PROTECTIVE EFFECTS OF ACUTE MODERATE EXERCISE ON VACCINATION INDUCED INFLAMMATION, ARTERIAL FUNCTION AND VACCINE EFFICACY

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Abstract

Acute induced inflammation, using vaccination, reduces flow-mediated vasodilation in the conduit artery in young healthy volunteers. However, this has not been shown in older adults. Immunosenescence with advancing age results in inadequate protection from disease because of ineffective responses to vaccination. An acute bout of moderate aerobic exercise improves arterial and endothelial function and may increase the efficacy of the vaccine in young individuals. Hence, this study sought to evaluate the effect of acute systemic inflammation on endothelial function and wave reflection in older adults. The second aim was to evaluate if acute moderate intensity endurance exercise immediately prior to induced inflammation can prevent the negative effect of acute systemic inflammation on vascular function while augmenting the efficacy of the vaccine. Fifty-nine healthy volunteers between 55 – 75 years of age were randomly allocated to an exercise or control group. Arterial function and inflammatory markers were measured at baseline, 24 hours and 48 hours after influenza vaccine and sham injections. Antibody titers were measured at baseline and 4 weeks following the Influenza vaccine. CRP increased when measured at 24 and 48 hours and IL-6 increased at 24 hours from baseline after the Influenza vaccine compared to the sham injection while unexpectedly, arterial function was unaltered. There were no significant correlations between changes in inflammatory markers and changes in arterial function. Fitness was related to endothelial function as baseline. Endothelial function was significantly higher in individuals classified as having good fitness compared to the poor fitness category. There was a significant decrease in the endothelial function at 48 hours after vaccination compared to baseline in the fair fitness while there was significant decrease in the endothelial function when measured at 24 and 48 hours as compared to the baseline in good fitness category group. The endothelial function was unaffected in the poor fitness group. There

were no differences in the levels of antibody titers against the H3N2 influenza strain between the men and women in exercise group as compared to the control group. However, women in the exercise group had a significantly higher antibody response for H1N1 influenza strain. In conclusion, there was dissociation between inflammation and endothelial function following induced acute systemic inflammation in older adults. The responses of endothelial function to induced acute systemic inflammation were related to fitness. Acute moderate aerobic exercise was not immune-stimulatory in healthy older men, but may serve as a vaccine adjuvant in older women.

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CHAPTER 1

INTRODUCTION

Acute systemic inflammation or infection transiently increases risk of cardiovascular events (91, 113, 130), but the underlying mechanisms are not fully understood. The negative effects of acute inflammation include increases in inflammatory markers such as C-reactive protein (CRP) and direct arterial effects of inflammation, including increased arterial stiffness and decreased endothelial function, all of which increase the risk for cardiovascular events (17, 60, 64, 132). Thus, there is clear evidence that inflammation decreases vascular function. With advancing age there are further impairments in cardiovascular (41) and immune function (including innate immunity) (16, 32, 59, 86). It is possible that acute inflammation, including that induced by influenza vaccination, produces greater decrements in arterial function in older individuals (33, 71, 77), thus increasing the risk of cardiovascular events (8, 132). Using influenza vaccination as a model to induce inflammation we have shown (in young healthy individuals) that induced inflammation decreases arterial function, consisted with an increase in risk(107).

The innate immune response is up-regulated following an acute bout of moderate intensity exercise in animals and humans (14, 137). Animal studies have consistently shown that administration of a moderate acute stressor just before antigen exposure results in an enhanced immune response (9, 25, 137). Recent human studies have shown acute exercise prior to an inflammatory stimulus induces immuno-enhancement and an anti-inflammatory response in young healthy individuals (31, 128, 137). Furthermore, an acute bout of moderate intensity exercise improves endothelial function and decreases arterial stiffness (65, 66, 104). Thus, one acute exercise bout of moderate intensity appears to exert a protective effect on arterial function. However, the effect of acute moderate intensity exercise administered before vaccination-

induced acute inflammation on vascular function in the elderly is unknown. Therefore, **the overall aim of this project is to investigate if *acute moderate intensity endurance exercise immediately prior to vaccination-induced inflammation can prevent the negative effect of acute systemic inflammation on vascular function in older adults.***

Immunosenescence with advancing age results in inadequate protection from diseases because of ineffective responses to vaccination (48). Although acute moderate exercise prior to vaccination can act as an adjuvant and increase the efficacy of the vaccine in young individuals (31), it is unknown if older individuals will show a similar positive response. Thus, a second aim of this project is to investigate if ***moderate acute aerobic exercise immediately prior to an influenza vaccine can increase the efficacy of the vaccine in older adults.***

It has been shown that acute exercise prior to an influenza vaccine produced greater antibody responses in young women compared to young men (31). However, other studies (68, 69) have not reported any sex differences; hence, an exploratory aim is to investigate if ***acute exercise immediately prior to an influenza vaccine produces different effects on antibody response in older men and women.***

The primary aims of the present investigation are as follows:

Aim 1: To examine the effect of acute exercise immediately prior to an acute systemic inflammatory stimulus on vascular function in a randomized, double-blind, sham procedure-controlled crossover design in older adults.

Hypothesis: We hypothesize that the acute systemic inflammatory response will be lower in the exercise group as compared to the control group thus preventing or reducing the negative effects of inflammation on the vascular function.

Aim 2: To evaluate the effect of acute moderate aerobic exercise immediately prior to an influenza vaccine on vaccine efficacy.

Hypothesis: We hypothesize that the acute exercise immediately prior to the vaccination will improve vaccine efficacy as measured by hemagluttination inhibition (HI) antibody titers in older adults.

Aim 3: To evaluate if acute exercise immediately prior to an Influenza vaccine produces different effects on antibody response in men and women.

Hypothesis: We hypothesize that women will show a higher antibody response to Influenza vaccine as compared to men.

Significance

Cardiovascular disease continues to be the leading cause of death in the United States and the risk of CV events increases with age. It is well established that influenza vaccination has obvious beneficial outcomes in older individuals, a small but significant portion of middle aged and older individuals experience an increase in cardiovascular events thought to be mediated by the inflammatory response following vaccination. Furthermore, a significant portion, up to one third, of older individuals receive no or greatly reduced protection from the influenza vaccination. If an acute bout of moderate intensity aerobic exercise decreases the subsequent acute systemic inflammatory response to influenza vaccination in older individuals, it is possible that an acute moderate exercise bout may also exert a protective effect on the vascular function which could potentially provide a cardioprotective effect. Furthermore, it is possible that acute moderate exercise may increase vaccine efficacy, which could have potentially large public health benefits. Thus, if our hypotheses are supported, it is possible that one bout of acute

aerobic exercise will reduce the immediate risk of the effects of influenza vaccine dose, while enhancing the long term protection of vaccination by improving the efficacy of the vaccine. Findings from our study will provide new insight regarding potential stress-immunological mechanisms relating inflammation to vascular function and vaccine efficacy in older individuals. Our study will provide the foundation for understanding these relationships in human aging and the foundation for potential future interventions.

CHAPTER 2

LITERATURE REVIEW

The vascular endothelium provides a vasodilatory and anti-atherogenic influence on the cardiovascular system (8). Endothelial cells receive signals not only from chemical stimulation (50) or environmental changes, but from mechanical stimuli as well (8, 103, 114). These signals are further translated to the smooth muscle around the artery. The relaxation of the smooth muscle results in dilatation of the artery(114). This process of endothelial dependent vasodilatation has been attributed for the most part to nitric oxide (NO) which is produced by the endothelium (103, 114). A constant production of NO is essential for the basal dilator effect in the arterial system of humans. Along with maintaining the dilator vascular tone, NO plays a very important role in maintaining the vascular wall in a quiescent state by inhibiting inflammation, thrombosis and cellular adhesion and proliferation (22, 56). Hence, reduction or loss of NO results in shifting of the vascular wall towards an inflammatory and pro-artherogenic phenotype (17).

Endothelial stunning and induced inflammation:

A very brief exposure to any endotoxin or certain cytokines impairs the endothelial function for days and this effect is called “Endothelial Stunning” (8, 60). It is further accentuated in the presence of other underlying risk factors (8). One of the primary risk factors related to endothelial dysfunction is inflammation (60, 130, 132). There is a strong link between chronic, low grade inflammation and the progression of atherosclerosis (76). Hence, introduction of an acute inflammatory stimulus in the presence of low grade chronic inflammation, there is a transient increase in the risk of cardiovascular events (8, 60). Endothelial stunning as a result of

inflammation affects the process of endothelium dependent relaxation. In addition, infection of the endothelial cells with viruses further increases the expression cell-surface adhesion molecules and pro-coagulant activity (130, 131). During inflammation or vascular injury, there are important interactions between leukocyte-leukocyte, leukocyte-endothelium and leukocyte-vascular smooth muscle cell (61). The proteins causing these interactions are known as adhesion molecules and they are differentiated into selectins (P-selectin and E-selectin), selectin ligands, integrins (β1 and β2) and some immunoglobulins (ICAM and VCAM) (61). The primary functions of the adhesion molecules are to promote leukocyte recruitment, leukocyte rolling along the endothelial surface, adhesion and activation to the endothelium. E-selectin helps in rolling of the leukocyte along the endothelial wall when activated, this is followed by leukocyte adhesion and transmigration into the tissue by the integrins and ICAM-1 & VCAM-1 (61). It has been observed that following a vascular injury, there is an increase in the expression of ICAM-1 and VCAM-1 on the endothelial cells, macrophages and smooth muscle cells (61). The expression of VCAM-1 is increased in fibrous or lipid-containing plaques. Hence, any infection or inflammation to the endothelial cells triggers a pro-atherogenic cascade via cell-surface adhesion molecules.

To mechanistically study the idea that transient or induced inflammation can bring about negative changes in the endothelial function and increase the risk of acute cardiovascular event, Bhagat et. al. (1996) hypothesized that administration of systemic inflammation (administration of bacterial endotoxin) would impair endothelial dysfunction in young participants (8). It was found that a brief exposure of bacterial endotoxin impaired endothelium-dependant vasodilatation for several days. The study also reported that the endotoxin reduced the vasodilator response to bradykinin and arachidonic acid (8). This suggests that endotoxin

affected the L-arginine – Nitric oxide pathway (8). Importantly, the results from this study suggested that the transient decrease in the endothelial function due to the endotoxin would not only result in decreased vasodilatation but also shift the vascular wall towards a pro-inflammatory and pro-atherogenic phenotype which increases the chances of acute cardiovascular events.

