date have been primarily cross-sectional and only investigated associations. To our knowledge, no study has examined the effects of acute, induced inflammation on arterial and ventricular function in older adults using a prospective design.

Therefore, the overall aim of our project is to examine the effects of aging on vascular and ventricular coupling following induced acute systemic inflammation.

Regular physical activity reduces inflammation and oxidative stress [18], improves vascular function in older and younger adults [19] and attenuates the effects of aging on the vasculature and the heart [3, 20, 21]. Physical activity also reduces cardiovascular disease risk, and these benefits appear to be dependent on activity intensity and volume [22, 23]. It is not currently known if physical activity also protects against acute inflammatory insult, nor is it certain if levels (volume and intensity) of physical activity are associated with inflammation and inflammation-induced cardiovascular changes. Thus, a second aim of our project is to evaluate whether or not fitness, as well as volume and intensity of physical activity, affect vascular and ventricular function and VVC following induced inflammation in young compared to older individuals.

Significance and Relevance of the Research

Subclinical levels of inflammation are common in older adults and are linked to decreased vascular and LV function and, importantly, to cardiovascular mortality [24, 25]. An acute, inflammatory insult transiently increases the risk of cardiovascular events in both older and younger adults [13-15]. Cross-sectional data in older adults and prospective data in young adults suggest LV performance is decreased, combined with a decrease in endothelial function, an increase in arterial stiffness, following induced inflammation [13, 17]. Specific, causal relationships are not clearly established in older adults.

Aging alters the components of VVC [6]. Acute inflammation may also more profoundly affect VVC in older adults, which may affect cardiac energetics, coronary perfusion, and exercise capacity. This is perhaps another mechanism by which acute inflammation evokes cardiovascular events, although no studies to date have directly investigated this concept.

Physical activity has protective effects in both older and younger adults, related to its beneficial effects on the heart and vasculature [26, 27]. However, specific information regarding the impact of physical activity volume and intensity on acute inflammation and subsequent cardiovascular responses are scarce [22] or speculative in older adults. The contribution of this project will be to gain new insight regarding the effect of acute inflammation on heart and vascular responses, and how aging and physical activity modulate these responses.

This contribution is important; it will lead to a better understanding of the cardiovascular effects caused by acute inflammation, and potential protective effects of physical activity, in

older (and younger) adults. **Thus, this project may significantly advance the field and lead to the development of more specific and noninvasive, preventive strategies for decreasing inflammation-induced cardiovascular events.** As such, this study has large public health implications as many co-morbidities associated with aging, such as arthritis, illness, and stress, are characterized by transient increases in systemic inflammation [28, 29].

Chapter II: Literature Review

Aging and the Cardiovascular System

Aging and Arterial Stiffness

Normal, non-pathological aging is associated with increased arterial stiffness in men and women [1]. It is also linked with widening of the pulse pressure, increases in the aortic root and large artery diameters and augmented aortic input impedance [30]. Although smooth muscle relaxation is relatively preserved past age 30, individuals under 30 years of age have significantly more smooth muscle vasorelaxation after sodium nitroprusside administration [31], indicating differences in smooth muscle responsiveness or structural composition of the smooth muscle in older individuals.

Others have observed this impairment in endothelium-independent dilation in hypertensive patients and have shown that it is linked to increased muscle sympathetic nerve activity, a vasoconstricting signal whose influence is also augmented in older adults [32-35]. Aging also causes an increase in collagen and a decrease in elastin content of the medial arterial wall, resulting in less compliant arteries in older adults [36, 37]. Collectively, these factors cause an augmentation of arterial stiffness in older adults.

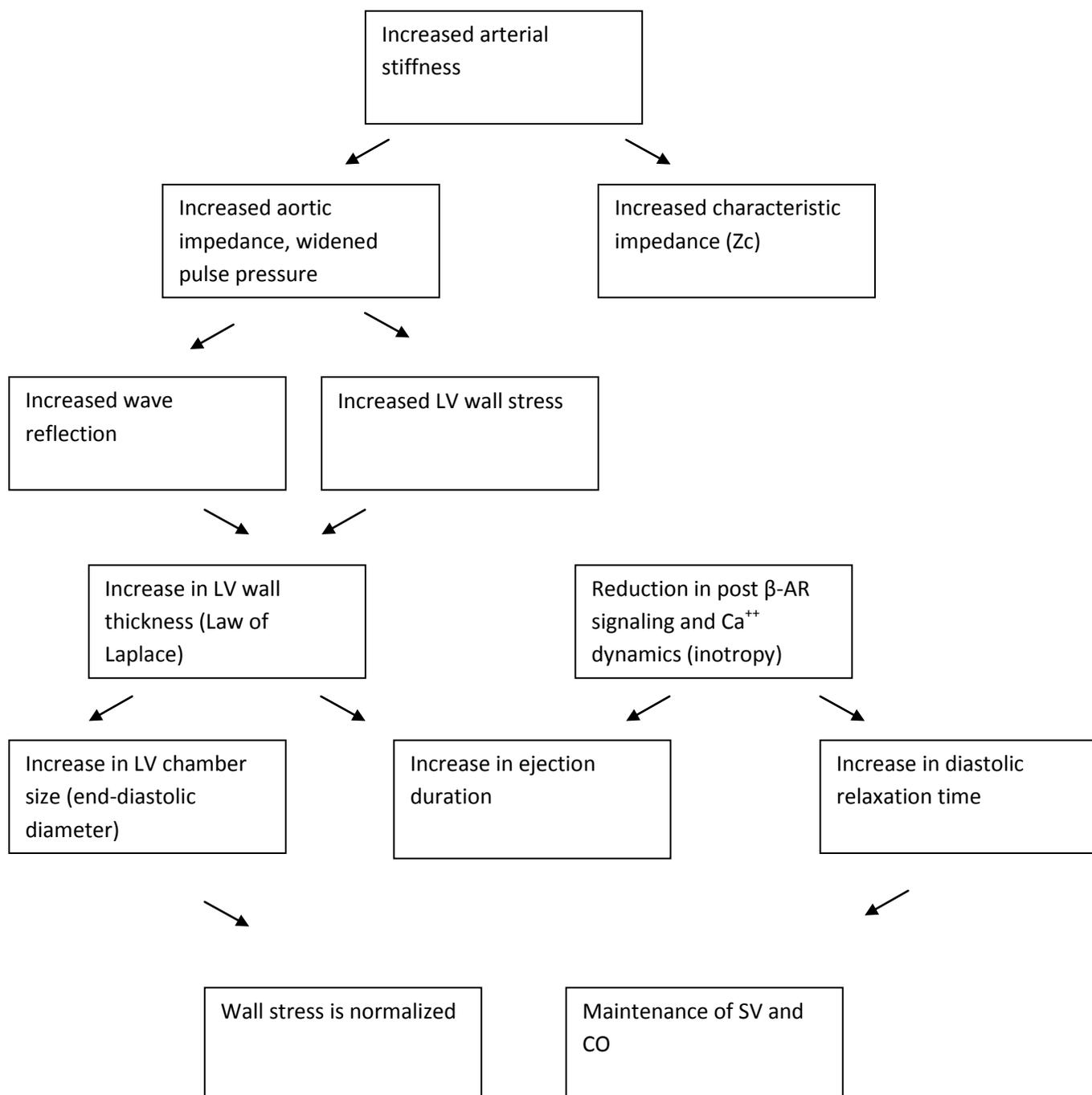
Various stimuli, such as shear stress and local metabolites, “turn on” nitric oxide (NO) signaling, not only causing endothelium-dependent vasorelaxation but also the scavenging of reactive oxygen species (ROS) [38]. The scavenging of ROS inhibits undesirable arterial remodeling, which is characterized by an increase in collagen in the medial wall and fraying of elastin. In this way, the ROS:antioxidant ratio is a crucial regulator of vascular stiffness that is disrupted in aging [39].

Providing further support for this notion, previous studies show that a pro-oxidant state (characterized by reduced bioavailable NO and more common in aged individuals [2]) increases production of vascular cellular adhesion molecule (VCAM), matrix metalloproteinases (MMPs), and intracellular adhesion molecule (ICAM) [40]. Additionally, exposure of the vasculature to high glucose loads, more common in older adults, causes the formation of irreversible, covalent proteoglycan bonds called advanced glycation end-products (AGEs) that also cause arterial stiffening [41].

Furthermore, increased age is a risk factor for vascular calcification, or the inappropriate deposit of bone within the artery. Indeed, calcified regions in the vasculature are increased in individuals over age 30, and are almost ubiquitous by age 70-80 [42, 43]. As a result, arterial stiffness and systolic blood pressure rise, and calcified regions of the vasculature become visible with some imaging techniques. Importantly, the process of arterial calcification is related to cardiovascular morbidity and mortality [44].

Decrements in bioavailable NO and associated accumulation of the aforementioned molecules are extremely important as they often precede and/or contribute to atherosclerotic plaque formation and arteriosclerosis that leads to exacerbation of arterial stiffening [9]. Collectively, all of these factors that contribute to a stiffer arterial system are increasingly common in aged individuals and lead to structural alterations and remodeling that increase arterial stiffness and also affect the left ventricle, Figure 1.

Figure 1. Age-related arterial stiffening and ventricular consequences.



Effect of Physical Activity and Exercise Training:

Regular physical activity offsets age-associated increases in arterial stiffness [20, 26, 45]. Active, post-menopausal women retain central pulse wave velocity and aortic pulse pressure (cPWV and aPP) values that are similar to those found in premenopausal women, indicating that higher levels of activity counters the effects of aging in regards to arterial stiffness in women [20]. Additionally, regular physical activity attenuates the decrease in carotid artery compliance in men [26]. Physical activity may protect arterial compliance due to its ability to preserve NO and attenuate that pro-oxidant milieu that stimulates the atherosclerotic process and causes vascular remodeling [9]. Physical activity can also cause a reduction in other risk factors that may indirectly improve arterial compliance. For example, hyperlipidemia causes an increased ratio of low-density lipoprotein cholesterol (LDL-C) to high-density lipoprotein cholesterol (HDL-C) that can contribute to coronary heart disease and atherosclerosis but can be beneficially modified in physically active individuals [43].

Even the adoption of a short-term exercise intervention improves carotid artery compliance in older men, but it does not reach the same level as younger individuals [26]. This suggests that, while exercise and physical activity are effective treatments for arterial stiffening, they are more efficacious as preventive measures.

Aging and Endothelial Function

Aging also results in decreased endothelium-mediated nitric oxide (NO) responsiveness [31]. Both men and women over age 55 have blunted flow-mediated dilation (FMD) compared to younger adults (aged 35) but preserved smooth muscle function [2]. These changes are not abolished by estrogen therapy in older women [46]. Taken together, these findings reveal an age-associated decrease in endothelial function and resultant vascular responsiveness in both men and women.

In older men and women, vessel diameter is negatively correlated with flow-mediated dilation (FMD). Exposure to high pressure or flow (as in hypertension) over time causes remodeling, which increases vessel diameter, as well and causes endothelial damage [39]. As a result of the widening of the vessel, shear stress is reduced and endothelial function is further impaired. Hence, arterial diameter increases while FMD is attenuated in aging. Smooth muscle function is correlated to body mass index (BMI) in men and risk factor score in women [46], suggesting a potential for divergent modulation of vascular smooth muscle reactivity with aging between sexes

Other sources of impaired endothelium-dependent dilation (EDD) specifically related to aging have been postulated and include: i.) reduced bioavailable NO due to increased oxidative stress, Figure 2, ii.) increased endothelin-1 (ET1), iii.) decreased mitochondrial biogenesis, iv.) increased advanced glycation end-product (AGE) formation, v.) estrogen bioavailability of the arterial wall, vi.) lifestyle/risk factors [2].

Reductions in NO not only potentiate arterial stiffening as discussed above, but also incur an inability to vasodilate in response to various stimuli, such as shear and metabolic stress [2].

This is important, as this property of NO is protective and allows for the matching of metabolic supply with demand in the local circulation [47]. Bioavailable NO is greatly impacted by the level of oxidative stress in the endothelial environment [2]. Heightened oxidative stress leads to the uncoupling of NO and its precursors, tetrahydrobiopterin (BH₄) and endothelial nitric oxide synthase (eNOS) [40]. As a result of augmented oxidative stress, peroxynitrate (ONOO⁻) is formed in place of NO, and vasoactive effects of NO are lost [40]. Additionally, ET-1, a potent vasoconstrictor, is formed. Its actions and availability directly oppose NO [48]. Thus, the effect of the age-related reduction in NO is two-fold: it causes augmentation of ET-1 and its constrictive action, as well as attenuation of vasorelaxant, NO signaling [48].

A major source of ROS is by-products of inefficient mitochondrial metabolism. Ineffective metabolic processes, such as overload of the electron transport chain, cause oxidant species to accumulate and impair NO signaling [49, 50]. Increased mitochondrial number and enzymes reduce the amount of oxidant production and thereby improve vascular function. Aging is characterized by reduced cytochrome c oxidase and mitochondrial biogenesis, thus leading to compromised metabolism and ensuing impairment in endothelial responsiveness [50].

AGE formation causes reductions in vascular health via two mechanisms. As previously mentioned, the formation of these protein-glucose molecules causes irreversible arterial stiffening [41]. Furthermore, the receptor for AGE (RAGE) triggers inflammation by causing nuclear factor kappa beta (NF- κ B) translocation and up-regulation of pro-inflammatory cytokines in a feed-forward manner [41]. The production of these cytokines compromises NO availability and decreases endothelial reactivity. In older adults, states of high blood glucose (due to reduced insulin sensitivity) causes the formation of AGE more readily than in younger adults [51].

Additionally, AGE's build up over time, making their vascular effects more pronounced in older people [4]. The role of NF- κ β in vascular damage is described below, Figure 2.

Figure 2. *Role of NF- κ B and pro-inflammatory cytokines in inflammation-mediated vascular damage.*

TNF- α , IL-6 \rightarrow inhibition of NF- κ B by I κ B *turned off* by degradation of I κ B \rightarrow nuclear translocation of NF- κ B \rightarrow feed-forward up-regulation of pro-inflammatory cytokines \rightarrow up-regulation of MMP2 and MMP9, ICAM and VCAM, ET-1, and formation of ONOO- \rightarrow continued cycle of vascular damage

Women lose estrogen and its protection past menopause. Estrogen maintains vascular health by signaling through the β_2 receptors on the arterial wall to increase vasorelaxation via NO and adenylyl cyclase-cyclic AMP [33, 52, 53]. In fact, the effects of estrogen are so potent that young women experience no relationship between sympathetic (constrictor) signals and blood pressure because estrogen and β_2 .NO production counter these adrenergic, constrictor influences [33, 52]. Once estrogen is no longer available, older women are especially vulnerable to neural cues that increase vascular tone [33].

Effect of Physical Activity and Exercise Training:

Much of the vascular benefit associated with physical activity is purportedly due to a reduction in oxidative stress and improvement in NO bioavailability in exercising older adults, as endogenously-administered antioxidants rescue endothelial function in sedentary older adults but have no effect in exercising older adults [54].

Reduced oxidation in the endothelium is important; it leads to more bioavailable NO instead of peroxynitrite (ONOO-) which preserves its vasodilatory properties [39]. There are other notable differences in NO dilator function in aged individuals that may be corrected by physical activity: for example, although NO dilator function is blunted in older, sedentary women it is maintained in older, fit women [45]. However, NO dilator function (as assessed by FMD) is also reduced in men regardless of fitness/training status [45, 48].

Much of the benefits currently associated with adequate physical activity during the aging process include the modulation of risk factors. Indeed, many factors that contribute to decreased endothelial responsiveness can be placed into the lifestyle/risk factors category [55]. Obesity, more common in aging, is associated with increased inflammatory cytokines that oppose NO's

actions [56]. Along these lines, increased plasma low-density lipoprotein cholesterol (LDL-C) is associated with EDD in older but not younger sedentary men [55], which suggests that managing this risk factor with physical activity is important for EDD in older men (but not younger men). Furthermore, exercising older men with borderline/high LDL-C were protected against impairment of EDD [55], suggesting a role for physical activity in reducing the negative effects of LDL-C upon arterial function.

Maintaining a high fitness level or beginning exercise training reduces the impairment in NO-mediated vasodilation found in older adults [20, 45]. Along these lines, a previous study showed that 24 weeks of aerobic training led to improved FMD in older, sedentary women [45]. Others have also reported improvement in NO dilator function in response to exercise training in both sexes and during aging [57-59].

Aging and Ventricular Structure & Function

Normative values have been established for left ventricular mass, stroke volume [60], end-systolic volume (ESV), and ejection fraction (EF) based on age and sex [5]. Many of these variables are highly dependent upon body size, so scaling methods are frequently employed (Chirinos). Whether raw data, height-adjusted data, or BSA-adjusted data is used, women have reduced LV mass, end systolic volume (ESV), and end-diastolic volume (EDV) compared to age-matched men, but both sexes augment all previously mentioned volumes with aging [61]. The only exception to this trend was end-diastolic diameter (Edd), which was consistently higher in women [61]. However, EF is uniformly reduced in both men and women as they age [5].

With aging, the LV becomes more spherical, and this sphericity is associated with clinical conditions, such as dilated cardiomyopathy, and is a predictor of exercise capacity [62]. Women exhibit a decrease in LV length with age, but men do not compensate in this manner [62]. There is about a 9% reduction in long-axis length in women between 20 and 90 years of age, but it occurs concomitantly with an increase in LV mass, resulting in no net change in total mass [62]. Men reduce length ~ 11% over the same time period, however, there is no concurrent increase in wall thickness, resulting in an overall LV mass loss of 10% [62]. These structural changes occur without short-axis cavity dimension changes in either gender [62]. Taken together, these results indicate a lack of ability for myocyte hypertrophy to overcome the myocyte drop-out in men and altered ventricular structure in both sexes in aging [62].

Ventricular function is similarly impacted by aging; older women without diagnosed CVD have reduced diastolic and increased systolic function compared to younger women [63]. Older men and women both experience an age-related reduction in the ability to maintain stroke

volume with exercise at intensities $>70\%$ of VO_2 max, and this effect of aging was more pronounced in the oldest subset of women [64]. This at least partially explains the age-related loss in work capacity.

Left ventricular function is similarly compromised during aging, and this can be at least partially attributed to reductions in compliance and accumulation of fibrotic tissue [21, 27]. Additionally, post β_1 -receptor signaling is impaired, causing reduced contractility [4]. Calcium handling, which is pivotal to effective contractile ability, is similarly influenced by aging. Reduction in phospholamban and sarco-endoplasmic reticulum calcium 2A (SERCA 2A) cause an inability of the aged heart to sequester intracellular calcium and appropriately contract. These structural and functional impairments affect both systolic and diastolic function in aging [65, 66].

Effect of Physical Activity and Exercise Training:

Right ventricular (RV) and LV hypertrophy are observed in the hearts of individuals who have engaged in intense, long-term (multiple years) training [67]. However, overall arrhythmia occurrences are reduced in exercise-trained men, most likely due to the reduction in CV risk factors incurred by regular exercise [67]. Additionally, there is less collagen formation and cross-linking in the hearts of life-long exercisers, suggesting that habitual activity inhibits undesirable ventricular remodeling [21, 27]. In contrast, sedentary aging results in a loss of ventricular compliance and failure of the Starling mechanism: increases in end-diastolic volume no longer result in increases in stroke volume. Rather, the ventricle grows larger to accommodate more volume in order to preserve stroke volume despite reductions in contractility [3, 27].

Other studies indicate that life-long physical activity protects the Starling mechanism by reducing the age-associated augmentation of ventricular stiffening [3, 27]. Importantly, this results in lesser blood pressure lability and greater exercise tolerance in these active older adults. Additionally, habitual physical activity in older adults reduces ventricular myocyte atrophy and apoptosis [68].

In habitually endurance-trained individuals, neither age nor gender impacts sub-maximal Qc-VO₂ relationships [64]. However, others report a favorable change in LV systolic reserve post-training (HR controlled) in men but not women [66]. This indicates that older men can improve LV filling dynamics, but women cannot [66]. Systolic reserve is important; it provides a buffer to protect the individual from operating at near-maximal capacity and is linked to improved exercise tolerance [69]. Exercise training results in augmented maximal capacity in men through increased SV and peripheral vasodilator function, while only peripheral function (a-vO₂ difference) is improved post-training in older women [70, 71]. This is a crucial limitation, as there is clearly a ceiling in peripheral O₂ extraction that may thus limit the ability of older women to improve exercise tolerance. Diastolic function is also improved or maintained in older adults who are habitually active, as evidenced by a preservation of Starling forces [3]. Furthermore, older men who embark upon an exercise program later in life are also able to improve filling dynamics [72, 73].

Aging & Ventricular-Vascular Coupling

Ventricular-vascular coupling (VVC) refers to the appropriate matching of arterial and ventricular elastance in order to maintain cardiac metabolic efficiency and efficacy [74]. This matching is crucial; without physiologically advantageous VVC, small changes in volume lead to large changes in pressure and, possibly, in cardiac perfusion [75]. Although combined arterial and ventricular stiffening can still result in acceptable VVC, cardiovascular reserve and exercise capacity are greatly diminished by tandem stiffening [76].

In aging, widened pulse pressure (PP) due to a reduction in aortic compliance results in increased reliance upon systolic pressure to aid in coronary perfusion. In a compliant system, ~70% of coronary perfusion occurs in diastole because of the recoil action of the large proximal vessels [75]. In contrast, stiffer systems rely upon systolic pressure for up to 50% of coronary perfusion [75]. Therefore, any drop in systolic pressure in a stiffened system (perhaps due to the onset of diuretic usage or dehydration) can lead to enhanced cardio-depression and increase the size and functional consequence of an ischemic bed [4]. Hence, VVC has been linked to post-MI survival [7].

Older age and female sex are associated with increases in both ventricular and vascular stiffness, even without CVD, and this may contribute to the augmented incidence of heart failure with preserved ejection fraction (HFpEF) in older women [6]. Others also report increased end-systolic ventricular elastance in older women that is matched by tandem increases in arterial elastance, resulting in appropriate coupling ratios that are not different from older men despite differences in each component [63].

EaI is significantly higher in women than men, and PP increases to a greater extent in women than men in normal aging [77]. There are age and gender differences in EF and a reduction in EF reserve with aging; in men, this is because of an age-related loss of LV contractility (Elv); in women, the culprit is increased arterial load (Ea) [77]. These age-associated changes in VVC in both men and women result in impaired exercise performance and an increase in overall cardiovascular outcomes [77].

Effect of Physical Activity and Exercise Training:

The dynamic Starling mechanism is reduced with aging, possibly because of ventricular and vascular stiffening [3]. Although this maladaptation may be minimized by consistent training, it seems as though the extent to which exercise acts as a preventative depends upon age at the onset of regular physical activity [3]. Intensity of training may also be important as studies reporting preservation of Starling curves in older, exercising adults have studied Master's athletes [27]. In contrast to aged adults, younger sedentary and athletic adults have similar Starling curve, suggesting that sedentary aging causes the loss of Starling function [3, 78].

However, beginning an exercise training program later in life does not improve VVC in healthy, older adults [78]; it seems that exercise training must begin by early middle age in order to preserve VVC.

Aging and the Immune System

Aging and Inflammation

Strong acute-phase responses to injury have been pivotal to human survival and antibody formation (Figure 3), however, the resultant cumulative inflammation over time has a role in immune dysregulation with aging [79, 80]. The term “inflammaging” has been coined to refer to the increase in pro-inflammatory cytokines, reduction in growth hormone (GH), sex steroids, and vitamin D that occurs in normal, non-pathological human aging [8]. The state of low-grade inflammation common in aging is associated with this phenomenon and is hypothesized to be a correlative or causal factor in age-related immunosenescence [80, 81]. Due to this de-regulation of immune responses, as well as reductions in T cell numbers and naiveté, autoimmune diseases (such as arthritis) are more common, and ability to produce antibodies are attenuated in aged individuals [80, 82].

Figure 3. *Cytokine cascade and development of protection following an immune challenge.*

1. Recognition of foreign antigen by Toll-like receptor, B cells, or macrophage
2. increase in anti-inflammatory (IL-4 and IL-10) OR pro-inflammatory cytokines (IL-6, IL-1 β , TNF- α , INF- γ)
3. antigen presentation to B cells
4. formation of antibody (Th2 response) OR up-regulation of natural killer (NK) cells and macrophages (Th1 response)

Indeed, advanced age is associated with a two to four-fold increase in both circulating concentration and cellular production of cytokines, especially interleukin-6 (IL-6) [83]. This is attributed not only to aging itself, but also to co-morbidities that tend to occur in conjunction with aging, such as: increased adiposity, presence of sub-clinical infections, poor nutrition, and decreased sex hormone production [83].

The process of inflammaging affects cardiovascular function, as both IL-6 and C-reactive protein (CRP) are associated with regional or global ventricular dysfunction in a symptom-free cohort [10, 25]. Higher CRP was associated with reduced ventricular function in men but not women, who had higher levels of CRP than the men [10]. Elevated levels of homocysteine (Hcy) are also associated with regional LV dysfunction in both sexes [84]. Additionally, higher levels of CRP are related to increased arterial stiffness [11].

Systemic markers of inflammation have proven to be clinically relevant, as elevated preoperative CRP is predictive of early events following coronary artery bypass graft (CABG), and IL-6 can predict both early and late events after CABG [85]. High-sensitive CRP (hsCRP) is related to CV morbidity and mortality and is inversely related to fitness level in sedentary men [86]. IL-6 is a stronger independent predictor of ventricular dysfunction, and it has been deemed the “cytokine of aging” by some research groups [10, 25]. More specifically, significant inverse correlations were found in asymptomatic men and women between IL-6 concentration and left ventricular systolic function, linking inflammation and ventricular dysfunction in individuals free of overt cardiovascular disease [25]. Others postulate that IL-6 production becomes uncontrolled during aging and accounts for the “phenotype” of old age: frailty, osteoporosis, augmentation of CRP, and lymphoproliferative disorders [87].

Aging is associated with a pro-inflammatory phenotype of the vascular endothelium, including disrupted NF- κ B pathways [49]. A pro-inflammatory endothelium is characterized by a reduction in I κ -B mediated inhibition of NF- κ B-triggered inflammation [38]. This causes a feed-forward up-regulation of inflammation, increasing production of cytokines such as tumor necrosis factor alpha (TNF- α) and IL-6. Importantly, tumor necrosis factor alpha (TNF- α) overproduction is associated with CV injury and vulnerable phenotype, including: extracellular matrix changes/stiffening, wall thinning, and myocardial wall changes [88] and is up-regulated in a feed-forward manner by NF- κ B signaling.

Many of the inflammation-induced decrements in arterial health are attributable to resultant reduction in NO bioavailability [2, 9]. Both TNF- α and IL-6 uncouple tetrahydrobiopterin (BH₄), an essential cofactor involved in eNOS synthesis, and lead to the formation of peroxynitrate (ONOO⁻) instead of NO. This results in a reduction in vasorelaxation, increase in adhesion molecules (ICAM, VCAM), potent vasoconstrictors (such as ET-1), and degradation of elastin and accumulation of collagen in the medial space through MMP-2 and MMP-9 activation [40].

Effect of Physical Activity and Exercise Training:

Regular physical activity and higher levels of fitness modulate the presence of inflammation [80]. Cross-sectional data report a reduction in inflammatory biomarker content in individuals who report more frequent and more intense PA [83]. In fact, the NHANES III, Health ABC study, and CHD study all highlight an inverse dose-response relationship between

level of physical activity and plasma CRP concentrations, although CRP was up-regulated with aging [83].

In men, this relationship persists even when BMI is considered [89]. Data from another interventional study shows that activity-induced changes in CRP were not associated with changes in body weight or body fat, suggesting that physical activity can modify inflammation independent of obesity [90]. Thus, the effects of physical activity upon inflammation do not appear to be mediated by reductions in adiposity. This reduction in inflammation is important, as regular physical activity has also been shown to reduce the negative effects of inflammation on the vasculature, blood pressure, LV function in older adults [19, 80, 91-93].

When physical activity was assessed categorically, men completing the highest levels of activity had the lowest CAD risk, and every 50 MET-h/wk was associated with a 26% reduction in CHD risk [22]. Intensity also predicted CHD risk: men who exercised >6 METS had a RR of 0.72 compared to the group exercising <4 METS [22]. Thus, the “dose” of physical activity appears to be an important factor in its effect on cardiovascular health and clinical outcomes. However, more exact guidelines for physical activity frequency and intensity related to cardioprotection following acute inflammatory insult have not been elucidated.

Physical activity reduces CRP, white blood cell count, and fibrinogen activity [94, 95] and elevates endogenous antioxidant activity in older adults [96]. This attenuation of inflammation and ROS is one mechanism by which PA purportedly exerts its beneficial effects on vascular health in aging. Further support for this notion is provided by Seals, et al, who showed that active men maintained youthful levels of vascular reactivity due to a more favorable

oxidative environment compared to their non-exercising peers. BH₄ availability, and, thus, NO formation, is also favorably preserved in active older men [97].

There are a number of other potential mechanisms postulated to incur the vascular benefits of exercise training, such as; reductions in adiposity and associated macrophage accumulation and phenotype, increase in muscle-produced IL-6, or increased parasympathetic/sympathetic balance [80]. Dose does seem to be important, as moderate exercise improves inflammatory/immune profiles, while inactivity and extreme training do not confer these benefits [91].

Acute Inflammation and the Cardiovascular System

An acute inflammatory insult results in a combination of vascular effects; a short-term (~8 hr) decrease in wave reflection (Aix) and blood pressure, peripheral vasodilation, impaired endothelial function, and increased arterial stiffness [17]. This transient reduction in endothelial function has been deemed “endothelial stunning” by Bhagat and colleagues as it is a very robust decrement in function that occurs without concomitant alteration in structure but persists for up to 7 days post-inflammatory stressor [13]. This effect of acute exposure to endotoxin was abolished by hydrocortisone administration, indicating that dampening the associated inflammation rescues endothelial function [13].