Even though Bhagat et. al. noted the transient changes in the endothelial function, the result could be due to an effect of a strong endotoxin. Endotoxins {e.g. lipopolysaccharide (LPS)} are a component of the cell wall of gram-negative bacteria. Endotoxins elicit a strong immune response by inducing the release of pro-inflammatory cytokines. Although, there is low-grade inflammation with aging, exposure to endotoxin results in much higher levels of inflammation. Hence, a model of induced inflammation with an endotoxin may not be able to accurately represent mild systemic inflammation. This study was later followed up by Hingorani et. al. (2000) with induction of mild systemic inflammation (administration of vaccine) (60). In this study, Salmonella Typhi vaccine was administered to young healthy individuals and assessment of the resistance vessels (forearm blood flow) and conduit vessels (brachial artery dilatation) was performed. The forearm blood flow was measured in response to intrabrachial infusions of bradykinin (BK), acetylcholine (ACh), nitroglycerin (NTG) and verapamil. The brachial artery dilatation was measured in response to ischemic response and NTG. In addition, pro and anti-inflammatory cytokines were measured. The study noted that administration of intramuscular vaccine (typhoid) caused a systemic inflammatory response (increase in IL-6 levels) and a temporary decrease in response to BK and ACh. However, the decrease in the endothelial-dependent dilation was not seen in the control group. Similarly, there was no change in the response to NTG in the brachial artery even though there was a reduction in the FMD. This

suggests that vaccination induced inflammation was specific only to the endothelial agonists while there was no effect on the NO donor drug (NTG) and verapamil vasodilatation. The results suggest that vaccination temporarily reduces the ability of the vascular endothelium to produce the endogenous vasodilators (60).

Acute systemic inflammation also temporarily increases arterial stiffness while decreasing wave reflection in healthy young individuals (132). Vlachopoulos et. al. were the first to note that 8 hours post salmonella typhi vaccination there is a significant increase in the carotid-femoral pulse wave velocity (PWV), suggesting an increase in aortic stiffness (132). There was also a significant decrease in augmentation index (AIx) which suggests a decrease in wave reflections at 8 hours and 32 hours post vaccination. This study reported a differential change in the measures of AIx and PWV. AIx is a measure of wave reflection and commonly accepted as a measure of the enhancement of the aortic pressure by a reflected pulse wave. Change in the timing of the reflected wave can cause a ventricular-vascular uncoupling. PWV is based on the time of transmission of the pressure wave between points (carotid-femoral) with the help of ECG-gating. Hence, it is accepted as a measure of arterial stiffness. In certain cases change in PWV would affect the timing of the incident and reflected wave thus causing a change in AIx because The reflections of pressure and flow waves occur when there is a change in impedance (49). This can happen with a change in stiffness or lumen diameter or a combination of both (49). However, in other cases where there is a change in the timing of the wave-reflection without any change in the PWV e.g. effect of vasoactive drugs, AIx and PWV may not work in tandem. Most importantly, AIx has been shown to be affected by height and cannot be controlled between subjects where upon such is not the case with PWV (139). In the study by Vlachopoulos et. al., the mechanism for the dissimilar changes in AIx and PWV has been thought to be a result

of vasodilatation of the small and medium peripheral muscular arteries and arterioles (132). It has been noted from various animal studies that immediately following an infection or acute inflammation there is increased vasodilatation as a result of higher production of NO or prostanoids from de novo synthesis of inducible nitric oxide synthase (iNOS) or cyclooxygenase II (COX II) in response to circulating cytokines (51, 82, 130). eNOS and COX-I however are down regulated. Interestingly, this immediate response is followed by "Endothelial stunning" for several days in response to an acute inflammation or endotoxin. The study also reported that the changes in PWV and wave reflection were completely abrogated by ingestion of 1200 mg aspirin pre-treatment. This suggests that arterial stiffness was a result of acute systemic inflammation which was reversed by preventing the release of IL-1 (aspirin dose) (64, 132). However, the aspirin dose may have also blocked the prostaglandin – prostacyclin vasodilatation by blocking the COX-1 and COX-2 enzymes. Prostacyclin (PGI2) is produced in the endothelial cells from the prostaglandin (which is derived from Arachidonic acid in presence of COX) in the presence of prostacyclin synthase (43). In contrast to NO, PGI2 does not contribute to maintenance of basal vascular tone (43). Interestingly, within the endothelial cells with an increase in intracellular calcium, NO is released continuously while PGI2 is released only in a transient manner (81). PGI2 and NO tend to work in synergistically to certain extent, as PGI2 facilitates the release of NO and in turn the action of PGI2 in the VSMC is facilitated by NO (112). Hence, even though the researchers may have blocked the release of IL-1 and thus prevented arterial stiffness, the aspirin dose may have also prevented vasodilation via prostragladins that can occur even in the low presence of NO.

We have shown that influenza vaccination-induced acute inflammation causes an increase in blood pressure and arterial stiffness using a randomized double blind sham placebo-controlled

cross over design(106). Nineteen healthy subjects (male 10, female 9; age 24±4 yrs) received influenza or a sham vaccine (normal saline). The influenza vaccination caused a significant increase in blood CRP (1.42±0.6 at baseline, 2.81±1.0 after 24 hours, 5.0±1.3mg/L after 48 hours, $p<0.05$) and IL- 6 (1.12±0.3, 2.56±0.4, 2.26±0.6pg/mL, $p<0.05$) but not TNF- ∝ compared with sham injection. Endothelial function decreased following the vaccination, but this decrease occurred before significant changes in circulating levels of CRP. Central systolic blood pressure (98.0±7.4, 104.5±10.8, 100.7±8.4mmHg) and PWV (7.8±0.9, 8.6±1.5, 8.7±1.3m/s) were also significantly increased after the influenza vaccination but not following sham vaccination. These findings support a link between inflammation and arterial dysfunction. These findings also suggest that an acute inflammation causes a temporary increase in central blood pressure and arterial stiffness in young people (106).

In another study, using a randomized sham placebo-controlled, double blind design, 24 healthy subjects (age 24.8±3.5 yrs) were injected with an influenza vaccine as a model to generate a systemic inflammatory response (62). Heart rate recovery after maximal treadmill exercise as an index of autonomic nervous system function was calculated as the difference between maximal heart rate during the test and heart rate 1 (HRR 1) and 2 (HRR 2) minutes after cessation of exercise. Both blood analysis and HRR were measured at before each vaccination and 48 hours after each vaccination. C-reactive protein (1.87±1.2 to 2.75±1.3 mg/L, $p<0.05$) was significantly increased after an influenza vaccine, but not tumor necrosis factor-α (2.01±0.1 to 2.00±0.19, p=NS). HRR1 was significantly attenuated after an influenza vaccination but not sham vaccination. However, HRR 2 was not significantly attenuated after an influenza vaccination. These findings suggest that inflammation alters autonomic function consistent with an increase in cardiovascular risk (62).

Vascular Ageing and inflammation:

With advancing age, there is a rise in systolic pressure accompanied by a decrease in diastolic pressure, thus widening the pulse pressure and a higher prevalence of isolated systolic hypertension (12, 53). Arterial stiffening has been epicenter for the widened pulse pressure in older adults (93). Studies have noted that ageing primarily affects the large elastic arteries (80, 115) while the smaller arteries are seldom affected (83, 110, 126, 127). The primary changes in the large arteries include increased stiffness, increased lumen diameter and increased wall thickness (53). The increase in arterial stiffness is usually associated with degeneration of the medial layer of the artery (87). The repetitive pulsatile stress over years results in thinning, breaking or splitting of the elastic fibers of the medial layer (4). This causes an increase in collagen and ground substance content within the layer (4). The age-dependent chronic cyclic stress results in loss of the elastic lamellae, thus causing structural stiffening(4). This process is referred to as "Arteriosclerosis" (53).

Arterial properties can be measured using ultrasound or pulse waveform analysis (PWA). This technique typically reflects the overall or systemic properties of the arterial tree. When a pressure wave is generated following cardiac ejection, it travels along the arterial tree forwards all the way up to the resistance arteries in the periphery from where it is reflected back towards the heart. When the speed of this wave is high enough to return to the central aorta, the reflected wave overrides the incident wave to create an augmented waveform. Aortic AIx has been accepted to be the measure of wave reflection and is measured non-invasively by estimating the aortic waveform from the radial waveform with a generalized transfer function. Studies have shown that in individuals over the age of 40years, the reflected wave augments the blood

pressure in late systole and causes a positive aortic AIx (53). There are pathological increases in AIx that can occur in this age group as a result of either increase in magnitude or hastened timing of the reflected wave or both (53, 54, 110). Numerous studies have shown the clinical significance of AIx in predicting cardiovascular risk (74, 108, 134). Also, the timing of the wave reflection is very important as it is related to cardiac function. In young healthy adults the wave reflection returns to the heart in early diastole thus augmenting the diastolic pressure and increasing the coronary blood flow (53). However, with advancing age, this coupling is disturbed. Increase in the stiffness of the large arteries causes an early return on the reflected wave and an increase in the incident wave (53). This causes a significant systolic pressure augmentation and a decrease in diastolic pressure (87). The decrease in diastolic pressure further results in the reduced coronary blood flow and predisposing the individual to myocardial ischemia. Chronic activation of this cycle results in systolic hypertension, left ventricular remodeling and diastolic dysfunction (49). All of these factors are known to exponentially increase the mortality rate due to cardiovascular disease in older adults (49).

In one of the first studies, O'Rourke reported that with progressive aortic degeneration as seen with ageing, the systolic pressure increases indirectly by the early return of the wave reflection (5). It has been suggested that changes in large artery pulse contour seen with aging are not only related to alterations in central artery stiffness, but changes in the peripheral vasomotor modulation of smaller resistance vessels (87).

In addition to the structural changes, there are biochemical changes associated with aging. The most commonly used approach to measure some of these changes is to measure the endothelial dependent and independent vasodilatation. In one of the early studies by Ludmer et. al. they assessed and reported decrease in the Endothelial dependent dilation (EDD) in the coronary

arteries of patients with heart disease (75). However, most researchers perform the EDD measurement in the peripheral arteries to assess the arterial endothelial function. In general, the brachial artery FMD is used as surrogate for measurement in the coronary artery because assessing FMD in epicardial arteries is more invasive as compared to brachial artery FMD. Previous studies have shown that a significant number of patients with unstable angina had endothelial dysfunction of the brachial artery as tested by FMD. Hence most researchers use brachial artery FMD to predict the clinical outcome (2, 36, 63, 73, 85).