This inflammation-mediated alteration in cardiovascular performance has been hypothesized to be a mechanism by which acute infection results in myocardial infarction [13]. This net systolic response of the LV to acute inflammation has also been characterized and includes reductions in contractility, stroke work, and ejection fraction [98]. Furthermore, the arterial system is also adversely affected by inflammation: central arterial stiffness increases, endothelial function is reduced, and Aix is attenuated following acute inflammation [15, 16].

These responses have the potential to impact preload and stress imposed upon the LV, as well as the heart-large artery interaction. Neither the effect of acute, inflammatory stimulus or aging on VVC has ever been explored. There is a large public health outcome in studying a clinically relevant variable (VVC) in the context of a common, physiological stressor.

Summary: Aging, Physical Activity, and Cardiovascular Function Following Acute Inflammation

Aging results in increased vascular stiffness and reduced endothelium dependent dilation following acetylcholine administration [1, 31]. It is hypothesized that chronic, low-grade inflammation plays a role in attenuating nitric oxide (NO) function and resultant endothelium dependent dilation, and that this vascular phenotype becomes more common with aging [38].

In addition to augmented baseline levels of inflammation in older adults, acute systemic inflammation further impairs NO-dependent endothelium dependent dilation and vascular reactivity [99, 100]. An acute inflammatory stimulus results in dysfunction of the endothelium for up to 48 hours after the introduction of the stressor [13]. In young, healthy individuals, both CRP and IL-6 are up-regulated following vaccine injection [17]. C-reactive protein (CRP) and interleukin 6 (IL-6), markers of systemic inflammation that are elevated in older individuals, are also associated with augmented arterial stiffness, impairment in LV function, and decreased endothelial function [11, 17, 101]. Indeed, CRP is directly correlated with increases in central pulse pressure, augmentation index (AIx), and central systolic pressure [12]. Other inflammatory cytokines associated with acute immune insult, tumor-necrosis factor α and interleukin 1 β , also induce endothelial dysfunction up to 24 hours following vaccine injection [102].

Regular exercise can attenuate age-related vascular changes due to both a reduction in CVD risk factor number and severity and also by allowing endothelium dependent dilation to be maintained by preserving NO responsiveness and reducing the age-related increase in arterial stiffness [2, 20, 26, 45]. Even in young adults, endurance exercise training increases arterial distensibility compared to strength-trained young adults or sedentary controls [103]. Regular

physical activity also preserves ventricular compliance and helps to retain pressure-volume relationships more similar to those found in younger adults. Hence, physical activity may be an effective strategy for the prevention of inflammation-induced decrements in cardiovascular function, although no study to date has tested this concept.

In this context, we aimed to determine how acute inflammation altered VVC in older versus younger adults and whether or not physical activity affected these responses in both age groups.

Specific Aims:

Aim 1: To determine the effects of aging on VVC following acute, induced inflammation.

We hypothesized an increase in VVC due to a reduction in Elv that would be more pronounced in older compared to younger adults.

Aim 2: To determine the effects of aging on large artery and endothelial responses to acute, induced inflammation. We hypothesized an increase in arterial stiffness and a reduction in endothelial function that would be more pronounced in older compared to younger adults.

Aim 3: To determine the association of physical activity on VVC and large artery and endothelial function following acute, induced inflammation. We hypothesized that physical activity would be cardioprotective and that higher levels of physical activity (both intensity and volume) would be associated with a lesser reduction in the VVC, as well as arterial and endothelial function, following inflammation, especially in older adults.

Chapter III: Effect of Aging and Physical Activity on Ventricular-Vascular Coupling Following Acute, Induced Inflammation

Introduction

Overall arterial load, or resistance to ventricular ejection, can be quantified by calculating arterial elastance (E_a) [65, 104]. This measure incorporates both pulsatile and steady components of the arterial system, as well as characteristic impedance [65]. Similarly, net ventricular stiffness or contractility can also be measured using ventricular elastance (E_{lv}) [27, 69]. Importantly, both arterial and ventricular elastances are characterized in the time domain, allowing for the direct comparison of how changes in volume and pressure affect each other in these two, inter-related systems [65]. Taken together, they reflect both the stiffness and reserve capability of the cardiovascular system [65]. Hence, the evaluation of both E_a and E_{lv} allow for evaluation of how either determinant of cardiac output (the arterial system or the left ventricle) affects the other and impacts hemodynamic lability, cardiac output, post-myocardial infarction survival, and exercise tolerance [7, 69, 76].

Aging is accompanied by structural and physiological changes that lead to perturbations in cardiovascular function [4]. With aging, the left ventricle and arteries both become stiffer due to a decrease in the elastin:collagen ratio and loss of bioavailable nitric oxide (NO) [4]. Hence, both E_a and E_{lv} increase with aging [6, 75]. Both of these types of age-related changes are associated with chronic, low-grade inflammation that is now recognized as a hallmark of the aging process [40]. This tandem rise in E_a and E_{lv} is important, as the maintenance of the ratio of E_a/E_{lv} is imperative for maintaining ejection fraction and cardiac output without large increases in cardiac wall stress [105]. Individual characterization of both E_a and E_{lv} is

necessary as a concomitant rise in both E_a and E_{lv} may allow the ratio remain the same but lead to a loss of cardiovascular reserve [4].

Acute inflammation is accompanied by a series of cardiovascular responses, including: increased peripheral vasodilation, reduced endothelium-dependent dilation, and augmented arterial stiffness, leading to reductions in augmentation index and blood pressure [13, 100, 102]. The left ventricle is also affected, and systolic function is impaired [98]. Importantly, many comorbidities associated with aging are also associated with acute augmentation of inflammation, including: arthritic flare-ups, caregiver stress, vaccination, and bouts of illness [28, 106-108]. A transient but real increase in systemic inflammation in the face of augmented baseline levels of inflammation may impact resultant cardiovascular changes and has the potential to cause further or exponentially large perturbations in cardiovascular homeostasis. However, this has not been tested in humans to date, and most studies examining changes in ventricular function following acute inflammation utilize large, robust inflammatory stressors, such as sepsis or *E.coli* endotoxin in animal models [109, 110], or are cross-sectional evaluations of the response to sepsis in humans [111, 112] that are less clinically relevant than the smaller acute increases in inflammation associated with aging. Furthermore, all previous data using a prospective design in humans have been collected solely on arterial function and only in younger adults [13, 17].

Regular physical activity reduces inflammation in aging and preserves the compliance of the ventricle and arterial system [18, 22, 26, 113]. Evidence also exists for a dose-response relationship between physical activity volume and intensity and coronary heart disease (Tanasescu et al., 2002). Therefore, higher amounts of physical activity (volume and intensity) may provide protection against post-inflammation perturbations in cardiovascular performance,

particularly in older adults, who are already predisposed to augmented baseline levels of inflammation [9, 18].

Accordingly, our aim was to determine the response of Ea and Elv as surrogates for net heart and arterial function in older (OA) versus younger (YA) healthy adults at 24 and 48 hours following acute inflammation induced by an influenza vaccine. We hypothesized an increase in Ea and concomitant decrease in Elv that would lead to an increase in the coupling ratio (and reduction in ejection fraction) following induced inflammation. Furthermore, we hypothesized that older adults would experience a more significant disruption of cardiovascular homeostasis following the induction of inflammation compared to young adults, but that physical activity would be associated with reduced cardiovascular responses to inflammation, especially in OA.

Methods

Subjects. Sixty-two healthy volunteers between the age of 18-35 or 55–75 years participated in the study. Twenty-four older adults (m=8, f=16) and 38 younger adults (m=22, f=16) completed all study visits and are included in analysis. Subjects were recruited using flyers, email announcements, and word of mouth from the Champaign-Urbana and Chicago areas.

All subjects were free from diagnosed cardiovascular or respiratory disease, and only non-smokers were eligible for participation. Additional exclusion criteria included: diagnosed uncontrolled hypertension, stroke, or myocardial infarction within the 6 months prior to the study, diabetes mellitus, inflammatory diseases (Crohn's disease, rheumatoid arthritis and systemic lupus erythematosus), bleeding disorder, allergy to eggs, a history of adverse reaction to influenza vaccination, or use of medications known to affect inflammation (aspirin, steroids). Participants who had suffered from a common cold, influenza, bacterial or viral infection or upper respiratory tract infection 2 weeks before testing or had already received the influenza vaccination for the current season were also excluded. All subjects were recruited from the local community and provided written informed consent prior to participation. The study was approved by the Institutional Review Board of the University of Illinois at Urbana-Champaign and the University of Illinois at Chicago.

Study Design. All subjects completed baseline cardiovascular testing and a blood draw. Premenopausal women were tested in the early follicular phase (days 1-5) of menstrual cycle to control for the influence hormone fluctuations. Subjects were asked to report to the laboratory following an overnight (~12 hr) fast and did not consume caffeine, alcohol or exercise for 12 hours prior to testing. They rested in a supine position for 10 min prior to baseline testing. They then received a standard dose influenza vaccine (Fluvarix, Glaxo-Smith-Kline), which has been

shown to reliably increase systemic inflammation [17, 108, 114]. The study took place during 2 successive flu seasons, and the strains of virus used in the vaccine (2 A subtypes: H1N1, H3N2; 1B subtype) and vaccination dosage were the same in both seasons. All the measurements for every visit were performed between 05.00-11.00 hr to control for diurnal variation. At 24 and 48 hr following the vaccination, baseline testing and blood draw procedures were repeated under the same conditions. After the final testing session, subjects were given an accelerometer (ActiGraph 594, Ft. Walton Beach, FL) with instructions to begin wearing the device seven days post-vaccination and to wear the device continuously while awake for 1 week.

VO₂ Peak Test. Participants were asked to perform a maximal aerobic capacity test (VO₂peak) using a modified Balke Treadmill protocol. Speed was maintained at a comfortably brisk pace at ~70% of each participant's age-predicted maximum heart rate (HR). This speed remained constant throughout the test while the grade increased by 2% every 2 minutes. The starting grade on the treadmill was 0% for older adults and 10% for younger adults. Prior to the test, the subjects were fitted with head gear and a mouthpiece to collect expired gases. Open-circuit spirometry was used to estimate VO₂ peak (Parvo-Medics Inc., Sandy, UT) by sampling and analyzing expired air. Data were inspected using 30 sec averages, and the highest value achieved was considered VO₂ peak. VO₂ peak was defined as maximum effort after a minimum of two of the following criteria were met: (i) a rating of perceived exertion score of ≥ 17 on the Borg scale (scale 6–20), (ii) a respiratory exchange ratio > 1.1 , (iii) a plateau in HR with a change in workload, (iv) no change (increase of no > 150 ml) in oxygen uptake with an increase in workload, (v) volitional fatigue, defined as a desire of the subject to stop the test. All subjects achieved at least two of these criteria.

Brachial Blood Pressure. Resting systolic and diastolic BP (DBP) were measured in duplicate in the upper arm at heart level using an automated oscillometric cuff (HEM-907 XL; Omron, Shimane, Japan) after a 10 minute supine resting period in a quiet, dimly-lit room. If the two values of either systolic or diastolic pressure were not within 5 mm Hg of each other, another measurement was taken until 2 values within 5 mmHg of each other were obtained. Values within 5 mm Hg of each other were averaged and used for analyses.

Pulse contour analysis. Radial and carotid artery pressure waveforms were obtained in the supine position from a 10-s epoch using applanation tonometry (Millar Instruments, Houston, TX) and calibrated using the brachial systolic and diastolic BP [115]. Using a generalized validated transfer function [116], a central aortic pressure waveform was reconstructed from the radial artery pressure waveform (SphygmoCor; AtCor Medical, Sydney, Australia) in order to determine sub-endocardial viability ratio (SEVR) and end-systolic pressure (ESP). Aortic mean arterial pressure was determined from the integration of the reconstructed aortic pressure waveform using the SphygmoCor software. This technique has been validated and is reliable [116, 117]. In our lab, the day to day coefficient of variation is ~ 5% for these variables. An in-device quality rating of $\geq 80\%$ was required for all radial artery recordings used in analysis.

Cardiac Echocardiography. Cardiac output (CO), SV, EDV [10] and end systolic volume (ESV) were assessed at rest using two-dimensional echocardiography using an Aloka Alpha-7 system (Hitachi-Aloka, Tokyo, Japan). With subjects in the left lateral position, measurements were obtained using the four-chamber apical view. The interior endocardial border of the left ventricle was manually traced during both end systole and end diastole. Volumes were measured using Simpson's rule. Stroke volume was calculated by subtracting EDV from ESV. CO was calculated as HR multiplied by SV. EF was calculated from the ventricular volumes and

expressed as a percentage. Three beats were measured and the average of the 3 measurements was used in analysis. Values were indexed to body surface area (BSA) by dividing SV and ESV by BSA as determined by Mosteller's formula in order to make comparisons between groups despite differences in body size [118].

Tissue Doppler imaging in the 4-chamber apical view was used to determine S' (rate of systolic contraction) and E' (rate of diastolic relaxation) tissue velocities with the cursor placed on the lateral wall at the level of the mitral annulus. Standard pulsed wave Doppler velocity was used to determine early (E) and late (A) ventricular filling velocities at the tips of the open leaflets of the mitral valves. The average of three beats was used for analysis.

Left ventricular mass (LVM) and fractional shortening were measured using M-mode view of the left ventricle from the parasternal long axis with the reference cursor placed at the tips of the open mitral valves. Fractional shortening was expressed as a percentage using the formula: $(LVED - LVES)/LVED \times 100$. In order to account for differences in this measure attributable to loading conditions, stress-corrected fractional shortening was determined from the ratio of FS to SV[6]. LVM was derived using the Teicholz formula [119].

Three heartbeats were analyzed for each measure at each time point, and their average was used for analysis. All data were collected and analyzed by a single technician, and the coefficient of variability was ~10% for these measures.

Calculation of Ea/Elv. Arterial elastance was calculated using the equation $Ea = ESP/SV$ and ventricular elastance was calculated as $Elv = ESP/ESV$ [65]. The value of ESP used for these calculations was determined from applanation tonometry, as this method of obtaining ESP has been shown to be more reliable than using a calculation based solely upon brachial SBP [120].

Pulse Wave Velocity. PWV was measured using techniques described in detail elsewhere [121]. Distances from the suprasternal notch to the femoral artery and from the carotid artery to the suprasternal notch were measured as straight lines with a tape measure and recorded to the nearest mm. The distance from the carotid artery to the suprasternal notch was then subtracted from the distance between the suprasternal notch and femoral artery to account for differences in the direction of pulse wave propagation. Using a high-fidelity strain-gauge transducer (SphygmoCor; AtCor Medical), pressure waveforms were obtained first at the right common carotid artery and then at the right femoral artery for central PWV (cPWV). PWV was calculated from the distances between measurement points and the measured time delay between 10 proximal and distal waveforms. The peak of the R wave simultaneously recorded from the ECG was used as a timing marker.

Markers of Inflammation. After a 12 hr fast, venous blood samples were collected using a butterfly needle inserted into the antecubital vein. Samples were centrifuged at 4° C for 15 min at 1100g and were stored at -80° C until analyzed. Serum concentrations of C-reactive protein (CRP) and interleukin-6 (IL-6) were measured in duplicate to determine levels of systemic inflammation. High-sensitivity Quantikine enzyme-linked immunosorbent assay (ELISA) kits (R&D systems, Minneapolis, MN) were used to measure serum high-sensitivity IL-6 and CRP, respectively. The average inter-plate coefficient of variation was 8% for IL-6 and 7% for CRP blood analyses.

Accelerometry. Subjects are asked to wear a tri-axial accelerometer (Actigraph 594, Ft. Walton, FL) for 7 days, beginning one week after vaccination in order to control for any effect of inflammation upon voluntary physical activity. The average of all days with wear times over 10 hrs was used for analysis, with a minimum of 4 days being required to accurately determine

physical activity level [122]. This method has been validated for use [123, 124]. We used established criteria to determine total vector magnitude and percentages of total time spent in either sedentary/light or moderate-vigorous physical activity [125] .

Statistical Analysis. Normality of the variables of interest was assessed using Shapiro-Wilk tests. Non-normally distributed variables were log transformed before further analysis. A repeated measures, 2 x 3 (age x time point) ANOVA was performed to test for differences between time points and age in variables of interest. Post-hoc t-tests were performed only if initial analysis yielded significance. Correlational analyses (Pearson's or Spearman's, as appropriate) were performed to determine relationships between changes in inflammation and changes in cardiovascular function post -inflammation. In order to determine whether or not differences in body size played a role in our findings, we included statistics on indexed values where appropriate. Results are reported as mean \pm SEM. Significance was declared in all measures if $p < 0.05$. Statistical Software for the Social Sciences (SPSS, Chicago, IL) version 18.0 was used.

Results

Subject Characteristics. All demographic information is shown in Table I. OA had a lower VO_2 peak, total physical activity magnitude, and percentage and total amount of time spent in moderate-vigorous activity compared to YA, $p < 0.05$ for all. OA had higher blood pressure, age, body mass index, and time spent in sedentary-light activity compared to YA, $p < 0.05$ for all.

Medication types and frequencies of our subjects are shown in Table II.

Table I. SUBJECT CHARACTERISTICS (mean \pm SEM)

	Older Adults (OA)	Younger Adults (YA)
Age (yrs)	63 \pm 1 *	25 \pm 1
BSA (m ²)	2.06 \pm 0.05	2.00 \pm 0.05
BMI (kg/m ²)	29 \pm 1 *	24 \pm 1
VO ₂ peak (ml/kg/min)	22.8 \pm 1*	45.3 \pm 1
PA (vector magnitude)	457,960 \pm 26,672*	665,633 \pm 41,151
Time Spent in Sed-Light Activity (%)	96 \pm 0.5*	92 \pm 1
Time Spent in Mod-Vig Activity (%)	4 \pm 5*	8 \pm 6
Time Spent in Mod-Vig Activity (min)	36.64 \pm 4 *	66.94 \pm 5
SBP (mmHg)	120 \pm 3	115 \pm 2
DBP (mmHg)	71 \pm 2 *	65 \pm 1
LVMI (g/BSA)	92 \pm 4*	80 \pm 2
MAP (mmHg)	88 \pm 2	83 \pm 2

Table Legend. Age=age in yrs, BMI=body mass index, VO₂peak=maximal aerobic fitness score, PA=physical activity, Time spent in Sed-Light Activity=percentage of total time spent in sedentary, light, or lifestyle activity (METs<3), Time spent in Mod-Vig Activity=percentage of time spent in moderate, vigorous, or very vigorous physical activity (METs>3), Min Spent in Mod-Vig Activity=min per day spent in activity > METs, SBP=systolic blood pressure, DBP=diastolic blood pressure, LVMI=left ventricular mass indexed to body size, and MAP=mean arterial pressure, *denotes a significant difference from YA, p<0.05.

Table II. MEDICATION TYPES AND FREQUENCIES (n)

Medication Type	OA	YA
<i>Anti-hypertensive Medications</i>	13	0
β-blocker	4	0
Diuretic	8	0
Ca⁺⁺ channel blocker	1	0
<i>Other Medications</i>	19	7
HMG-CoA reductase inhibitor	7	0
Thyroid replacement	6	0
Hormone replacement	1	0
Anti-histamine	3	1
Oral Contraceptives	0	6
Antidepressants	1	0
Osteoporosis Drugs	1	0

Table Legend. Medications are divided by type. β-blocker=beta-adrenergic receptor blocker (anti-chronotropic agent), Diuretic=total blood volume reducing agent, Ca⁺⁺ channel blocker=calcium channel blocker (inotropic agent), Hormone replacement=endogenous female or male hormone replacement drug, Thyroid replacement=supplemental thyroid hormone, Anti-histamine=histamine blocker, HMG-CoA reductase inhibitor=statin therapy, oral contraceptives=any oral birth control agent, Antidepressant Drugs= anti-anxiety or anti-depressant pharmacotherapy, Osteoporosis Drugs=osteoclast inhibitors.

Arterial and Ventricular Elastances and the Coupling Ratio. There were significant overall time as well as interaction effects at 24-hr post-inflammation for ESP ($p=0.02$, $F=10.323$ for whole cohort reduction in ESP and $p=0.02$, $F=5.482$ for interaction effect).

The decrease in E_a was not significant ($p=0.09$, $F=2.969$), but E_{lv} was reduced ($p=0.02$, $F=5.700$) in the total cohort without a significant interaction effect ($p=0.188$, $F=1.773$ for interaction effect) at 24 hr post-inflammation. E_{lv} returned to baseline at 48 hr post-inflammation. The coupling ratio was unchanged, ($p=0.233$, $F=1.450$ for time and $p=0.186$, $F=1.792$ for interaction effects), Figures 4-6.

These findings persisted when indexed values were used: (time and interaction effect for E_{aI} were non-significant, from 2.5 ± 0.1 to 2.6 ± 0.1 to 2.6 ± 0.1 mmHg/ml/m² in YA and from 4.0 ± 0.2 to 3.8 ± 0.2 to 4.0 ± 0.3 mmHg/ml/m² in OA, $p>0.05$, and the time effect was significant for E_{lvI} , from 4.6 ± 0.2 to 4.5 ± 0.3 to 5.1 ± 0.4 mmHg/ml/m² in YA and from 4.9 ± 0.3 to 4.2 ± 0.3 to 4.4 ± 0.3 mmHg/ml/m² in OA, $p=0.03$, $F=4.86$), indicating that differences in body size did not affect elastance responses to induced inflammation in our study. At each time point in our study, OA had higher E_a , E_a/E_{lv} , E_{aI} , and E_{aI}/E_{lvI} ($p<0.05$ for between group differences) and the same E_{lv} and E_{lvI} values as YA ($p>0.05$ for between group difference).

Figure 4. Arterial elastance response to acute, induced inflammation.

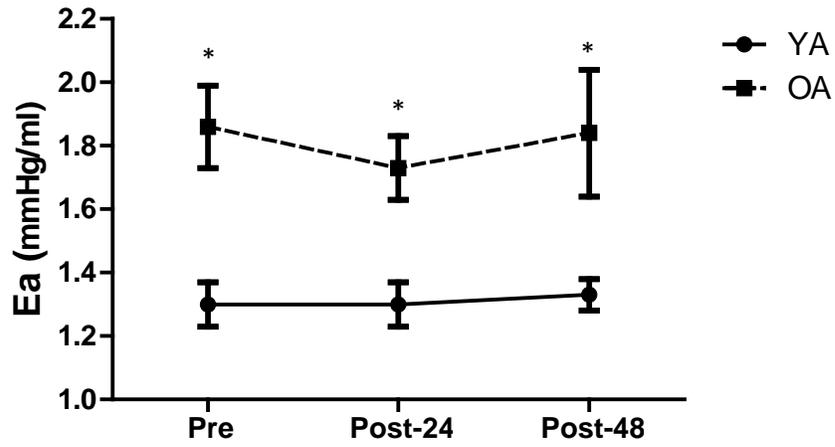


Figure 5. Ventricular elastance response to acute, induced inflammation

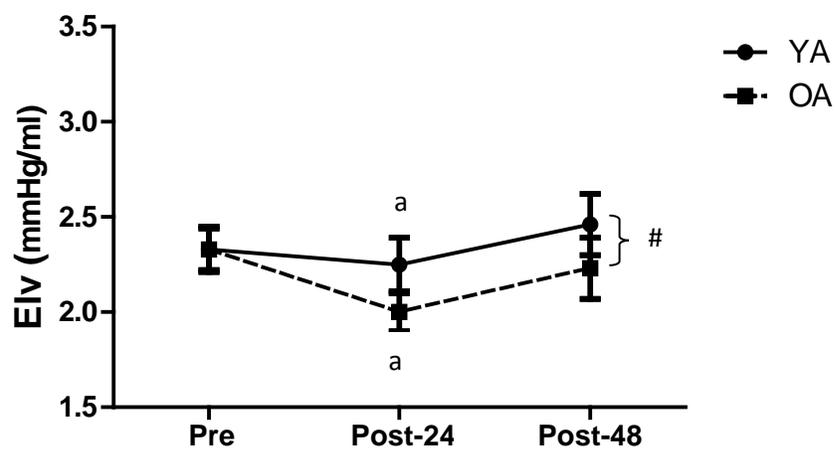


Figure 6. Coupling response to acute, induced inflammation

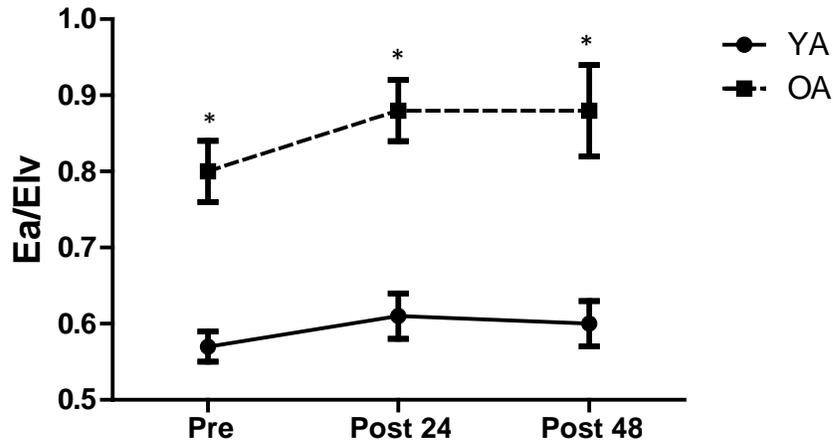


Figure 4-6 Legend. Elastance responses to acute, induced inflammation in OA and YA. * denotes a significant difference from YA at that time point, and ^a signifies a significant difference from baseline value in that age group, [#] indicates an overall time effect of vaccination, $p < 0.05$ for all. Although OA had higher Ea at each time point, there was no time or interaction effect for Ea. There were no between-group differences in Elv, but both OA and YA reduced Elv at 24 hr post-inflammation. OA had higher Ea/Elv at each time point, but there was no effect of inflammation on Ea/Elv.

Central Arterial Stiffness, Aortic Blood Pressure, and TPR. There was no change in cPWV, from 7.3 ± 1.8 to 7.9 ± 2.0 to 7.6 ± 1.3 m/s in OA, and from 5.5 ± 1.1 to 5.7 ± 0.8 to 5.7 ± 0.9 m/s in YA, ($p=0.315$, $F=1.030$ for time and $p=0.691$, $F=0.160$ for interaction effects). There was also no change in cPWV controlled for aortic MAP ($p=0.10$, $F=2.840$ for time and $p=0.578$, $F=0.313$ for interaction).

Aortic MAP (aMAP) was reduced with an interaction effect ($p=0.03$, $F=5.153$) following vaccination, ($p=0.006$, $F=7.959$ for time effect). Vaccination caused a reduction in aMAP ($p<0.01$, for difference between baseline versus 24 and 48 hr time points) in OA but not in YA ($p>0.32$, for difference between baseline and 24 and 48 hr time points), Table III. OA had higher cPWV, cPWV controlled for aMAP, and aMAP than YA at each time point, $p<0.05$.

TPR was reduced ($p=0.02$, $F=6.161$) without interaction ($p=0.22$, $F=1.514$), as was aortic diastolic blood pressure, (aDBP, $p=0.01$, $F=7.145$ for time and $p=0.23$, $F=1.459$ for interaction effect), Table III. OA had higher TPR at baseline and higher aDBP at each time point versus YA, $p<0.05$ for all.

Echocardiographic Variables, SEVR and Heart Rate. There was a significant increase in HR following vaccination ($p=0.03$, $F=4.898$ for time effect and $p=0.184$, $F=1.807$ for interaction). There was no interaction effect for SV ($p=0.359$, $F=0.855$), and although the change in SV reached significance ($p=0.04$, $F=4.569$ for time effect). SV indexed to body size approach a significant decline ($p=0.054$, $F=2.876$).

There were similar increases in CO ($p=0.004$, $F=8.838$) and COI ($p=0.01$, $F=6.472$) in OA and YA following vaccination without interaction effects ($p=0.26$, $F=1.304$ for interaction). There was no change in EF post-vaccination ($p=0.06$, $F=3.753$ for EF time effect following

vaccination), while the YA group exhibited higher EF than the OA group at each point ($p < 0.05$, Table III).

There was no significant change in ESV ($p = 0.13$, $F = 2.345$ for time and $p = 0.218$, $F = 1.551$ for interaction), or ESVI post-vaccination, ($p = 0.07$, $F = 3.392$ for time and $p = 0.46$, $F = 0.553$ for interaction effect). EDV was attenuated ($p = 0.012$, $F = 6.682$ for time $p = 0.255$, $F = 1.319$ for interaction), and EDVI was also significantly attenuated without interaction effect following vaccination in the total group, ($p = 0.008$, $F = 5.597$ for time and $p = 0.681$, $F = 0.171$ for interaction effect), Table III. ESV and ESVI were greater in OA than YA at all time points, while SV and SVI were lower in OA than YA at 48 hr post-vaccination, $p < 0.05$ for all, and there were no between group differences for baseline and post-24 hr SV and SVI or EDV and EDVI at any time point in OA compared to YA ($p > 0.05$ for all, Table III).

There was a significant time and interaction effect for SEVR (OA: 155 ± 23 , 153 ± 19 , $155 \pm 24\%$, and YA: 185 ± 58 , 164 ± 35 , $178 \pm 39\%$, ($p = 0.013$, $F = 6.478$ for time and $p = 0.048$, $F = 4.087$ for interaction) showing that SEVR was reduced in YA but not in OA at 24 hr post-vaccination. SEVR was higher in YA at baseline and at 48 hr post-vaccination ($p < 0.05$ for difference between age groups).