In humans, the EDD is measured using 2 different methods, namely pharmacological stimulus (agonist of NO) is infused into the brachial artery or evoking a mechanical stimulus by inflating a cuff on the upper arm to a supra-systolic pressure for 5 minutes followed by the rapid release (45). In the later method, the dilatation of the resistance arteries distal to the occlusion produces a temporary increase in blood flow known as reactive hyperemia while it causes a temporary vasodilatation in the proximal conduit artery known as flow mediated vasodilatation (FMD).

It has been shown by numerous studies that FMD, both in the arms and legs is impaired with aging (28, 35, 44, 92, 110). Eskurza et. al. studied FMD in 3 groups of men; young healthy individuals, old sedentary individuals and old endurance-exercise trained individuals. They found that at baseline, the old sedentary individuals had a 45% lower FMD as compared to the young sedentary adults (35). However, the exercise trained older adults demonstrated similar FMD to the young sedentary men. In another study by Parker (97)et. al. studied FMD in both brachial and popliteal arteries of young (20-30 years) and older (60 to 79 years) women. They reported that older women had about 50-60% lower FMD in both brachial and popliteal arteries. Additionally, they noted that the endothelial-independent dilatation, as assessed by administration of nitroglycerin was blunted by 45-65% in the older women. They concluded that

the age-associated decline in FMD may be partly due to the decreased responsiveness of smooth muscle (97).

Recently, there has been an increased emphasis on understanding the mechanisms of vascular ageing so as to reduce the cardiovascular mortality in older adults. Studies have shown that the impaired EDD with aging is primarily a function of reduced NO bioavailability (30, 84, 110). In humans, this is supported by a study performed by Taddei et.al. (124) where the authors evaluated age-related endothelial dysfunction and if it was a result of changes in L-arginine-NO pathway in hypertensive and normotensive individuals. They measured the forearm blood flow following intrabrachial dose-dependent infusion of Sodium nitroprusside and ACh. ACh was then infused in presence of L-NMMA, vitamin C and both. The authors noted there was reduced vasodilation to acetylcholine as compared to sodium nitroprusside in hypertensive patients compared with control subjects. Interestingly, even in normotensive subjects, the inhibitory effect of L-NMMA on response to acetylcholine decreased in parallel with advancing age, whereas vitamin C increased vasodilation to acetylcholine in only the oldest group. This suggests that the NO production is lower in older adults as compared young adults due to the reduced vasoconstriction in response to L-NMMA (124). Even though NO bioavailability is reduced with aging interestingly, Donato et. al. reported in healthy humans the eNOS protein expression was higher in older adults as compared to young adults (28). This result was accompanied by a decrease in EDD in the older adults. This may seem to suggest that in healthy older adults the increase in protein expression (eNOS) could be to compensate for the reduced NO bioavailability (28, 110).

Another biochemical mechanism that was reported by Donato et. al. was a significant increase in plasma concentrations of Endothelin-1 (ET-1) in older adults as compared to the young

individuals (28). ET-1 is considered to be the most potent vasoconstrictor released by the vascular endothelial cells. ET-1 acts through specific receptors called the ET_A and ET_B receptors (123). The function ET-1 via ET_A receptors on smooth muscle cells is mediating contractions or vascostriction (125). However, the function of ET_B receptors is a little more complex and is based on the location of the ET_B receptors i.e. endothelium or smooth muscle. ET_B receptors on smooth muscle evoke contraction whereas the ones on endothelium cause relaxation by production of endothelium derived relaxing factors (EDRF) (125). Overall, ET-1 is very important as it causes a direct vasoconstrictor tone on the vasculature via ET_A and ET_B of the smooth muscle and helps in counterbalancing the vasodilatory effects of NO or other EDRF on ET_B receptors of the endothelium. Hence, the increased concentration of ET-1 was related to the decrease in EDD. Taken together the study suggests that changes in ET-1 and NO bioavailability but not eNOS expression contribute to the vascular endothelial dysfunction in older adults.

Inflammation and Oxidative stress in aging:

Inflammation has been suggested as one of the primary mechanisms of reduced arterial function with ageing. There is substantial amount of clinical and animal study data to show that ageing is associated with chronic low grade inflammation (11, 26, 96). In 126 centenarians, the plasma levels of TNF-α were positively correlated with IL-6, sTNFR-II and CRP. This suggests that there is a continuous interrelated activation of the entire inflammatory cascade (11). In yet another large study comprising of 1727 older adults (mean age = 70), there was a significant increase in the circulating plasma levels of IL-6 independent of disease status and other disorders of aging (11, 18). These studies suggest that the low-grade inflammation in older adults is due to

the dysregulation of cytokine production. This state is further exacerbated by other superimposed age-related pathologies.

It has been reported by Csiszar. et. al. that there is a cross-talk between oxidative stress, inflammation and endothelial dysfunction, and all of these factors are involved in the pathogenesis of cardiovascular disease (20). Ungvari et. al. reported that reactive oxygen species (ROS) can act as the signaling molecules for activating the pathways that regulate the inflammatory processes in the arteries of aged mice (129). The oxidatively damaged molecules tend to induce the inflammatory processes which in turn cause more cellular oxidative stress. This creates a vicious cycle which tends to contribute to endothelial dysfunction.

In a recent study, Miles et. al. studied 162 healthy male individuals between the age group of 18-84 for cytokines, chemokines and adhesion molecules (78). They noted that there is a significant positive correlation between age and several other cardiovascular risk factors (e.g. BMI, hypertension, total cholesterol, LDL and HDL concentrations) with the plasma concentrations of sVCAM-1, IL-6, MCP-1, IL-18 and sE-selectin. Also, the correlations with age remained significant for sVCAM-1, IL-6, MCP-1 even when other cardiovascular risk factors were controlled for. This suggests that the age associated inflammation may not be triggered by other known risk factors (78). Several other studies conducted in animals and primates have reported that even with normal/healthy ageing (20, 21, 129), there is a shift towards the proinflammatory zone in vascular gene expression coupled with an increase in inflammatory cytokines/markers, including TNF-$\propto$, IL-6, MCP-1, VCAM-1 and inducible nitric oxide (iNOS).

Donato et. al. studied the expression of inflammatory proteins in vascular endothelial cells of peripheral veins obtained from 24 young (mean age = 23) and 36 healthy older adults (mean age= 63) (27). They noted that even the healthy older adults had a lower endothelial dependent

vasodilatation and significantly higher levels of plasma C-reactive protein (CRP) and IL-6. Interestingly, they also reported higher total and nuclear expression of nuclear factor B (NF-kB), which is a pro-inflammatory gene transcription factor, in the endothelial cells of the older adults (27). The endothelial cells of the older adults also had a lower expression of the NF-kB inhibitor factor. Hence, the authors concluded that the impaired endothelial function present in healthy older group is associated with a progression of pro-inflammatory phenotype in the vascular endothelium (27). This cycle of activation of pro-inflammatory gene expression in the vascular endothelium by the NF-kB and in turn increased levels of NF-kB due to chronic inflammation leads to faster progression of atherosclerosis. Hajra et. al. in a mice model have shown that regional activation of NF-kB in the vascular endothelium may contribute to the localization of atherosclerotic lesions at the sites (52). Also, the increased expression of iNOS in coronary vessels, carotid arteries and aorta is due to the prolonged increase in the NF-kB binding in older adults (20, 129). Studies have reported that this activity results in increased vascular peroxynitrite production (129). This suggests that mitochondrial oxidative stress regulates the activity of endothelial NF-kB and hence with aging the vascular inflammation increases, in part due to decline in the mitochondrial function.

Hence, taken together these data show that that oxidative stress and inflammation occur concurrently with ageing which affect the vascular tree in a deleterious manner.

Acute exercise and IL-6 as an anti-inflammatory tool:

The normal cytokine cascade as seen with sepsis is TNF-$\propto$ $\rightarrow$ IL-1β $\rightarrow$ IL-6 $\rightarrow$ IL-1ra $\rightarrow$ sTNF-R $\rightarrow$ IL-10 (11, 101). Out of these TNF-$\propto$ and IL-1B are known to pro-inflammatory cytokines, however IL-6 has properties of both pro and anti-inflammatory cytokine (101). Interestingly, exercise elicits a slightly different cascade of cytokines as compared to sepsis.

With exercise, the pro-inflammatory cytokines (TNF-∝ and IL-1β) do not increase; however, IL-6 is the first circulating cytokine (101). Studies have shown that IL-6 increases up to 100 times during exercise and decreases in the post-exercise period (40, 98, 101, 122). Apart from IL-6, studies have reported an increase in IL-1ra and sTNF-R which are known cytokine inhibitors and other anti-inflammatory cytokines like IL-10 (94, 95). Numerous studies have noted an increase in the circulating levels of plasma IL-6 after exercise without significant muscle damage (15, 29, 90). It has also been noted that the levels of plasma IL-6 increase with exercise are related the intensity, duration and mass of muscle recruited during exercise (40, 101). Steensberg et. al. have shown that IL-6 is released from contracting skeletal muscle during exercise unlike TNF-∝ (118). Fishcer et. al. have shown that even moderate exercise increases the IL-6 levels by approximate 20-fold in young adults (42). Using the same exercise model Pedersen et. al. showed that the increase in plasma IL-6 levels is even higher in older adults (99).

In one of the early studies, Schindler et. al. reported that IL-6 inhibits TNF-∝ production induced by LPS in human monocytes (109). In addition, Starkie et. al. reported that infusion of rhIL-6 in healthy humans results in inhibition of endotoxin induced TNF-∝ production (116). It has been shown that the production of IL-1ra and IL-10 is stimulated by IL-6 (117). Knowing that IL-1ra and IL-10 exert anti-inflammatory properties, the stimulation of these cytokines by IL-6 seems to suggest that IL-6 creates an anti-inflammatory environment (101).