The E/A ratio was similarly reduced in YA and OA at 24 hr post vaccination ($p = 0.04$, $F = 4.467$ for time effect) without an interaction effect ($p = 0.23$, $F = 1.502$). E' velocity was also significantly reduced post-vaccination, ($p = 0.01$, $F = 7.234$) for time effect, without an interaction effect, ($p = 0.255$, $F = 1.325$). Both of these measures of diastolic function were attenuated in OA versus YA at each point in the study ($p < 0.05$ for all).

There were both time and interaction effects for the reduction in FS post-vaccination ($p=0.001$, $F=12.339$ for time and $p=0.03$, $F=5.077$ for interaction effect) showing that OA (but not YA) decreased FS at 24 hr post-vaccination. There was no significant change in tissue Doppler derived S' wave velocity in either group following vaccination ($p=0.13$, $F=2.385$ for time and $p=0.119$, $F=2.503$ for interaction effect). Stress corrected FS (FS/SV), was significantly reduced 24 hours post vaccination, ($p=0.05$, $F=4.077$ with a significant interaction effect ($p=0.03$, $F=4.819$, Table III).

Similar to the change in FS following vaccination, OA reduced FS/SV while YA maintained pre-vaccination values. At baseline and 48 hr post-vaccination, OA had reduced S' and OA had reduced FS and FS/SV at 24 hr post-vaccination compared to YA, $p<0.05$ for all, Table III.

Table III. CARDIAC PARAMETERS BEFORE AND AFTER VACCINATION (mean \pm SEM)

	OA pre	OA post-24	OA post-48	YA pre	YA post-24	YA post-48
EF (%)	55 \pm 1*	55 \pm 2*	55 \pm 4*	64 \pm 1	62 \pm 1	64 \pm 2
CO (L/min) [#]	3.45 \pm 0.3*	4.07 \pm 0.3	3.64 \pm 0.2	4.05 \pm 0.2	4.42 \pm 0.2	3.97 \pm 0.2
COI (L/min/BSA) [#]	1.8 \pm 0.1	2.0 \pm 0.2	1.8 \pm 0.1*	2.0 \pm 0.1	2.2 \pm 0.1	2.0 \pm 0.1
HR (bpm) [#]	62 \pm 2	63 \pm 1	63 \pm 2	59 \pm 2	62 \pm 2 ^a	60 \pm 2
SV (ml) [#]	64 \pm 3	62 \pm 3	58 \pm 4*	70 \pm 4	68 \pm 3	68 \pm 3
SVI (ml/BSA)	31 \pm 1*	31 \pm 2	28 \pm 1*	35 \pm 1	34 \pm 1	34 \pm 1
ESV (ml)	49 \pm 3*	52 \pm 2*	46 \pm 3*	39 \pm 2	40 \pm 1	38 \pm 2
ESVI (ml/BSA)	23 \pm 1*	25 \pm 1*	22 \pm 1*	20 \pm 1	20 \pm 1	19 \pm 1
EDV (ml) [#]	118 \pm 6	114 \pm 7	108 \pm 5	110 \pm 3	109 \pm 4	108 \pm 4
EDVI (ml/BSA) [#]	54 \pm 3	55 \pm 4	52 \pm 2 ^a	56 \pm 1	55 \pm 1	54 \pm 2
S' (cm/s)	8.2 \pm 0.2*	8.3 \pm 0.3	7.8 \pm 0.2*	9.1 \pm 0.2	9.2 \pm 0.3	9.0 \pm 0.2
FS (%) ^{# §}	26 \pm 1	22 \pm 1 ^{*a}	24 \pm 2	28 \pm 2	26 \pm 1	28 \pm 1
FS/SV (%/ml) ^{# §}	0.47 \pm 0.05	0.39 \pm 0.03 ^{*a}	0.42 \pm 0.04	0.43 \pm 0.02	0.42 \pm 0.3	0.41 \pm 0.02
ESP (mmHg) ^{# §}	103 \pm 3*	98 \pm 2 ^{*a}	98 \pm 2 ^{*a}	87 \pm 1	83 \pm 2 ^a	86 \pm 2
aMAP (mmHg) ^{# §}	90 \pm 2*	86 \pm 2 ^{*a}	87 \pm 2 ^{*a}	80 \pm 2	79 \pm 2	79 \pm 2
aDBP (mmHg) [#]	72 \pm 2*	69 \pm 2 ^{*a}	70 \pm 2 ^{*a}	65 \pm 1	64 \pm 1	64 \pm 1
TPR (MAP/CO) [#]	25 \pm 2*	23 \pm 2	24 \pm 2	21 \pm 2	20 \pm 2	21 \pm 2
E/A [#]	1.30 \pm 0.07*	1.18 \pm 0.05 ^{*a}	1.18 \pm 0.06 ^{*a}	2.09 \pm 0.09	1.98 \pm 0.06 ^a	2.09 \pm 0.08
E' (cm/s) [#]	10.0 \pm 0.4*	9.9 \pm 0.4*	9.7 \pm 0.3*	17.5 \pm 0.5	16.0 \pm 0.6 ^a	16.3 \pm 0.5 ^a

Table Legend. EF=ejection fraction, CO=cardiac output, COI=cardiac output indexed to body surface area, HR=heart rate, SV=stroke volume, SVI=stroke volume indexed to body surface area, ESV=end-systolic volume, ESVI=end-systolic volume indexed to body size, EDV=end-diastolic volume, EDVI=end-diastolic volume indexed to body size, S'=S wave velocity, and index of systolic contractility, FS=fractional shortening, FS/SV=fractional shortening indexed to stroke volume, ESP=end-systolic pressure, aMAP=aortic mean arterial pressure, aDBP=aortic diastolic blood pressure, TPR=total peripheral resistance, E/A=early-to-late diastolic filling ratio, E'=E wave velocity, and index of early diastolic relaxation. * denotes a significant difference from YA at that time point, ^a signifies a significant difference from baseline value in that age

group[#] indicates a significant time effect of inflammation, and [§] signifies a time by age group interaction, $p < 0.05$ for all.

Markers of Inflammation. OA had higher IL-6 and CRP than YA at baseline (1.2 ± 0.2 in YA versus 2.9 ± 0.5 pg/ml in OA for IL-6, $p=0.01$, $F=11.09$; and 1.13 ± 0.2 in YA and 2.78 ± 0.4 mg/L in OA, $p=0.03$, $F=5.084$ for difference in CRP between groups). IL-6 was increased in the entire cohort and peaked at 24 hr post vaccination. The increase in IL-6 was significant in YA but not OA at 24 hr, ($p < 0.001$ for both time ($F=53.26$) and interaction ($F=22.82$) effects, Figure 7) effects. There was no difference in IL-6 concentration between age groups at 24 hr post-vaccination, $p=0.167$, $t= -1.419$.

CRP was increased without interaction ($p=0.19$, $F=1.806$ for interaction effect) in the entire cohort at the 24 hr and 48 hr time point, ($p < 0.0001$, $F=29.005$ for overall time effect). YA and OA both significantly increased CRP at 24 hr and at 48 hr post-vaccination, Figure 8.

Because the study took place over the course of 2 flu seasons, we compared the inflammation responses between season 1 and season 2. We found no difference between IL-6 ($p=0.45$, $F=0.576$) or CRP ($p=0.16$, $F=2.03$) levels post-vaccination between seasons. This indicates that the inflammatory stimulus (influenza vaccine) evoked the same response in both seasons.

Figure 7. IL-6 responses to acute, induced inflammation.

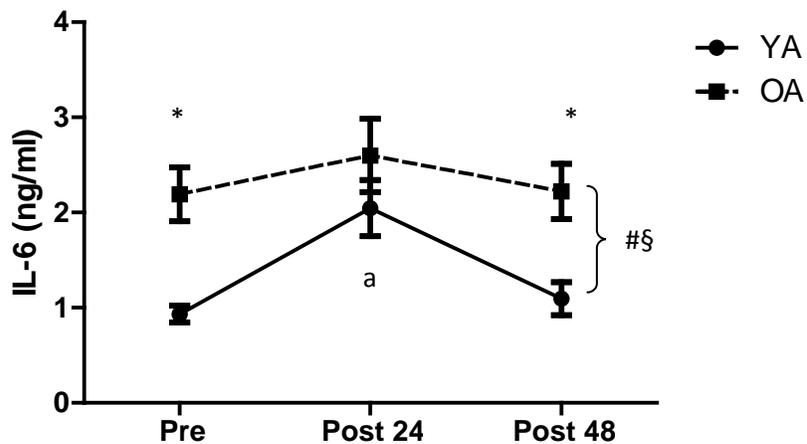


Figure 7 Legend. IL-6 increases after acute, induced inflammation in OA and YA. * denotes a significant difference from YA at that time point, and ^a signifies a significant difference from baseline value in that age group, # indicates an overall time effect of vaccination, § indicates an interaction effect, $p < 0.05$ for all. OA had higher IL-6 at each time point, but YA increased IL-6 at 24 hr post-inflammation and returned to baseline at 48 hr post-vaccination, whereas OA did not augment IL-6 following inflammation. At 24 hr post-vaccination, there was no difference in IL-6 concentration between groups.

Figure 8. CRP responses to acute, induced inflammation

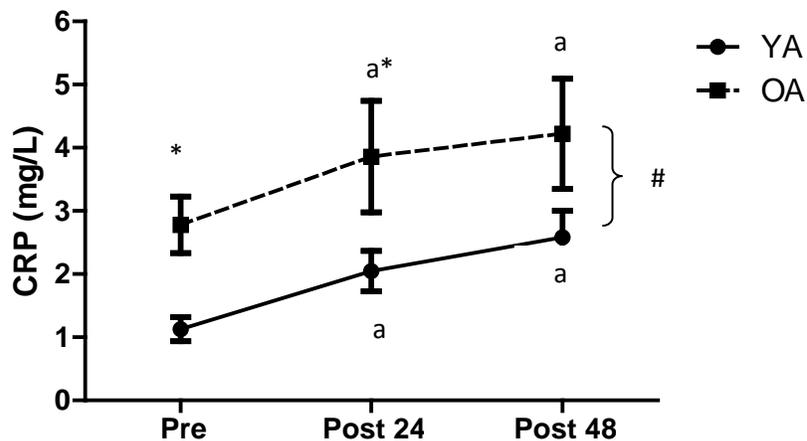


Figure 8 Legend. CRP increases after acute, induced inflammation in OA and YA. * denotes a significant difference from YA at that time point, and ^a signifies a significant difference from baseline value in that age group, # indicates an overall time effect of vaccination, $p < 0.05$ for all. OA had higher CRP at each time point, but YA increased CRP at 24 and 48 hr post-inflammation, whereas OA did not change CRP concentration following vaccination. At 48 hr post-vaccination, there was no difference in CRP concentration between OA and YA.

Correlations. We found an inverse relationship between time spent in higher-intensity physical activity (min spent in moderate-vigorous activity, $p=0.04$, $r= -0.289$) and change in Elv but no relationship between physical activity (volume or intensity) and change in concentration of inflammatory markers post-vaccination ($p>0.05$ for all).

In YA, but not in OA, the change in IL-6 was related to the change in PWV at 24 hr, ($p=0.04$, $r=0.351$). Physical activity volume ($p=0.01$, $r= -0.447$) and intensity/percent of time in moderate-vigorous activity ($p=0.03$, $r=0.379$) were related to the change in PWV at 24 hr.

Discussion

The major finding of this study was that both YA and OA have similar composite (Ea and Elv) cardiovascular responses to acute, induced inflammation. These changes in cardiovascular function include a reduction in left ventricular contractility, SV and ESP and an increase in HR and CO. Taken together, these reciprocal changes appear to be targeted to maintain blood delivery and the maintenance of the coupling ratio. However, the augmentation of central factors (CO) was unable to overcome the hypotensive responses to inflammation caused by changes in the periphery (as evidenced by the reduction in TPR). As a result, post-inflammation blood pressure was attenuated. This mismatch between central and peripheral adaptations to acute, induced inflammation have been shown to be ubiquitous in cases of extreme systemic inflammation, i.e., sepsis, [111] but were also evident in our physiologically and clinically relevant model of mild induced inflammation in both OA and YA.

The seemingly paradoxical tandem decrease in both peripheral resistance and cardiac function is interesting and related to inflammation. Traditionally, attenuation of TPR is thought to enhance left ventricular ejection, and less resistance to ejection should therefore yield an increase in SV and EF [126]. However, arachadonic acid products formed via the cyclooxygenase and lipoxygenase pathways in response to inflammation act on group IV afferents to increase arterial blood pressure by stimulating the sympathetic nervous system [127]. Stimulation of these nerve fibers also causes substance P, a potent vasodilator and sympathetic activator, to be released. The sympathetic activation leads not only to vasodilation, but also to a rise in HR. Hence, the rise in HR we observed may have preserved some indices of contractility (S' wave velocity) by influencing chronotropic properties of the myocardium [128].

Indeed, in early stages of induced inflammation, catecholamine release may have inotropic effects and preserve cardiac contractility. However, the increase in cytokine (TNF- α , IL-2 and IL-6) concentration following inflammation results in the formation of inducible nitric oxide synthase (iNOS) 4-6 hours post-inflammatory stressor [129]. Cytokine-dependent iNOS formation exacerbates peripheral vasodilation and ultimately has negative inotropic effects on the heart [129]. Along these lines, systemic levels of IL-6 and CRP have been correlated to LV dysfunction in large, cross-sectional studies [10, 25]. Consistent with these findings our OA group had higher baseline levels of systemic IL-6 and CRP coupled with lower EF compared to YA. Importantly, our data provide evidence that, in healthy, older adults, a “basement” has not been reached for the effect of inflammation on cardiac function (considering the higher baseline levels of inflammation in OA), since acute increases in inflammation had an additive, detrimental effect on ventricular performance. This may have important implications when treating patients for conditions characterized by acute, systemic increases in inflammation.

The vaccination induced a reduction in Elv. The reduction in FS also provides further support for an inflammation induced reduction in ventricular performance. Intriguingly, other measures of systolic function (EF, S' wave velocity) were not changed following inflammation. It is important to note that most clinical measures of ventricular function are dependent on loading conditions, such as EDV as well as HR. Others also report that controlling for stroke volume is crucial when evaluating contractility, as loading conditions may mask actual inotropic capability [78]. Our findings similarly highlight the necessity of interpreting contractility in light of loading conditions: when we analyzed a load-corrected value of FS we still found a decrease in OA but not YA at 24 hr post-inflammation, indicating that the contractile function of the older heart following inflammation was compromised. However, other indicators of load dependent

ventricular systolic function such as EF and S' velocity were not altered by inflammation. Since the greatest change in load corrected FS occurred 24 hrs after vaccination, while there were no changes in ventricular volumes at that time point, it is unlikely that volume loading per se significantly impacted the reduction in FS and SV. Considering the reduction in TPR and the fact that ventricular vascular coupling did not change (the reduction in Elv was coupled with a similar but non-significant reduction in Ea) it is unlikely that afterload per se substantially impacted the reduction in FS and SV. This is further substantiated by the reduction in ESP. Thus other factors, perhaps a failure of the Starling mechanism [3], or reduced contractility caused by arachadonic acid products or iNOS production [129-131] may have caused the reduction in FS and SV.