Immunoscenescence:

Each year Influenza affects a wide number of people of all age groups. However, the influenza virus can cause a disproportionate increase in serious illness and deaths in the age group of 65 years and older. The most effective way of avoiding influenza is the Influenza vaccine. However, the vaccine is ineffective in about 25% of the older population (47, 138). Numerous reasons have

been speculated for the lower influenza vaccine response in the older adults namely, vaccination history, exposure to influenza viruses, living situation, dietary factors and most importantly immunoscenescence (7, 47). Aging is often associated with a decline in the immunological system known as immunosenescence. Hence, in the recent years there has been increase in studying the role of exogenous and endogenous adjuvants to increase the efficacy of the vaccine.

Acute stress (exercise) as an adjuvant:

It has been shown that high levels of chronic stress (psychological or physical) are detrimental to the immune system and function (18, 46). In regards to influenza vaccine titers, chronic stress has been shown to reduce the antibody titer in all age groups (79, 102). However, studies have shown that acute stress can be immunoenhancing (9, 24). The immunoenhancement due to an acute stressor has been looked at from an evolutionary point of view and is considered adaptive for survival (31). Dhabhar et. al. conclude that the immunoenhancement of an acute stressor is related the duration of the stressor, type of stressor and the temporal relationship between the stressor and the challenge (24). In terms of temporal relationship, they have shown that close proximity of the acute stressor prior to the antigen exposure seems to boost the immune system (24). In number of animal studies it has been reported that restraint stress and footshock prior to administering a vaccine increased the humoral immune response to the antigen exposure (100, 136).

However, in human studies the results have been mixed. One of the reasons being the limited novel psychological and physical stressors that can be introduced prior to the antigen. The consistent pattern seen in most of the human studies is the upregulation of the innate immune system by acute stress (31, 111). This includes an increase in the number of natural killer cells and neutrophils and increases in the cytokine production. In one of the first human studies with

exercise as a stressor, Eskola et. al. studied the effect of heavy (marathon) and moderate (35 mins of running) on the number and function of lymphocytes in eight young healthy men (34). They reported that even though the lymphocyte responsiveness is transiently depressed by heavy stress, the humoral immune functions are enhanced. They also noted that the recovery of the reduced lymphocyte functions occurs within 24 hours. The study reported that the 4 marathon runners had significantly higher antibody response to tetanus toxoid vaccination as compared to the fifty nine control participants (34). In a similar study with chronic stress Brunsgaard et. al. studied the effect of DPT (Diptheria, tetanus toxoid and pneumococcal) vaccine on 22 triathletes who completed a half-ironman, 11 non-competing triathletes and 22 sedentary controls (10). They reported no difference in the antibody response amongst the groups.

However, intense and prolonged exercise is associated with a post-exercise "open window" for infection because of a high plasma cortisol level, decrease in the activity of NK cells, decrease in granulocyte oxidative burst activity, decrease in mitogen-induced lymphocyte proliferation and a decrease in nasal mucociliary clearance (86, 88, 89, 137). Eskola et. al. also noted a transient decrease in lymphocyte function for 24 hours following marathon running and antigen exposure. Interestingly, there is some evidence that exhaustive exercise in rats prior to the exposure of LPS caused a decrease in stimulation of TNF-$\propto$ in plasma (6, 137).

The underlying mechanisms regarding the immunoenhancing effects of acute stress remain a debatable topic. There are 2 key possibilities namely, glucocorticoids and IL-6. Dhabhar et. al. in late 90's reported that infusion of glucocorticoids (corticosterone) in mice augmented the delayed type hypersensitivity (DTH) response (24). The dose of corticosterone was of the same level found during acute stress in adrenalectomized mice. Thus, the dose mimicked acute stress in intact mice. In this study, they provided some evidence that increase in the levels of

glucocorticoids in close temporal proximity of antigen exposure, provides immunoenhancement. IL-6 has been shown to provide both pro and anti-inflammatory effects. In 1999, Lee et. al. reported that when IL-6 gene is administered concurrently with the influenza vaccine in mice, the animals are completely protected from any subsequent viral challenge (72). In a human study implicating IL-6, it was reported that healthy adults who were antibody responders to Franscisella tularensis had higher levels of IL-6 prior to immunization as compared to non-responders (70).

Recently, Edwards et.al. used an acute psychological stressor and an acute physical stressor in close proximity to the administration of influenza vaccination (31). They examined the effects of these stressors to the antibody response to the all the 3 vaccine strains. They studied 31men and 29 women who were randomly assigned to either dynamic exercise, mental stress or control group. Dynamic exercise included a four step incremental cycle ergometer, while the mental task included mental arithmetic task. Plasma cortisol and IL-6 were measured at baseline, after the task and 60 minutes of recovery, while the antibody titers were measured 4 weeks and 20 weeks post vaccination. The antibody titers for A/Panama strain were higher in women for both mental and exercise group as compared to the control group. Interestingly, men did not show any difference between groups. Also, the plasma levels of IL-6 at the 60 minute recovery was noted to be a significant predictor of A/panama antibody titers in women. The study seems to provide evidence that physical as well as psychological stressor can be immunoenhancing at least in women (31).

However, in a more recent study Campbell et. al. studied 156 healthy participants to evaluate if exercise can augment the immune response to vaccination and if the temporal relationship of exercise and vaccination would affect the efficacy of the vaccine (13). They noted that the

eccentric exercise did not further augment the antibody titer as compared to the control group. The authors concluded that as the participants were healthy the immune responses to the vaccine could be at a maximal extent and hence, there would be limited room for immunoenhancement by exercise.

Taken together, these studies suggest that acute stress prior to the exposure of antigen can be immune-enhancing based on the duration and intensity of exercise and temporal proximity to the antigen. Acute psychological stress too seems to be immune-enhancing and potentially may affect women differently as compared to men (31). However, in healthy humans if the immune responses are at the maximal extent there is a very small margin for immune-enhancement by exercise.

Chapter 3

Effect of Acute Moderate Exercise on Vaccination Induced Inflammation and Arterial Function in Older Adults

The vascular endothelium provides a vasodilatory and anti-atherogenic influence on the cardiovascular system (8). Endothelial dependent vasodilatation has been attributed mostly to nitric oxide (NO), which is produced by the endothelium (103, 114). NO also has an important role in maintaining the vascular wall in a quiestant state by inhibiting inflammation, thrombosis and cellular adhesion and proliferation (22, 56). Hence, reduction or loss of NO results in shifting the properties of the vascular wall towards an inflammatory and pro-artherogenic phenotype (17).

A brief exposure to certain cytokines impairs endothelial function for days and this effect is called "Endothelial Stunning" (8, 60). It is further accentuated in presence of other underlying risk factors (8), particularly inflammation (60, 130, 132). There is a strong link between chronic, low grade inflammation and the progress of atherosclerosis (76). There is also a substantial amount of clinical and animal data showing that ageing is associated with chronic low grade inflammation (11, 26, 96). In addition, an acute inflammatory stimulus in the presence of low grade chronic inflammation transiently increases the risk of cardiovascular events (8, 60).

Acute induced inflammation, using Salmonella typhi vaccination, reduced flow-mediated vasodilation in the conduit artery in young healthy volunteers (60). We have previously shown similar results using the influenza vaccine in young healthy adults (39). Also, acute systemic inflammation induced by salmonella typhi vaccination increases aortic arterial stiffness while decreasing wave reflection in healthy young individuals (132). However to our knowledge, there

is no study showing the effect of induced acute systemic inflammation on endothelial function in healthy older adults.

An acute bout of moderate aerobic exercise reduces both central and peripheral femoral artery stiffness (66) while increasing endothelial function (105, 133). Acute moderate aerobic exercise is also anti-inflammatory, potentially due to the release of IL-6, which can inhibit TNF-∝ production (109) while stimulating the production of anti-inflammatory cytokines such as IL-1ra and IL-10 (117), creating an anti-inflammatory environment (101). Therefore, it is possible that acute moderate intensity exercise administered before vaccination-induced acute inflammation may have a protective effect on vascular function particularly in older individuals, but this has never been investigated. Consequently, the overall aim of this study was to first evaluate the effect of acute systemic inflammation induced by influenza vaccination on endothelial function and wave reflection in older adults. Furthermore, we sought to evaluate if acute moderate intensity endurance exercise immediately prior to vaccination-induced inflammation can prevent the negative effect of acute systemic inflammation on vascular function.

METHODS

Subjects

Fifty-nine healthy volunteers (men and women) between 55 – 75 years of age (mean age = 67) were recruited. Twenty-three men and 32 women completed the study while 1 man and 3 women dropped out of the study following the initial visit. The reasons for drop-out were change in medication (1 male) and change of mind (3 women). All subjects were free of acute cardiovascular or respiratory disease and none smoked. A stress test was performed on visit 1 to exclude any participant with evidence of stress induced myocardial ischemia. Exclusion criteria included participants with diagnosed uncontrolled hypertension, stroke, or myocardial infarction within 6 months prior to the study. Participants were excluded if they had metabolic disease (diabetes mellitus – Type II), inflammatory diseases (rheumatoid arthritis and systemic lupus erythematosus), bleeding disorder, or were taking medications known to affect inflammation (aspirin), and any form of smoking. Participants who had suffered from the common cold or influenza or upper respiratory tract infection 2 months preceding testing were also excluded. Participants who had already received a flu shot for the season, who were taking thyroid medications, or allergy medications on a regular basis were excluded as well. Subjects were also excluded if they were taking over the counter pain/anti-inflammatory medications. All subjects were recruited from the local community and provided written informed consent prior to participation. This study was approved by the Institutional Review Board of the University of Illinois at Urbana-Champaign.

Study Design

This was a randomized, double-blind, sham procedure-controlled crossover trial. The administration of vaccine and sham was counterbalanced within the groups. All subjects reported to the laboratory for a total of 9 days of testing. Before the vascular measures, subjects did not consume caffeine, alcohol or exercise for 12 hours prior to testing. All the participants were asked to fast for at least 10 hours. All the measurements for every visit were performed between 6.30am to 9.30am to control for diurnal variation. Participants rested in the supine position for a period of 10-min in a temperature-controlled room prior to testing. The sequence of vascular measures was as follows: blood pressure measurement, carotid artery tonometry, radial artery tonometry, brachial artery reactivity and carotid artery ultrasound imaging. The vascular measures were followed by a blood draw.

Study design:

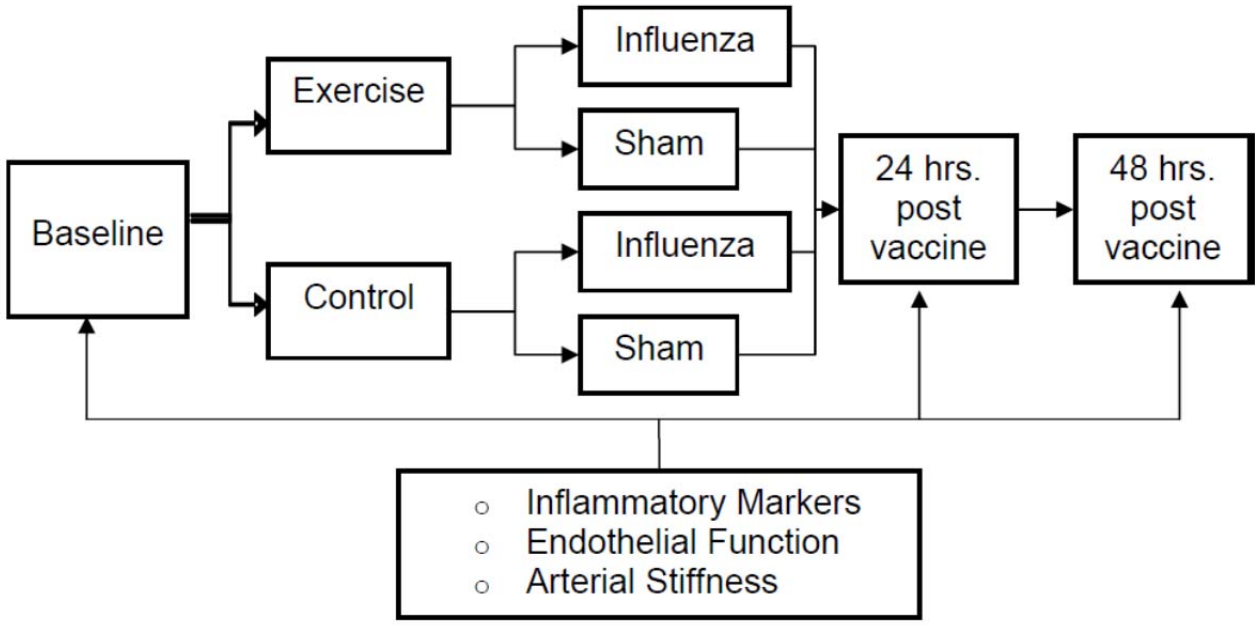

Figure 3.1: Participants were evaluated for inflammation, endothelial function and arterial stiffness in baseline condition, then randomized to exercise or control group. All the participants in both groups received influenza vaccine and sham injections followed by repeat evaluations at

24 and 48 hours post-injection. All the participants in both the groups were counterbalanced for the order in which the injections were administered.

Visit Details:

On Visit 1, all the included participants were asked to perform a peak aerobic capacity test (VO2peak) in presence of a physican using a modified Balke Treadmill protocol. Speed was maintained at 3.0 mph throughout the test while the grade increases by 2% every 2 minutes. The starting grade on the treadmill was 2%. Following the stress test the participants were randomly assigned to either the exercise group or the control group.

Participants received vaccine or the sham (normal saline – 0.5ml with sodium chloride 0.9% wt/vol into the deltoid muscle (shoulder) of their non-dominant arm on the 2^{nd} and 5^{th} visit, Subjects randomized to the exercise group performed a 40 minute moderate intensity aerobic exercise bout at an intenisty of 55 – 65% of their maximum HR immediately preceeding the both the vaccination and sham injection. Those randomized to the non-exercise group did not perfrom any physical activity preceeding the vaccination and sham injection. The participant and the researchers were blinded to when they received the vaccine or sham injection. Vascular measurements and blood draws were conducted at 24 and 48 hours following vaccination. The study design is represented in Figure 3.1.

Anthropometrics: Height and weight was measured using a stadiometer (to the nearest 0.5 cm) and a beam balance platform scale, respectively. BMI was calculated as weight (kg) divided by height (m) squared.

Brachial BP: Following 10 minutes in the supine position, resting systolic and diastolic BP was measured in the supine position using an automated oscillometric cuff (HEM-907 XL, Omron

Corporation, Japan). All BP measurements were made in duplicate and the average of the two values was recorded.

Carotid BP: Carotid artery pressure waveforms were attained using applanation tonometry (Millar Instruments, Houston, TX) and calibrated against brachial mean arterial and diastolic pressure.

Wave Reflection and Aortic BP: Applanation tonometery was performed using a high-fidelity strain-gauge transducer (SphygmoCor, AtCor Medical, Sydney, Australia) on the radial artery to obtain pressure waveforms. Using a generalized validated transfer function, a central aortic pressure waveform was reconstructed from the radial artery pressure waveform. Augmentation index (AIx) was calculated as the ratio of amplitude of the pressure wave above its systolic shoulder (i.e., the difference between the early and late systolic peaks of the arterial waveform) to the total PP and was expressed as a percentage. Because AIx is influenced by varied HR, AIx values were also normalized to an HR of 75 bpm.

Intima-media Thickness (IMT): The carotid artery was imaged with ultrasound (Aloka, SSD-5500, Tokyo, Japan) using a 7.5 MHz linear-array probe. IMT of the common carotid artery was defined as the distance between the leading edge of the lumen-intima interface to the leading edge of the media-adventitia interface of the far wall of the carotid artery. All measurements were made at end diastole. The IMT of the common carotid artery was determined from an average of 5 measurements obtained 20 mm proximal to the carotid bifurcation (37, 55, 57).

Carotid Artery Stiffness: The carotid artery was imaged with ultrasound (Aloka, SSD-5500, Tokyo, Japan) using a 7.5 MHz linear-array probe. Carotid BP was measured with applanation tonometery. Heart rate was recorded with a single lead electrocardiogram (ECG). β-stiffness

index (β), which adjusts arterial compliance for changes in distending pressure, was then calculated as follows:

$$\beta = \frac{\log P_1/P_0}{(D_1 - D_0/D_0)}$$

where D_1 and D_0 are the maximum (systolic) and minimum (diastolic) diameters, and P_1 and P_0 are the highest (systolic) and lowest (diastolic) carotid pressures. Our previous studies have successfully used this technique (37, 38, 55, 57)

Flow Mediated Vasodilatation (FMD): was assessed non-invasively using ultrasonography (Aloka, SSD 5500 Japan). The subjects were in supine position with their right arm outstretched. A rapid release cuff (Hokanson DE) was placed below the elbow joint on the widest part of the forearm. The brachial artery was imaged in longitudinal section, 5–10 cm proximal to placement of a blood pressure cuff, just below the antecubital fossa, using a high frequency (5–13 MHz) linear array probe. Once the image was obtained the arm was maintained in a stable position using a custom design immobilizer cushion, while the ultrasound transducer was stabilized using a clamp. Split screen was used to measure the arterial diameter (B-mode) on the left side of the screen while Doppler velocity was measured on the right side of the screen. The flow signals were corrected at an insonation angle of 60 degrees. The sample volume was placed in the middle of the artery with a large sample. Baseline measurement was recorded for 30 seconds followed by cuff inflation of 250 mmHg. The images were recorded at 5 frames/second using Vascular Tools (MIA). The ischemic stimulus was maintained for 5 minutes. Image capture was restarted 30 seconds prior to cuff deflation and continued until 180 seconds post-deflation.

Image Analysis: Analysis of the brachial artery diameter was carried out using an automated edge detection software system (Medical Imaging Applications, Iowa) as an off-line analysis.

The vertical and horizontal calibration was set based on the ultrasound settings for each individual participant. Following the calibration, region of interest (ROI) was set on the portion of the artery where the walls were most clear. The technician only edited the images where the intima lumen interface was not detected correctly by the software. The baseline and peak dilation were recorded at end-diastole of the cardiac cycle. The data is presented as percent change from baseline diameter.

Blood velocity image analysis: Similar to the arterial diameter, following the calibration, ROI was set around the Doppler waveform and the velocity-time integral (automated) was used to calculate the mean velocity. This method conforms to the guidelines set out for the ultrasound measurement of endothelium-dependent FMD of the brachial artery (19).

Brachial artery shear stress: Brachial artery shear stress was calculated using the formula: (8*Vm) / D, where Vm is the mean blood velocity and D is the arterial diameter. Following this, shear stress AUC was calculated using GraphPad (v. 4.03, La Jolla, CA) above the baseline to time to peak dilation. Normalization of FMD to shear stress was calculated by FMD/SS (AUC).

Peak Aerobic Capacity and Stress Test:

Peak oxygen consumption ($VO_{2\ peak}$) was assessed using a modified Balke protocol until volitional fatigue in presence of a physician. Expired gases were analyzed using a Quark b^2 breath-by-breath metabolic system (Cosmed, Rome Italy). 12 lead EKG was recorded throughout the test. Following the test, all participants underwent 3 minutes of active recovery followed by 5 minutes of passive recovery.

Blood analysis: Following an overnight fast, blood samples were collected using a butterfly needle inserted into the antecubital vein. Samples were collected into 10 ml tubes containing

EDTA (anticoagulant and chelating agent). Samples were separated by centrifugation 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 to assess systemic inflammation. High-sensitivity Separate Quantikine enzyme-linked immunosorbent assay (ELISA) kits (R&D systems, Minneapolis, MN & Abnova, Taipei, Taiwan) were used to measure serum IL-6 and CRP respectively.

Power analysis / Justification of sample size

We calculated the minimum sample size necessary for our design using our pilot data and other relevant studies (39, 60, 132). With a total sample size of 60 total subjects (n= 30 per group), we achieved a power of 80% for detecting the effects that we anticipate at a significance level of $p< 0.05$.

We performed sample size calculation using our pilot data for IL-6 and CRP as inflammatory markers. We have shown a 58% difference in the CRP values in the vaccination group after 48 hours as compared to the sham group generating an effect size of 0.50. Thus, we estimated that a total sample size of 33 subjects would provide 80% power at the 5% level of significance with a two tailed test. We have reported a 54% increase in IL-6 48 hrs. after vaccination also generating an effect size of 0.50. Hence, we estimated that a total sample size of 33 subjects would provide for achieved 80% power at the 5% level of significance. To estimate the effect size of the effect of acute exercise on vaccination induced inflammation, we used the data from Starkie et. al. (116). They showed that that TNF-α increased significantly more in a non-exercised group compared to an exercise group following an endotoxin infusion. Based on their data we estimated that a total of 13 subjects will be needed for 80% power at the 5% level of significance to detect a difference in TNF-α values between exercise and control.