Prior work that examined ventricular elastance responses to acute inflammation only investigated time points up to 4 or 20 hr following induction of inflammation and found that Ea and Elv were unaffected at that time [110, 132]. The difference in timing is a crucial distinction because studies using endotoxin to induce inflammation in animals have shown a delay of up to 24 hours before cardiac dysfunction develops [133], and iNOS production does not occur until 4-6 hr post-inflammation. Thus, the timing of the measurements may explain the divergent findings.

Interestingly, although OA exhibited higher baseline Ea, reflecting augmentation of overall arterial load and resistance to ventricular ejection, baseline Elv was not different between groups. This is an interesting finding that underscores the importance of the manner in which Elv is interpreted. Elv can reflect both ventricular stiffness and ventricular contractility [4, 69]. In order to correctly ascertain the significance of this variable, other factors should be considered. For example, in our cohort, OA had significantly higher Ea than YA, leading to an

augmentation of E_a/E_{lv} and a reduction in EF in OA compared to YA. Baseline blood pressures, cPWV, and ESP were also higher in OA. In light of this information, we believe that the similarity in baseline E_{lv} between groups reflects: i) contractile ability in YA and ii) contractile ability and ventricular stiffness in OA. This argument is strengthened by the main group differences in EF as well as LV mass, again indicating enhanced contractility in YA versus stiffness in OA. In addition, both E_a and E_{lv} increase with aging, although it is possible to maintain a reasonably stable E_a/E_{lv} ratio [75]. Nevertheless, our OA group exhibited a greater E_a/E_{lv} ratio than our YA group, even though the ratio was still in the optimal range [6, 65, 75]. This probably suggests that our OA group is close to maximizing stroke work at the expense of energetic efficiency. This becomes problematic when oxygen demands increase, such as during exercise, and reduction of reserve capacity may increase the risk of coronary event or reduce exercise tolerance [76].

The diastolic filling ratio (E/A) and ventricular diastolic velocity (E') were lower at baseline in the OA, but both OA and YA showed similar attenuation in diastolic function at 24 hr following inflammation. Additionally, the SEVR was also reduced in YA, suggesting that myocardial perfusion may be compromised in this group, however, this effect is most likely due to the increased HR in YA as they maintained aortic diastolic blood pressure. However, OA reduced aortic diastolic blood pressure following vaccination. Concomitant drops in diastolic filling and systolic pressures are especially concerning in a stiffer arterial system, as there is a greater reliance on systolic pressure to aid in myocardial perfusion [134]. Therefore, the drop in ESP and concomitant reductions in SV following inflammation may reduce coronary perfusion in OA and exacerbate the severity or size of previously existing ischemic beds, increasing the risk of ischemic events following acute inflammation [75]. Hence, during an acute inflammatory

insult there may be an augmentation of risk of major cardiac event in OA, who already demonstrate increased reliance upon systolic pressure for coronary oxygenation [4, 76].

Along these lines, diastolic performance itself has been linked to arterial stiffening [135] and vascular dysfunction [99]. In fact, this relationship is one of the proposed mechanisms by which HEFpEF develops, particularly in older women [136]. Abnormal ventricular-vascular interaction also drives the progression towards HEFpEF [137] and is influenced by inflammation. Collectively, these and our data provide additional support for the management of inflammation as a potential strategy to combat this pathology.

Correlates of Changes in Cardiovascular Function in OA and YA.

Interestingly, we found that change in inflammatory markers was related to change in arterial function in YA, and that physical activity intensity and volume were both correlated to the change in PWV. More active individuals may have a lesser change in PWV post-vaccination. These relationships did not exist in OA, but baseline levels of inflammation and arterial stiffness were higher in OA compared to YA. This may suggest that the additional baseline inflammation is sufficient to obscure these relationships during an inflammatory insult.

We found an inverse relationship between physical activity (moderate-vigorous intensity) and change in Elv. Thus, physical activity may have a protective effect following acute inflammation. This finding underscores the importance of engaging in higher-intensity physical activity, since total physical activity (vector magnitude) was not related to the change in Elv. Thus, physical activity guidelines for OA should emphasize the completion of more intense activity as a preventive measure should an illness or other episode characterized by an acute increase in inflammation occur.

Limitations.

This study utilized exclusively non-invasive measurements, and is thereby limited by error associated with the formulas used to estimate pressures and volumes [116, 119, 138]. However, all of the techniques we used have been validated using invasive measurements, and our method of quantifying ventricular volumes is the current clinical standard [116, 138]. We also required in-device quality ratings of >80% for applanation-tonometry derived ESP measurements and utilized a single technician to collect and analyze all of our images in order to minimize any error associated with the non-invasive nature of our study. Our CV values for ventricular volumes (~10%) also indicated that our measurements were reliable.

We included subjects who were taking anti-hypertensive medications and using HMG-CoA reductase inhibitors, which are known to reduce systemic inflammation. We believe these medications minimally impacted our findings, as we reported a robust decrease in blood pressure and increase in inflammation in OA despite medication use. We also only tested the elastance responses to acute, induced inflammation at rest. The use of exercise as an additional stressor may yield further insight, particularly regarding myocardial sensitivity of OA and YA to an additional catecholamine bolus post-vaccination.

Conclusions.

OA and YA had similar ventricular-arterial coupling responses to acute, induced inflammation. However, the individual facets of heart and artery function driving these total responses may differ in OA compared in YA. These divergent responses may contribute to the susceptibility of a major cardiac event during an acute inflammatory insult and highlight the role of chronic, low-grade inflammation in perturbing cardiovascular performance after an acute

stressor. Physical activity intensity helped to offset the decrements in Elv that occurred following acute inflammation in both OA and YA.

Chapter IV: Effect of Aging and Physical Activity on Blood Pressure and Endothelial Reactivity Following Acute, Induced Inflammation

Introduction

The aging process is characterized by a state of chronic, low-grade inflammation [41, 87, 139]. This inflammation leads to an increase in reactive oxygen species and reduction in nitric oxide, as well as structural changes including loss of elastin and increased collagen in the medial arterial wall [40]. Thus, aging results in stiffer arteries with impaired endothelial reactivity [1, 75]. Taken together, this indicates that the stiffer arterial system and attenuated endothelial function in aging are due, at least in part, to heightened oxidative stress and decreased bioavailable nitric oxide (NO) in older versus younger adults [2, 20, 27]. Consequently, aging is also associated with a reduction in exercise tolerance and increased overall and cardiovascular morbidity and mortality [69, 76].

Acute but transient increases in inflammation, common during acute bouts of illness, or in response to acute stress, also impact arterial function [16, 17, 28]. Specifically, acute inflammation leads to concomitant augmentation of arterial stiffness and decline in endothelial function via augmentation of oxidative stress and reduction in nitric oxide in adults younger than 40 years of age [13, 16, 17, 102]. This phenomenon has been proposed as a mechanism by which acute inflammation leads to cardiac events [13, 15, 140]. Considering older adults have heightened baseline levels of inflammation, oxidative stress and additional co-morbidities associated with inflammation such as arthritis [29], the connection between inflammation and cardiovascular function is especially relevant yet unexplored in this population.

Being physically active reduces the risk for overall and cardiovascular morbidity and mortality in older adults [23, 68, 141]. Additionally, physical activity is associated with attenuation of chronic, low-grade systemic inflammation in older adults, with evidence of a dose-response relationship [23, 142, 143]. Furthermore, physically active older adults preserve arterial compliance and reduce oxidative stress compared to sedentary older adults [26, 55].

To date, the influence of aging on the vascular response to acute (versus chronic) inflammation is unknown. However, acute, induced inflammation induces a milieu of cardiovascular alterations in younger adults, including impaired flow-mediated dilation (FMD) and augmented large artery stiffness [16, 17, 144]. Additionally, highly fit older adults seem to be protected against some of the perturbations in vascular function that are linked to acute inflammation [144].

Thus, the purpose of our study was to determine how acute, induced inflammation affected blood pressure, arterial stiffness and endothelial function in older versus younger adults. We hypothesized that older adults would experience a greater increase in arterial stiffness and decrease in endothelial function compared to younger adults. We also tested the hypothesis that more physical activity (both intensity and volume) would be inversely associated with inflammation-induced changes in cardiovascular function in older and younger adults.

Methods

Subjects. Fifty-three volunteers between the age of 18-35 or 55–75 years completed the study. Twenty-two older adults (m=8, f=14) and 31 younger adults (m=18, f=13) were included in analysis. Recruitment methods consisted of flyers, email announcements, and word-of-mouth in the Champaign-Urbana and Chicago areas.

All participants were free from diagnosed cardiovascular or respiratory disease and were non-smokers. Additional exclusion criteria included: diagnosed uncontrolled hypertension, stroke, or myocardial infarction within the 6 months prior to the study, diabetes mellitus, inflammatory diseases (Crohn's disease, rheumatoid arthritis and systemic lupus erythematosus), bleeding disorder, or use of steroidal medications known to dampen inflammation. Participants who had suffered from common cold or influenza and bacterial or viral infection or upper respiratory tract infection 2 weeks before testing, were allergic to eggs, had a prior adverse response to influenza vaccination, or had already received the influenza vaccination for the current season were also excluded. All participants were recruited from the local community and provided written informed consent prior to participation. The study was approved by the Institutional Review Board of the University of Illinois at Urbana-Champaign and the University of Illinois at Chicago.

Study Design. All participants completed a baseline cardiovascular testing and a fasting blood draw. Premenopausal women were tested during the first five days of menstrual cycle to control for the influence of hormonal variation. They then received an influenza vaccine for 2 A strains (H1N1 and H3N2) and 1 B strain of influenza virus (Fluvarix, Glaxo-Smith-Kline), which has been shown to reliably increase systemic inflammation [17, 114]. The study occurred during two successive flu seasons, and the vaccination dosage and virus strains were the same in both

seasons. Participants did not consume caffeine, alcohol or exercise for 12 hours prior to testing, and participants were asked to fast for at least 10 hours prior to their visit. All the measurements for every visit were performed between 05.00 to 11.00 hr and at the same time for each participant to control for circadian variation in these measurements. At 24 and 48 hr following the vaccination, baseline testing and blood draw procedures were repeated under the same conditions and at the same time as the previous testing session. After the third cardiovascular testing session, subjects were given an accelerometer (ActiGraph 594) and asked to begin wearing the device at 7 days post-vaccination and to wear the device continuously (while awake) for 1 week.

Anthropometrics. Standing height and weight measurements were obtained with participants wearing light-weight clothing with a stadiometer and digital scale.

Brachial artery BP assessment. Resting systolic BP (SBP) and diastolic BP (DBP) were measured at the brachial artery in a quiet, dimly-lit room using an automated oscillometric cuff (HEM-907 XL; Omron, Shimane, Japan). Measures were obtained in duplicate. If the two values were not within 5 mm Hg, another measurement was taken until 2 values within 5 mmHg of each other were obtained, and these values were averaged and used in analysis.

Inflammatory Markers. Following an overnight fast of ~12 hours, blood samples were collected with a butterfly needle inserted into the antecubital vein into a 10 mL tube. Samples were separated with centrifugation at 4° C for 15 min at 1100g and stored at -80° C until analyzed. Serum concentrations of C-reactive protein (CRP) and interleukin-6 (IL-6) were measured in duplicate to ascertain levels of systemic inflammation. High-sensitivity Quantikine enzyme-linked immunosorbent assay (ELISA) kits (R&D systems, Minneapolis, MN) were used to

measure serum IL-6 and CRP, respectively. Our intra-plate coefficients of variation were 8% for hsIL-6 and 7% for hsCRP.

Intima-Media Thickness. The carotid artery intima-media thickness (IMT) was imaged via ultrasonography with a 7.5 MHz linear array probe (Aloka α -7, Tokyo, Japan). The intima-medial space was defined as the distance between the leading edge of the lumen-intima border to the leading edge of the medial-adventitial border and recorded in millimeters. This measurement was obtained approximately 20 mm distal to the carotid bifurcation during diastole.

Pulse contour analysis. Radial artery pressure waveforms were obtained in the supine position with the right arm at heart level from a 10-s epoch using applanation tonometry (Millar Instruments, Houston, TX), calibrated using brachial mean and diastolic BP [115]. A central aortic pressure waveform was reconstructed from the radial artery pressure waveform (SphygmoCor; AtCor Medical, Sydney, Australia) using a generalized validated transfer function [116] in order to obtain central BP and augmentation index (AIx). Aortic mean arterial pressure was determined from the integration of the reconstructed aortic pressure waveform using the SphygmoCor software, and has been validated and is reliable for use [116, 117]. Heart rate was also obtained from this device. An in-device quality rating of $\geq 80\%$ was required for recordings to be used in analysis.

Brachial Flow-mediated Dilation. Brachial artery vasodilator function was measured through assessment of brachial artery dilatation using ultrasonography (Aloka, α -7, Japan). The brachial artery was imaged in longitudinal section, 5–10 cm proximal to placement of a blood pressure cuff, which was placed just below the antecubital fossa, using a high frequency (5–13 MHz) linear array probe. Flow was measured with the cursor in the center of the artery and sample

volume adjusted for arterial lumen diameter at a constant insonation angle of 55° for each subject. FMD was measured at a rate of 5 frames/s for 60 s at baseline (pre-inflation) and again for 3 min following ischemic stimulus (inflation of a blood pressure cuff around the forearm to 250 mmHg for 5 min).

Analysis of the FMD was carried out using a semi-automated edge detection software system, and vertical and horizontal calibration of the ultrasound settings were performed for each participant. Next, a region of interest (ROI) box was defined upon the portion of the artery where the wall was most clearly defined. Arterial wall diameter was found using the automated software, and images were edited if the software was unable to correctly discern the location of the intima-lumen interface. A single technician collected and analyzed all FMD data, and the intra-observer CV was 11%.

Arterial dilator response was then calculated as percentage change in brachial artery diameter from baseline and controlled for the shear stimulus created by the hyperemic flow. Mean blood velocity (V_m) was similarly measured using the same ROI box to find the velocity-time interval in each frame using automated software. This method conforms to the guidelines set out for the ultrasound measurement of endothelium-dependent FMD of the brachial artery [104].

Brachial Shear Stress. In order to account for the effect of differences in shear stress (SS) on brachial artery dilation, SS was calculated for each frame in the recording until peak diameter was reached with the formula:

$$SS=8*V_m/D$$

V_m is the average flow velocity at that frame, D is the arterial diameter and 8 is a constant. The area under the curve for these values was then calculated for each subject, and we adjusted for

this measure in our statistical analysis and report both FMD and FMD controlled for SS in our results.

Arterial Compliance and Beta-Stiffness Index. Carotid artery diameter was measured using ultrasonography (Aloka, α -7, Japan). The cephalic portion of carotid artery was imaged 1-2 cm proximal to the bifurcation in a longitudinal section, with a high frequency (7.5-13 MHz) linear array probe. Simultaneous blood pressure of the carotid artery was determined the using applantion tonometry (SphygmoCor, AtCor Medical, Sydney, Australia). Image analysis and calculation of arterial compliance (AC) was performed using automated wall detection echo-tracking software system. AC was calculated as previously described by our lab group [145, 146].