We used our own pilot data to estimate the effect size for flow-mediated dilation. FMD decreased by 2.04 units with a SD of 3.1 generating an effect size of 0.66 between vaccination and sham in our pilot study. Hence, we estimated that a total sample size of 21 subjects would provide 80% power at the 5% level of significance. PWV increased by 10.22% following vaccination compared to sham after 48 hours with and effect size of 0.91. Thus, we estimated that a total sample size of 12 subjects would provide 80% power at the 5% level of significance.

We then performed a power analysis to detect an interaction between vaccination (sham vs influenza) and exercise (exercise or no exercise) using an ANOVA model on the variables with smallest effect size. This analysis yielded an estimated a total sample size of 56 for this study. Nevertheless, because we used a novel design with variables not studied previously in this context our goal was a sample size of 60 for this study.

Statistical Analysis

All data are reported as means + SEM. A priori significance was set at $p < 0.05$. Normality of distribution was assessed. If the data were not normally distributed, outcome measures were logarithmically transformed. A 3-factor model Analysis of variance with repeated measures with condition (flu and sham) by time and the group factor was acute exercise. Analysis of variance with repeated measures was used to assess changes in other continuous outcome variables.

Based on cardiorespiratory fitness classifications in American College of Sports Medicine Guidelines (1), below 20^{th} percentile for age and sex is indicative of sedentary life style and increased risk of death from all causes and hence they were categorized as poor. Participants between 20^{th} to 50^{th} percentiles were categorized as Fair and everyone above 50^{th} percentile were categorized as Good based on age and sex. This categorization was done as our sample was not

large enough to categorize them based on 5 categories. A 3 by 3 (time * fitness category) ANOVA with repeated measures with fitness category as a between factor, and time of measurement (baseline, 24 hour and 48 hour post) was the within factor, was used to assess the effect of fitness on continuous outcome variables. Pearson's correlation coefficients were used to assess relationships between variables of interest. Data analysis was carried out using Statistical Package for the Social Sciences (SPSS, v 18, SPSS, Inc., Chicago, IL).

Results:

The baseline participant characteristics are presented in Table 3.1. There were no significant differences between the acute exercise and the control group at baseline (Table 3.1). There were also no significant effects of the acute exercise condition on any variables (Table 3.2), thus the data of the exercise and the control group were combined to evaluate the effect of influenza vaccination. There was a significant interaction (condition * time) in CRP and IL-6 for 24 and 48 hours after the Influenza vaccine compared to the sham injection. There was a significant increase in CRP at 24 hour and 48 hour time point (figure 3.2) and IL-6 at 24 hour time point (Figure 3.3) following the Influenza vaccine while there was no change in these variables following the sham injection. There were no significant differences in FMD or normalized FMD at 24 and 48 hours as compared to baseline following the Influenza vaccine (Figure 3.4). There were no significant differences in any blood pressure measures, AIx or carotid β-stiffness at 24 and 48 hours compared to baseline following the Influenza vaccine (Table 3.3). There were no significant correlations between change in inflammatory markers (CRP and IL-6) and change in FMD (data not shown).

There was a significant time by fitness category interaction for FMD. The baseline FMD was significantly higher in the good fitness category compared to the poor category (Figure 3.5). There was a significant decrease in the FMD at 48 hours compared to the baseline in both fair and good fitness category groups. Also, there was a significant decrease in the FMD at 24 hours compared to the baseline in the good fitness category group. There was a significant increase in CRP at 24 hours compared to the baseline in the individuals with poor fitness while the individuals with fair and good fitness did not show any significant changes at 24 hours (Figure 3.6).

Discussion:

The primary and novel finding of the study was that even though there was a significant change in the inflammatory markers following Influenza vaccination, there were no alterations in endothelial function or arterial stiffness in the older adults in this study. This finding was not consistent with our hypothesis that older adults would present with reduced arterial function 24 and 48 hours following an Influenza vaccine induced inflammation, as has been previously shown in younger populations (60). To our knowledge this is the first study to show a disassociation between inflammation and endothelial function in older adults. The second major finding of this study was that acute moderate aerobic exercise immediately before the Influenza vaccination had no effect on inflammation or arterial function. Our third major finding was that the FMD response to influenza vaccination was dependent on fitness.

Hingorani et. al. reported that there was a decrease in flow-mediated vasodilation in the conduit artery 8 hours after Salmonella typhi vaccination coupled with mild acute systemic inflammation in young healthy volunteers (60). Similarly, Vlachpoulos et. al reported a temporary increase in arterial stiffness following a similar vaccination also coupled with systemic inflammation in healthy young individuals (132). Although others have shown that more noxious stimuli such as endotoxin induced both inflammation and "Endothelial Stunning" (8), it is clear that a more mild stimulus such as Salmonella typhi vaccination also produces significant increases in inflammation and decreases in arterial function (60, 132). We have also reported similar findings in young participants, using influenza vaccination, which induced acute inflammation coupled with an increase in blood pressure and arterial stiffness (106). We have also shown a significant decrease in FMD following the Influenza vaccination in young healthy individuals (39). These findings demonstrate that influenza vaccination causes a temporary

decrease in endothelial function and an increase in central blood pressure and arterial stiffness in young healthy individuals, presumably as a result of the vaccination induced inflammation.

Acute submaximal exercise does not increase pro-inflammatory cytokines (TNF-∝ and IL-1 β) but IL-6 increases up to 100 times and decreases in the post-exercise period (40, 98, 101, 122). There are also increases in IL-1ra and sTNF-R which are known cytokine inhibitors and other anti-inflammatory cytokines like IL-10 (94, 95). Pedersen et. al. showed that moderate exercise increases the plasma IL-6 levels in older adults (99). Knowing that IL-1ra and IL-10 exert anti-inflammatory properties, the stimulation of these cytokines by IL-6 seems to suggest that IL-6 may create an anti-inflammatory environment (101) during moderate exercise. This anti-inflammatory environment could then prevent decreases in endothelial function. However, we found no effect of acute exercise prior to vaccination on either inflammation or endothelial function, following vaccination (Table 3.2). This may suggest that even though moderate exercise may enhance immune function immediately following the exercise bout, this had no lasting effect and did not affect the inflammatory or endothelial response 24 and 48 hours following vaccination. Hence, we decided to combine the data of the exercise and the control group to evaluate the effect of influenza vaccination.

To our knowledge this is the first study to use the model of induced inflammation in healthy older adults, and our data suggest that inflammation and reductions in FMD are uncoupled in this population. Even though we observed significant increases in inflammatory markers (Table 3.3) at 24 and 48 hours following the Influenza vaccine, we did not observe any significant changes in endothelial function, arterial stiffness, wave-reflection (AIx) or blood pressure. Also, there was no significant correlation between change of CRP and IL-6 and the change in FMD or any other measure of arterial function. Taken together, these data support the notion of dissociation

between the inflammatory changes and endothelial function in older adults. Consequently, the reduction in arterial and endothelial function due to vaccination is apparently only observed in young healthy individuals, suggesting that aging may affect the acute inflammation-vascular function interaction, or that the changes in FMD in young individuals may actually be unrelated to changes in inflammation.

There may be several reasons for the difference in our findings compared to previous studies. We speculate that one of the reasons may be that the regular seasonal Influenza vaccine may not be a strong enough stimulus to result in arterial dysfunction in older adults. As endothelial dysfunction may be dependent on inflammatory changes we compared the percent change in CRP from baseline at 24 to 48 hours between older adults to data previously published for young healthy adults (39). Young adults increased CRP 139 and 358% at 24 and 48 hours compared to 52 and 67% respectively for the older adults in our study. The study design and the equipment used were similar in both of these studies. Thus, the older individuals in our current study exhibited an attenuated change in inflammation. Furthermore, the older individuals exhibited higher baseline values for CRP compared to younger adults, consistent with previous studies showing low-grade inflammation with aging. Taken together this seems to suggest that the older individuals already have low grade inflammation and the Influenza vaccine may not cause a large enough inflammatory response to affect arterial function.

Another potential reason could be that older adults already present with lower endothelial function and higher wave reflection at baseline. Numerous studies have shown FMD to be impaired with aging (28, 35, 44, 92, 110). Parker (97) et. al. reported that older women had about 50-60% lower FMD in both brachial and popliteal arteries and hence they concluded that the age-associated decline in FMD may be partly due to the decreased responsiveness of smooth

muscle (97). In addition, studies have shown that in individuals over the age of 40 years, the reflected wave augments blood pressure in late systole and causes a positive aortic AIx (53). There are pathological increases in AIx that can occur in this age group as a result of either increase in magnitude or hastened timing of the reflected wave or both (53, 54, 110). O'Rourke reported that with progressive aortic degeneration as seen with ageing, the systolic pressure increases indirectly by the early return of the wave reflection (5). Hence, it may be possible that induced inflammation could not further deteriorate what may have been persistent arterial dysfunction.

Fitness also affects FMD, especially in older adults (35). Exercise trained older adults have demonstrated similar endothelial function to young sedentary men. Others have shown similar results (23). Hence, as our sample had both sedentary and physically active older adults we compared the endothelial function based on fitness categories constructed from the VO2peak data generated from the exercise tests (poor, fair and good). As our sample size was not large enough, we combined the percentiles as mentioned in the analysis section, based on American College of Sports Medicine (ACSM) guidelines (1). Consistent with previous literature (129), FMD was significantly higher in individuals with good fitness compared to those with poor fitness at baseline (Figure 3.5). However, there was a significant decrease in the FMD at 24 hours and 48 hours following the Influenza vaccine only in individuals with good fitness (Figure 3.5) with a significant decrease at 48 hours in individuals with fair fitness. The individuals with poor fitness did not have any changes FMD following the Influenza vaccine. This supports our previous speculation that in some of the older adults who already have lower endothelial function, the Influenza vaccine may not be able to further perturb arterial dysfunction. Also, CRP

was higher at baseline in individuals with poor fitness. Interestingly, individuals with poor fitness showed the highest increase in CRP 24 hours later (Figure 3.6).

As the inflammatory markers were disassociated from the endothelial function in this group, we explored other factors that may have caused the differential changes in FMD based on the fitness of an individual. As previously shown, increases in the wall thickness and arterial stiffness may limit the extent of maximal dilatation (135). However, we found that the carotid β-stiffness (Table 3.4) was not significantly different between groups or at 24 and 48 hours. Hence, arterial stiffness is an unlikely reason for the differential effects. Furthermore, since blood pressure remained unchanged between groups and over time, it seems unlikely that blood pressure could affect the response. Another factor may be a change in baseline vasodilatation as a result of vaccination, which could have affected the change in FMD. We found a significant increase in baseline brachial artery diameter in the individuals with poor fitness at 24 and 48 hours compared to the individuals with fair and good fitness (Table 3.4). It is possible that the increased brachial artery diameter may have left little margin for vasodilatation in the individuals with poor VO2. However, we can only speculate on mechanisms involved as this was beyond the scope of this study.

There were several limitations to this study. Even though the study concentrated on the association between inflammation and endothelial following induced acute systemic inflammation in older adults, one of the limitations was the absence of young adults for comparison. Statins have been shown to affect post-vaccination changes in FMD and inflammation. Hence, this may be a confounding factor in this study with older adults. However, there were only 18 participants who reported using statins and importantly, as we show that there seems to be a disassociation between inflammation and endothelial function, the effect of statin

on inflammation may not have affected the results in the present study. Also, given the number of medications and factors we had controlled for in our exclusion criteria, it was impractical to also exclude older adults currently using statin drugs. Finally, although our sample size was much larger than similar studies in young adults, our sample may not be representative of other groups of older adults.

Conclusion: To our knowledge, this is the first study to demonstrate there is dissociation between inflammation and endothelial function following induced acute systemic inflammation in older adults. We also demonstrated that the response of FMD and inflammatory markers to induced acute systemic inflammation were dependent on fitness, but acute exercise had effect on either inflammation or arterial function.

Table 3.1: Comparison of descriptive variables between exercise and control group

	Exercise (n=28)	Control (n=27)
Age (years)	66 ± 0.93	67 ± 0.77
Height (cms)	168.54 ± 1.68	165.61 ± 2.28
Weight (kg)	79.96 ± 2.78	71.52 ± 3.49
BMI (kg/m^2)	28.07 ± 0.83	25.94 ± 0.98
VO2peak	25.90 ± 1.20	25.14 ± 1.29
IMT (mm)	0.67 ± 0.02	0.69 ± 0.01

Note: Values are mean ± SEM. BMI, Body Mass Index; VO2peak, peak aerobic capacity; IMT, Intima-media Thickness. There were no significant differences between groups

Table 3.2: Arterial function and inflammatory markers in exercise and control group following influenza vaccine and sham injection

	Exercise (n=28)					Control (n=27)				
		Flu		Sham			Flu		Sham	
	Baseline	24 hours	48 hours	24 hours	48 hours	Baseline	24 hours	48 hours	24 hours	48 hours
AIx75 (%)	24.11 ± 1.98	24.13 ±0.92	22.95 ± 1.17	24.38 ±0.87	24.07 ± 1.01	24.75 ± 1.68	25.43 ± 0.9	25.55 ± 1.15	25.78 ± 0.86	24.99 ±0.99
β-stiffness	12.67 ± 0.83	12.37 ± 0.75	12.39 ±0.66	17.55 ± 4.02	12.81 ± 0.62	12.70 ± 0.91	11.93 ± 0.75	12.01 ± 0.66	11.63 ± 4.02	12.01 ± 0.62
FMD (%)	5.59 ± 0.65	3.76 ± 0.69	3.53 ± 0.51	4.77 ± 0.62	4.86 ± 0.67	5.91 ± 0.71	5.46 ± 0.62	4.41 ± 0.46	4.82 ± 0.56	5.04 ± 0.61
NFMD (AU)	0.07 ± 0.02	0.10 ± 0.08	0.04 ± 0.05	0.06 ± 0.04	0.11 ± 0.03	0.10 ± 0.03	0.13 ± 0.07	0.13 ± 0.05	0.11 ± 0.03	0.04 ± 0.02
IL-6 # (ng/mL)	1.55 ± 0.24	2.09 ± 0.32	1.47 ± 0.26	1.35 ± 0.26	1.22 ± 0.15	1.34 ± 0.15	2.31 ± 0.41	2.05 ± 0.43	2.05 ± 0.43	1.87 ± 0.37
CRP # (mg/L)	2.51 ± 0.48	3.35 ± 0.53	3.42 ± 0.47	2.29 ± 0.39	2.18 ± 0.45	2.56 ± 0.60	2.74 ± 0.43	2.98 ± 0.49	2.29 ± 0.46	2.49 ± 0.51

Note: Values are mean ± SEM. AIx75, Augmentation Index at heart rate of 75bpm; β-stiffness, Beta stiffness; FMD, Flow mediated dilation; NFMD, Normalized flow mediated dilation; IL-6, Interleukin 6; CRP, C-reactive protein. # Significant condition (Flu vs. Sham) and time interaction ($p<.05$). There was no significant effect of exercise on any variable.

Table 3.3: Comparison of blood pressure, arterial function and inflammatory markers

		Flu (n=53)		Sham (n=54)	
	Baseline (n=54)	24 hours	48 hours	24 hours	48 hours
SBP (mmHg)	125.88 ± 1.87	125.25 ± 1.97	123.36 ± 1.98	125.69 ± 1.75	125.02 ± 1.72
DBP (mmHg)	71.62 ± 1.14	70.55 ± 1.19	80.87 ± 10.05	71.15 ± 1.25	71.59 ± 1.19
MAP (mmHg)	89.71 ± 1.87	90.45 ± 1.33	89.72 ± 1.40	91.22 ± 1.35	91.17 ± 1.27
aorMAP (mmHg)	91.30 ± 1.28	90.47 ± 1.33	89.70 ± 1.40	91.25 ± 1.35	91.11 ± 1.26
AIx75 (%)	24.43 ± 1.26	24.79 ± 1.25	24.28 ± 1.32	24.98 ± 1.24	24.39 ± 1.31
β-stiffness	12.68 ± .61	12.16 ± .61	11.95 ± .66	14.37 ± 2.63	12.26 ±.60
Arterial Compliance (mm^2/kPa)	.70 ± .03	.74 ± .03	.75 ± .04	.76 ± .04	.72 ± .04
FMD (%)	5.65 ± .48	4.57 ± .44	3.93 ± .33	4.85 ± .39	5.01 ± .42
NFMD (AU)	.08 ± .02	.10 ± .03	.08 ± .02	.07 ± .01	.08 ± .02
IL-6 # (ng/mL)	1.44 ± .14	2.20 ± .25 *	1.76 ± .25	1.70 ± .25	1.55 ± .20
CRP # (mg/L)	2.53 ± 0.37	3.05 ± 0.34 *	3.21 ± 0.33 *	2.29 ± 0.31	2.33 ± 0.34

Note: Values are mean ± SEM. # interaction between condition and time ($p<.05$). * differs significantly from baseline ($p<.05$).

Table 3.4: Comparison of blood pressure and arterial structure variables in fitness categories at baseline and 24 and 48 hours following influenza vaccine

	Poor			Fair			Good		
	Baseline	24 h	48 h	Baseline	24 h	48 h	Baseline	24 h	48 h
SBP	130.71 ± 3.85	128.57 ± 3.95	128.64 ± 3.82	123.06 ± 3.60	120.50 ± 3.69	116.56 ± 3.57	122.53 ± 3.72	124.93 ± 3.81	116.56 ± 3.57
MAP	93.21 ± 2.61	91.92 ± 2.61	93.64 ± 2.75	90.31 ± 2.44	87.93 ± 2.44	85.62 ± 2.57	89.53 ± 2.52	89.93 ± 2.52	90.66 ± 2.66
β stiffness	11.52 ± 1.15	11.29 ± 1.24	10.00 ± 1.27	14.52 ± 1.08	13.74 ± 1.16	13.79 ± 1.18	11.19 ± 1.11	12.05 ± 1.2	12.64 ± 1.22
Brachial Diameter	4.22 ± 0.16	4.44 ± 0.16 *	4.39 ± 0.15 *	4.28 ± 0.16	4.38 ± 0.16	4.39 ± 0.15	4.42 ± 0.18	4.48 ± 0.17	4.31 ± 0.17

Note: Values are mean ± SEM. SBP, Systolic Blood Pressure; MAP, Mean Arterial Pressure; β-stiffness, Carotid beta stiffness; * differs significantly from baseline ($p<.05$).

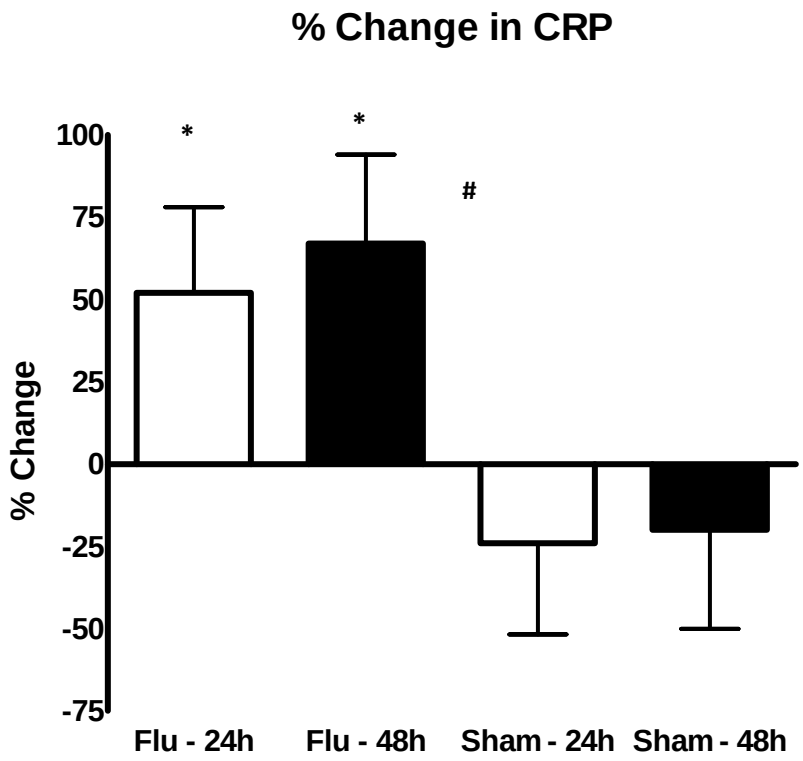

Figure 3.2: CRP changes in older adults from baseline following influenza vaccine and sham. * indicates there was a significant interaction between Flu and Sham ($p<0.05$). # indicates that the flu condition was significantly greater than the sham condition ($p<.05$)