β -stiffness index (β) was calculated in order to control arterial compliance for changes in blood pressure using methods described elsewhere [145, 146].

Accelerometry. Participants wore an accelerometer (Actigraph, Fort Walton Beach, FL) on the non-dominant hip for 1 week. Participants were instructed to begin wearing the device as soon as they awoke and to wear it throughout all of their daily activities except swimming and showering. Total displacement of the device (recorded as total vector magnitude) and amount of time in sedentary, light, and moderate to vigorous physical activities were recorded and used for analysis. The ability of the device to obtain these values has been validated, and 4-5 days of accelerometer data provide an accurate representation of physical and sedentary activity in both young and older adults [122, 124, 125, 147].

Statistical Analysis. Normality of the variables of interest was assessed using Shapiro-Wilk tests. Non-normally distributed data were log transformed prior to further analysis. A repeated measures, 2 x 3 (age group x time point) ANOVA was performed to test for differences between time points and age in normally distributed variables of interest. Post-hoc t - tests were conducted if initial ANOVA yielded significance. Pearson's or Spearman's correlations were performed as appropriate to determine relationships between variables of interest. An analysis of co-variance (ANCOVA) was performed when evaluating FMD adjusted for SS. Linear regression was performed to assess the contribution of physical activity to change in FMD. Results are reported as mean \pm SEM. Significance was declared in all measures if $p < 0.05$. Statistical Software for the Social Sciences (SPSS, Chicago, IL) version 18.0 was used.

Results

Subjects. The older adult group consisted of 14 women and 8 men, mean BMI=29±1 kg/m², and average age=62±1 yrs. The younger adult group contained 13 women and 18 men, mean BMI=24±1 kg/m² and mean age=25±1 yrs. Mean IMT was 0.66±0.2 in OA and 0.40±0.1 in YA. Age, BMI, and IMT were all significantly different between groups, p<0.05 for all.

Inflammatory Markers. OA had higher IL-6 and CRP than YA at baseline; 0.93±0.09 in YA versus 2.09±0.28 ng/ml in OA for IL-6, (p=0.01, F=11.09 for between group difference in IL-6) and 1.16 ± 0.2 in YA and 3.47±0.96 mg/L in OA, (p=0.03, F=5.084 for between group difference in CRP). There was a time and interaction effect for IL-6 at 24 hr post inflammation; YA increased IL-6 and OA did not, (p<0.001, F=22.82 for interaction effect). CRP was increased in both groups at the 24 hr and 48 hr time point without an interaction effect, (p=0.04, F=9.005 for time effect). The changes in inflammation are expressed as a percentage of baseline levels in OA and YA, Figure 9 and Figure 10.

The study took place over the course of 2 flu seasons, so we compared the responses to inflammation between season 1 and season 2 and found them to be the same: (p=0.45, F=0.576 for IL-6 and p=0.16, F=2.03 for CRP) for the interaction post-vaccination between seasons. This indicates that the influenza vaccine evoked the same response in both seasons.

Figure 9. Percent change in IL-6 at 24 and 48 hr post-vaccination in OA and YA.

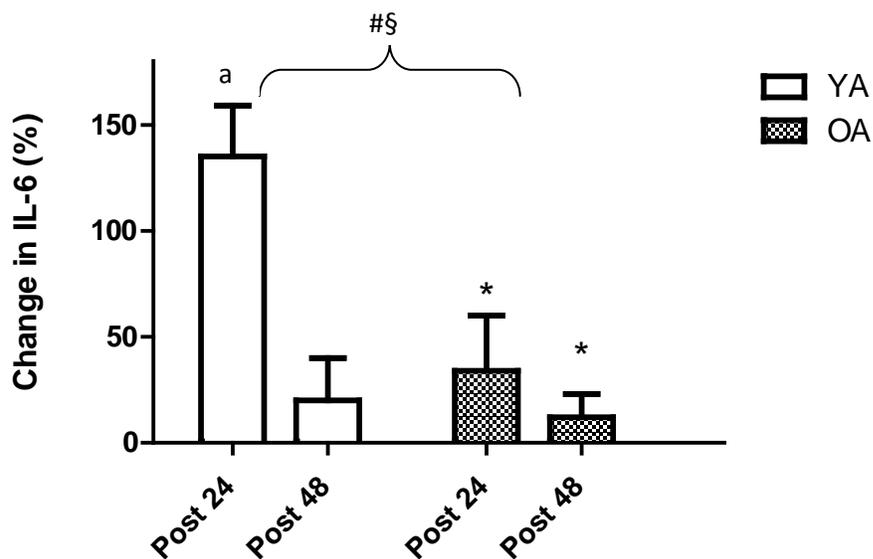


Figure 9 Legend. YA, but not OA, increase serum IL-6 concentration at 24 hr post-vaccination, ^a indicates a significant difference from baseline value in that age group, * indicates a significant difference from the YA value at that time point, # indicates an overall time effect of vaccination, § denotes a significant interaction effect, $p < 0.05$ for all.

Figure 10. Percent change in CRP at 24 and 48 hr post-vaccination in OA and YA.

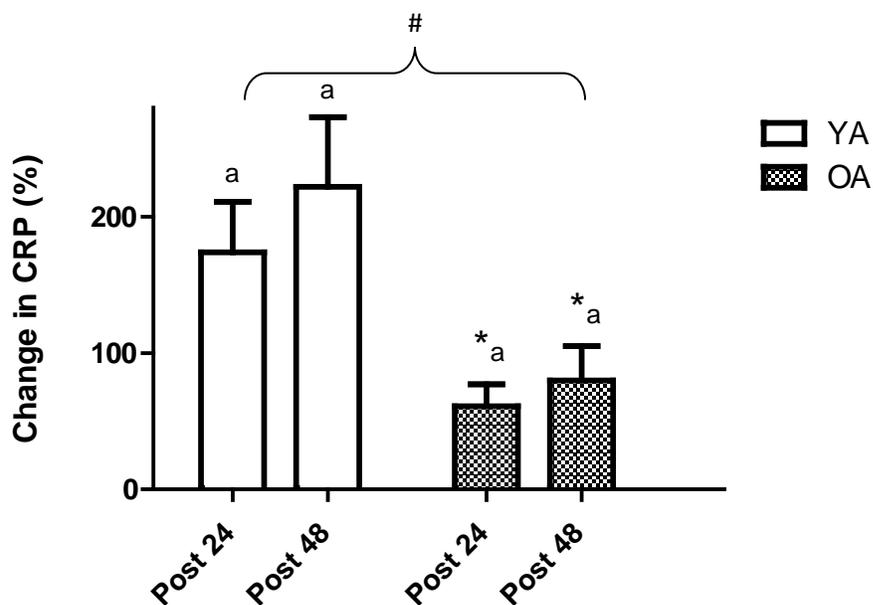


Figure 10 Legend. YA and OA increase serum CRP concentrations at 24 hr and 48 hr post-vaccination without interaction effect, ^a indicates a significant difference from baseline value in that age group, * indicates a significant difference from the YA value at that time point, [#] indicates an overall time effect of vaccination, $p < 0.05$ for all.

Peripheral Blood Pressure, Augmentation Index, and Heart Rate. OA had higher AIx, AIx@75, DBP and MAP than YA at baseline ($p < 0.05$). Older adults reduced SBP and PP post-vaccination while younger adults did not change SBP but increased PP after vaccination. Both groups reduced MAP and DBP following vaccination, Table IV.

There was a significant interaction effect for SBP ($p = 0.008$, $F = 7.652$ for interaction effect), Table IV. We also found a significant interaction effect for pulse pressure (PP, $p = 0.02$, $F = 5.426$ for interaction effect), Table IV.

There was a significant time but no significant interaction effect for the reduction in MAP ($p = 0.003$, $F = 9.559$ for time effect and $p = 0.08$, $F = 3.093$ for interaction effect, Figure 11), and DBP ($p = 0.001$, $F = 12.632$ for time and $p = 0.57$, $F = 0.325$ for interaction effect).

There was a significant time effect for AIx, ($p = 0.002$, $F = 10.4176$ for time and $p = 0.08$, $F = 3.181$ for interaction effect), Figure 12. We also found a significant decrease in AIx controlled for heart rate, AIx@75, but no interaction effect ($p = 0.007$, $F = 7.877$ for time and $p = 0.27$, $F = 1.258$ for interaction effect), Table V.

There was a time effect for the increase in HR following vaccination ($p = 0.03$, $F = 5.335$ for time effect and $p = 0.104$, $F = 2.757$ for interaction), from 59 ± 2 to 64 ± 2 to 62 ± 2 beats per minute in YA and from 62 ± 2 to 63 ± 1 to 63 ± 2 beats per minute in OA.

Table IV. BLOOD PRESSURE RESPONSES TO VACCINATION IN OA AND YA (mean \pm SEM)

	OA Baseline	OA Post 24 hr	OA Post 48 hr	YA Baseline	YA Post 24 hr	YA Post 48 hr
SBP (mmHg) §	120 \pm 3	115 \pm 2 ^a	115 \pm 3 ^a	115 \pm 3	116 \pm 2	115 \pm 3
DBP (mmHg) [#]	71 \pm 2*	68 \pm 2* ^a	70 \pm 2* ^a	65 \pm 1	62 \pm 1	64 \pm 1
MAP (mmHg) [#]	87 \pm 2*	80 \pm 4 ^a	82 \pm 4 ^a	82 \pm 3	80 \pm 2	82 \pm 2
PP (mmHg) §	49 \pm 2	49 \pm 2	43 \pm 2* ^a	49 \pm 2	53 \pm 2 ^a	52 \pm 2

Table Legend. Peripheral and central blood pressures post-vaccination in YA and OA, SBP=brachial systolic blood pressure, DBP=brachial diastolic blood pressure, MAP=brachial mean arterial pressure, PP=brachial pulse pressure, * indicates a significant difference from the YA value at that time point, ^a indicates a significant difference from baseline value in that age group, # signifies a significant over group time effect, § denotes a significant interaction effect, p<0.05 for all.

Figure 11. Change in MAP post-vaccination in OA and YA.

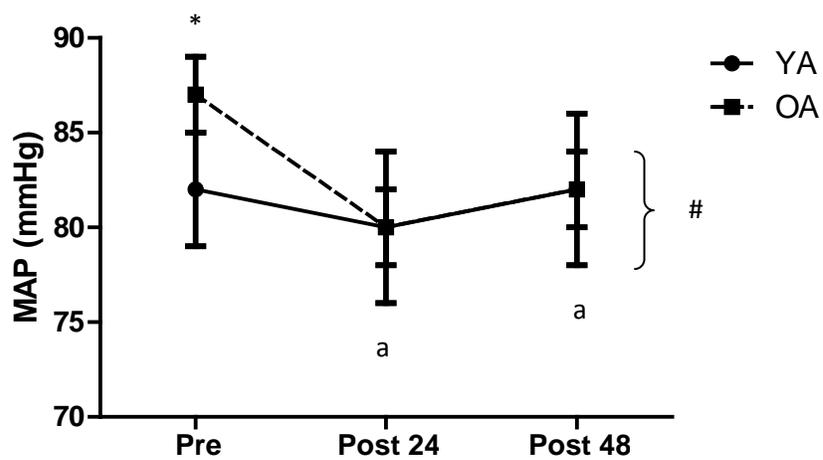


Figure 11 Legend. OA, but not YA, reduced mean arterial pressure post-vaccination, * denotes a significant difference from the younger group, ^a indicates a significant difference from baseline value in that age group, # indicates an overall time effect of vaccination, $p < 0.05$ for all.

Figure 12. Change in post-vaccination augmentation index in OA and YA.

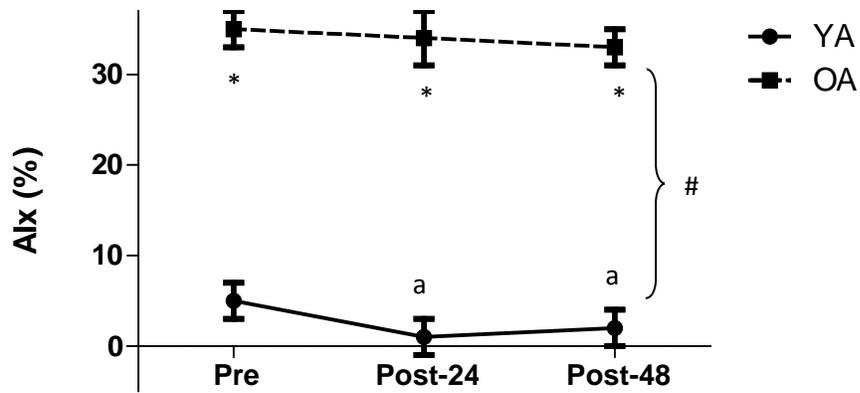


Figure 12 Legend. YA, but not OA, reduce AIx post-vaccination, ^a indicates a significant difference from baseline value in that age group, * indicates a significant difference from the YA value at that time point, # indicates an overall time effect of vaccination, § denotes a significant interaction effect, $p < 0.05$ for all.

Central Arterial Stiffness. There was a decrease in β -stiffness ($p=0.03$, $F=5.116$ for time and $p=0.10$, $F=2.877$ for interaction effect) and no change in arterial compliance ($p=0.11$, $F=2.745$ for time and $p=0.13$, $F=2.344$ for interaction effect) following vaccination, Table V. OA had higher β -stiffness at all time points and lower AC at baseline and post 48 hr compared to YA, $p<0.05$ for all.

Brachial Flow-mediated Dilation and Arterial Diameters. We found a significant interaction ($p=0.04$, $F=4.262$) effect for brachial FMD. There was a reduction in brachial endothelial reactivity in YA ($p<0.003$, for difference between baseline and 24 and 48 hr time points) but not OA ($p>0.44$, for difference between baseline and 24 and 48 hr time points) following vaccination, Figure 13. When we controlled for SS, the interaction effect remained significant ($p=0.04$, $F=4.456$). OA had lower FMD and FMD controlled for SS than YA at all time points, $p<0.05$ for all.

There was a time effect ($p=0.01$, $F=6.556$ for time and $p=0.09$, $F=2.970$ for interaction effect) for the change in resting brachial artery diameter, Table V. There were no between group differences for brachial diameter at any time point, $p>0.05$.

Correlational Analyses. In OA, we found that physical activity volume (VM) was inversely related to the percent change in CRP at 24 hr ($p=0.01$, $r = -0.574$) and 48 hr ($p=0.03$, $r = -0.501$) post-vaccination. There was no relationship between physical activity and change in FMD in either age group ($p>0.05$).

Table V. ARTERIAL RESPONSES TO VACCINATION IN OA AND YA (mean ± SEM)

	OA Baseline	OA Post 24 hr	OA Post 48 hr	YA Baseline	YA Post 24 hr	YA Post 48 hr
AC (mmHg)	0.91±0.1 *	1.2±0.1	0.94±0.1*	1.4±0.1	1.3±0.1	1.2±0.1
β-stiffness index #	9.8±1*	7.7±1*	9.6±1*	5.3±0.5	5.1±0.3	5.4±0.4
Diameter (mm) #	3.92±0.2	4.16±0.2 ^a	4.07±0.2 ^a	3.89±0.2	3.90±0.2	3.92±0.2
AIx@75 (%) #	29±2*	28±2*	26±2*	-4±2	-7±2	-7±2 ^a

Table Legend. Central arterial stiffness and brachial diameters pre- and post-vaccination in YA and OA. AC=arterial compliance, β-stiffness index=a pressure-independent measure of central (carotid) arterial stiffness, and Diameter=brachial artery diameter, AIx@75=augmentation index at a standardized 75 heart beats per minute, * indicates a significant difference from the YA value at that time point, ^a indicates a significant difference from baseline value in that age group, # signifies a significant over group time effect, p<0.05 for all.

Figure 13. Flow-mediated dilation post-vaccination in OA and YA.

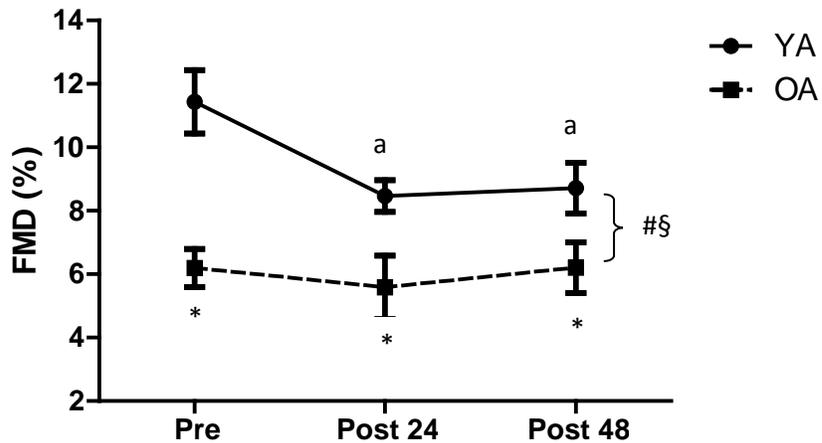


Figure 13 Legend. YA, but not OA, reduce FMD post-vaccination, ^a indicates a significant difference from baseline value in that age group, * indicates a significant difference from the YA value at that time point, [#] indicates an overall time effect of vaccination, § denotes a significant interaction effect, $p < 0.05$ for all.

Discussion

We found that acute, induced inflammation reduced endothelial function in YA but not OA. These findings are similar to those previously reported in YA, however, these are the first data to show that this effect of acute, induced inflammation does not occur in OA. Importantly, this study examined the effect of acute, induced inflammation in both YA and OA and thus specifically addressed the impact of aging on endothelial and arterial responses to acute, induced inflammation. Our results suggest that NO availability is attenuated in YA post-vaccination but unchanged in OA, who may already have attenuated amounts of bioavailable NO [48, 148]. Compared to YA, baseline levels of inflammation (IL-6, CRP) were elevated and baseline FMD was reduced in OA, and cross-sectional data has shown that systemic levels of inflammation are related to reduced endothelial reactivity in aging [38, 49]. We hypothesize that OA have already reached a level of inflammation sufficient to impair FMD, and that the additional increase in inflammation following vaccination was thus unable to further attenuate FMD.

These results were upheld when we controlled for shear. Shear stress is in part dependent on arterial diameter [149], and baseline arterial diameter increased in both groups following vaccination. However, the interaction effect remained significant when controlling for shear stress, showing that the effect of increased baseline brachial artery diameter did not alter our main finding of divergent FMD responses to acute inflammation in OA versus YA.

OA exhibited a decrease in blood pressure at both 24 and 48 hr following vaccination. We believe this is due to primarily to peripheral vasodilation following vaccination. Our arterial compliance results, as well as our findings regarding brachial diameter, support this hypothesis. However, it is also possible that a reduction in arterial stiffness may have contributed, since β -stiffness also decreased and β -stiffness is a less (but not totally) pressure-dependent measure of

arterial stiffness. Conversely, AC, a pressure dependent measure of arterial stiffness, did not change following vaccination. This is perplexing since a decrease in BP would be expected to also contribute to an increase in AC. Furthermore, others have shown increased arterial stiffness following vaccination in both young and old individuals (Jae et al, 2013; Vlachopoulos et al, 2005). Therefore, we hypothesize that the decrease in blood pressure in OA was caused primarily by peripheral vasodilation and less so by decreased arterial stiffness following vaccination.

Others have also reported post-inflammatory hypotension [132, 150]. This phenomenon is due to the formation of inducible nitric oxide synthase (iNOS), arachadonic acid / cyclooxygenase-2 (COX-2) products, and release of histamine (all potent vasodilators) following inflammatory stressor, which causes vasodilation [150, 151]. As a result, hypotension and impaired endothelial function occur. Nitric oxide antagonists and COX inhibitors have been suggested as a therapy to combat the effect of acute inflammation on blood pressure [151]. The effectiveness of this therapy stems not only from inhibition of vasodilators, but also by sensitizing the cardiovascular system to the constrictor influences of endothelin-1 and catecholamine's, which are up-regulated in a compensatory manner post-inflammation to oppose the overwhelmingly hypotensive milieu in an effort to rescue blood pressure [150, 151].

We speculate that the inhibition of ET-1 and sympathetic stimuli may account for the reduction in β -stiffness we observed. Older adults are more likely to have higher circulating levels of these vaso-constricting agents [48] and would thus be impacted more significantly by their down regulation. A potential inhibition of ET-1 did not, however, allow endothelial NO dilator function to be preserved in our study. We believe this is because iNOS production is a consumer of endothelial nitric oxide synthase (eNOS)'s essential co-factor, L-arginine [152], and

iNOS and eNOS are differentially affected by acute inflammation in the vasculature [153]. Up-regulation of iNOS has pleiotropic adverse effects in the vasculature discussed above, but increased eNOS protects the microcirculation and prevents end-organ ischemia following inflammatory stressor [153]. Hence, FMD (and presumably eNOS) was attenuated despite a possible reduction of the effects of ET-1 and reduction in β -stiffness we observed post-vaccination. Since we did not evaluate the potential contribution of ET-1, iNOS and catecholamines in our present study, future research is needed to further explore these potential effects.

Additionally, the therapeutic inhibition of iNOS and COX-2 production post-vaccination also rescues baroreflex sensitivity, providing further protection from large drops in blood pressure [151]. Interestingly, YA did not attenuate blood pressure but did increase heart rate after vaccination, and this may reflect intact defenses against inflammation-mediated hypotension in youth, including heightened baroreflex sensitivity or augmented responsiveness to catecholamines, compared to OA. Indeed, aging has been associated with reduced baroreflex sensitivity, and this may provide an explanation for the inability of OA to preserve blood pressure post-inflammation [154]. This effect of aging upon peripheral vasomotor tone following inflammation is important; in cases of more robust inflammation, uncontrolled dilation that is unresponsive to sympathetic stimuli may lead to cardiovascular collapse and can be fatal [111].

Similar to findings in previous studies focusing on younger adults [17], we found a reduction of AIx post-vaccination in our cohort. AIx is a measure of arterial wave reflection that is affected by arterial stiffness, peripheral vasomotion and heart rate. Our cohort also experienced an increase in heart rate post-vaccination. However, we still found a significant

reduction in AIx controlled for heart rate (AIx@75), indicating that other factors, such as the augmentation of arterial diameter and a reduction in arterial stiffness, and not just heart rate, potentially influenced AIx in our study.

In OA, we found a relationship between volume of physical activity and the percent change in CRP at 24 hr post-vaccination. This is an interesting finding as it shows that physical activity may have preventive properties regarding not only the state of chronic, low-grade inflammation seen in aging [18], but also in defense against inflammation following an acute stressor. Hence, regular physical activity may be an economical therapy for combating both chronic [155] and acute inflammation in aging.

We did not find any relationship between the changes in FMD or β -stiffness and any measure of physical activity. This is interesting as physical activity was related to change in inflammation. This may be further evidence for the idea that heightened baseline inflammation in OA (and adequate compensatory responses to acute inflammation in YA) cause a dissociation between markers of further, acute inflammation and subsequent endothelial and arterial repercussions. Hence, simply quantifying acute changes in inflammation may not adequately predict the actual cardiovascular response to this common stressor. Clinicians may find a very broad range of responses to acute inflammation in OA versus YA and should consider concomitant indicators of vascular and compensatory functions (such as blood pressure and endothelial function as well as heart rate), along with serum markers of inflammation, in order to best treat the net responses of their patients and avoid acute, unsafe drops in blood pressure.

Limitations.

There are some limitations to our study worth noting. All measurements were non-invasive in nature, though the techniques employed in this study have been validated against invasive methodology [116]. FMD measurements can be compromised by high variability, however, a single technician obtained and analyzed each recording and edge-detection software was utilized to reduce intra-observer error.

Our OA group included individuals taking prescription anti-hypertensive and anti-lipidemic medications. These medications obviously affect blood pressure but also have been known to reduce systemic inflammation [156, 157]. However, our study was acute in nature, and we did observe significant attenuation of blood pressure and augmentation of systemic inflammation. Thus, we believe that these medications minimally impacted our study's acute inflammation model.

Conclusions.

We found decreased endothelial reactivity but preserved blood pressures in YA post-vaccination. In contrast, OA showed no decrement in brachial endothelial function but significantly reduced peripheral and central blood pressures. This may be due to a decline or failure of post-inflammation defense mechanisms aimed at preventing unsafe drops in blood pressure, such as baroreflex function and/or catecholamine release or sensitivity. Previous physical activity does not affect this response to acute, induced inflammation in older or younger adults.

Chapter V: Summary of Results and Significance

We found that older and younger adults have similar composite (elastance) responses to acute inflammation. However, peripheral and central blood pressure, end systolic pressures, and some measures of systolic function were reduced in older but not younger adults. Conversely, endothelial reactivity (FMD) was attenuated only in younger (but not older) adults.

We believe that the maintenance of systolic function and blood pressure in younger adults represents intact compensatory mechanisms aimed at the prevention of potentially unsafe perturbations in cardiovascular homeostasis. This compensation may be mediated by the sympathetic nervous system or baroreflex function, both of which have been shown to be dysregulated in aging and important to rescuing homeostasis in other model of acute inflammation.

The attenuation of FMD in younger adults most likely occurs due to a reduction in bioavailable NO. Oxidative bursts from immune cells increase ROS, and NO scavenges these molecules instead of retaining its vasodilatory effects during acute inflammation. Interestingly, older adults maintained FMD post-stressor (vaccine). This may indicate that the immune/inflammatory dysregulation that occurs during aging is sufficient to impair FMD, and that further inflammation associated with an acute challenge cannot further impair endothelial reactivity.

Physical activity can modulate the left ventricular response to acute inflammation. The average time (min/day) spent in moderate-vigorous physical activity was inversely related to the reduction Elv (contractility). Thus, in order to offset undesirable cardiovascular responses to

acute inflammation, individuals should try to perform more physical activity that is adequately strenuous in nature.

This research may be important for clinicians, especially those who care for older adults. As aging is associated with a number of co-morbidities characterized by acute augmentation of inflammation (arthritic flare-ups, illness, periodontal issues, stress), older adults may be more likely to experience the aforementioned perturbations in cardiovascular performance. This may be another mechanism by which acute inflammation can induce cardiovascular events, particularly in older adults.

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Publications

Peer-reviewed Manuscripts

1. **Lane, AD**, Wu, P, Kistler, B, Fritschsen, P, Tomayko, E, Jeong, J, Chung, H, Yan, H, Ranadive, SM, Phillips, S, Fernhall, B, Wilund, KR, *Arterial Stiffness and Walk Time in Patients with End-Stage Renal Disease*, *Kidney Blood Press Res*, 2013;37:1-9.
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3. **Lane, AD**, Yan, H, Ranadive, SM, Kappus, RM, Sun, P, Cook, MD, Wilund, K, Woods, J, and Fernhall, B, *Sex Affects Wasted Left Ventricular Pressure Following Maximal Exercise*, *Int J Sport Med*, PMID:23526590, *in press*.
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Manuscripts in Review

1. **Lane, AD**, Yan, H, Ranadive, SM, Kappus, RM, Sun, P, Cook, MD, Wilund, K, Woods, J, Harvey, I, and Fernhall, B, *Sex Affects the Ventricular-Vascular Coupling Ratio Following Endurance Training*.
2. Yan, H, Heffernan, K, Ranadive, SM, **Lane, AD**, Kappus, RM, Sun, P, Cook, MD, Harvey, I, Wilund, K, Woods, J, and Fernhall, B, *Race, Sex and the Vascular Response to Exercise*.

Manuscripts in Preparation

1. **Lane, AD**; Bunsawat, K, Yan, H, Ranadive, S, Kappus, R, Baynard, T, Phillips, S, Woods, J, Motl, R; Fernhall, B, *Effects of Aging and Physical Activity on Ventricular-Vascular Coupling Following Acute, Induced Inflammation*
2. **Lane, AD**, Yan, H, Ranadive, Kappus, R, Bunsawat, K, Baynard, T, Phillips, S, Woods, J, Motl, R; Fernhall, B, *Effect of Aging and Physical Activity on Endothelial Function and Blood Pressure Following Acute, Induced Inflammation*

Book Chapter

1. Fernhall, B., **Lane, AD**, and Yan, H. Exercise for Special Populations. In: ACSM's Exercise For Older Adults (2012), Editor: Chodzo-Zajko, Lippincott, Williams, and Wilkins, Champaign IL.

Published Abstracts

1. **Lane, A**, Bunsawat, K, Kappus, R, Woods, J, Motl, R, Baynard, T, Fernhall, B, Effects of Acute, Induced Inflammation on Cardiovascular Function, *Med Sci Sports Exerc.*, 45(5S):1-210, May 2013.
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7. **Lane AD**, Yan H, Ranadive S, Kappus R, Cook, M, Wilund K, Woods J, Fernhall B, Sex Affects the Ventricular-Vascular Coupling Ratio Following 8 Weeks of Endurance Training *Med Sci Sports Exerc.* 44(5):S245, May 2012.
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Teaching Experience

2011- Department of Kinesiology & Community Health, *Clinical and Applied Exercise Physiology*, KIN 452 (3 credits) Course Instructor (Lecture and Laboratory), Enrollment: 25 students, University of Illinois, Urbana-Champaign

2010/2011- Exercise Physiology *Laboratory Experience* (week-long course), Enrollment: 24 students, Faculdade de Morticidade Humana, Lisbon, Portugal

2008- Department of Kinesiology, *Exercise Physiology, KIN 3433* (3 credits), Course Instructor, Enrollment: 36 students, University of Texas at San Antonio

Professional Memberships

2012- American Heart Association (AHA) member

2010- American College of Sports Medicine (ACSM) member