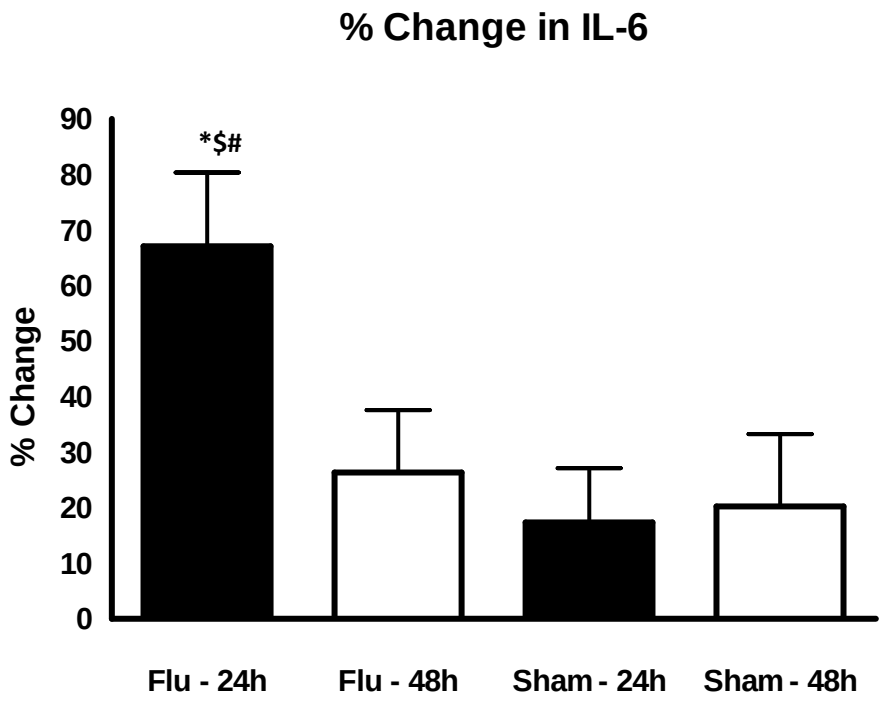

Figure 3.3: IL-6 changes in older adults from baseline following influenza vaccine and sham. # indicates there was a significant interaction between flu and sham injections ($p<.05$). * indicates that the flu injection was significantly greater than the sham injection. $ indicates that the 24 hour time point was significantly greater than the 48 hour time point for the flu injection ($p<.05$).

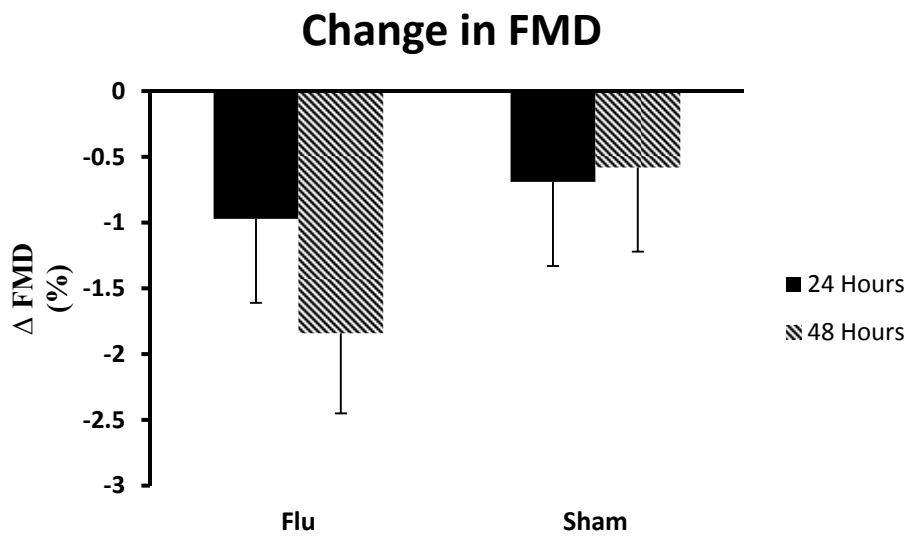

Figure 3.4: Changes in flow mediated dilation (FMD) from baseline following influenza vaccine and sham. There were no statistically significant effects.

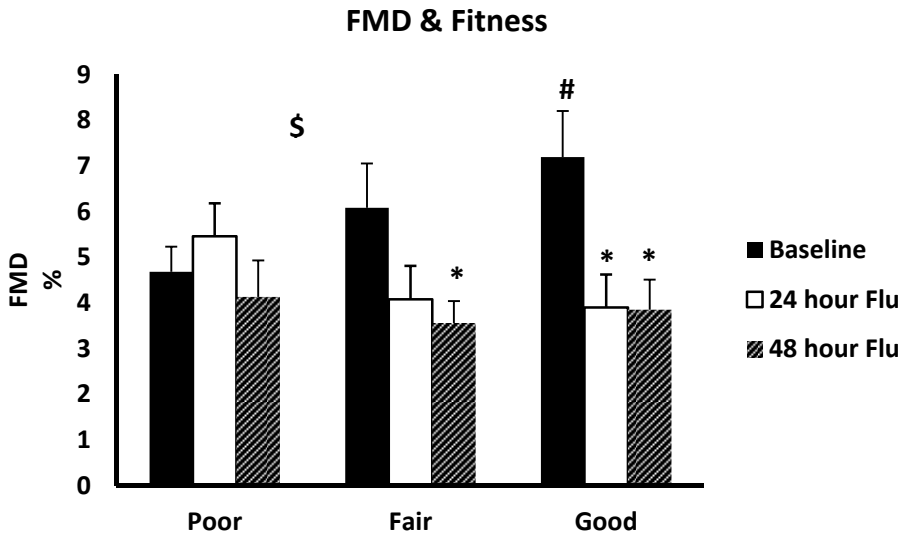

Figure 3.5: Flow mediated dilation (FMD) at baseline, 24 hours and 48 hours following influenza vaccine based on the fitness categories of poor (n= 20), fair (n=15), good (n=10). $ indicates a significant fitness group by time interaction ($p<0.05$). # indicates significantly different from the baseline values of the Poor group ($p<0.05$). * indicates significantly different from baseline within groups ($p<0.05$).

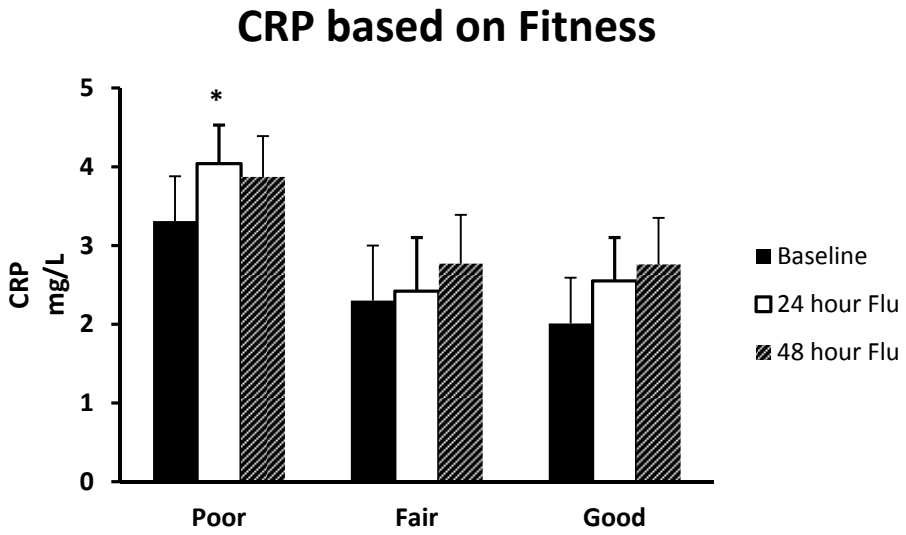

Figure 3.6: CRP values at baseline, 24 hours and 48 hours following influenza vaccine based on the fitness categories of poor (n= 22), fair (n=12), good (n=9). There was a significant interaction between group and time ($p<.05$). Flu vaccination increased CRP only in the poor fitness category. * indicates significantly different from baseline ($p<0.05$)

CHAPTER 4

Effect of Acute Moderate Exercise on Vaccination Induced Inflammation and Vaccine Efficacy in Older Adults

The influenza virus can cause a disproportionate increase in serious illness and deaths in individuals 65 years of age and older (138). The most effective way of avoiding influenza is through Influenza vaccination. However, the vaccine is ineffective in about 25% of the older population (47, 138). Numerous reasons may cause the lower influenza vaccine response in the older adults including, vaccination history, and exposure to influenza viruses, living situation, dietary factors and immunoscenescence (7, 47). Hence, studying the role of exogenous and endogenous adjuvants to increase the efficacy of the vaccine is important.

It has been shown that high levels of chronic stress (psychological or physical) are detrimental to immune system function (18, 46). Chronic stress has been shown by to reduce influenza vaccine antibody titers in all age groups (79, 102). However, studies have shown that acute stress can be immunoenhancing (9, 24, 100, 136).

An acute psychological stressor and an acute physical stressor in close proximity to the administration of influenza vaccination (31) induced higher antibody titers for A/Panama strain in women compared to an unstressed control group. It has been shown that acute exercise prior to an influenza vaccine produced greater antibody responses in young women compared to young men (31). However, other studies (68, 69) have not reported any sex differences. In a more recent study, Campbell et. al. noted that that the eccentric exercise in young healthy adults did not further augment the antibody titer in the exercise group as compared to the control group (13). Thus, it is currently unclear if acute exercise can enhance the efficacy of influenza vaccination. The primary aim of this study was to evaluate the effect of acute moderate aerobic

exercise immediately prior to administration of the influenza vaccine on vaccine efficacy in older adults. A second exploratory aim was to investigate if acute exercise immediately prior to an influenza vaccine produces different effects on antibody response in older men versus women.

Methods:

Subjects

Fifty-nine healthy volunteers (men and women) in the age group of 55 – 75 years (mean age = 67) were recruited. Twenty three men and 32 women completed the study while 1 man and 3 women dropped out of the study following initial visit. The reasons for drop-out were change in medication (1 male) and change of mind (3 women). All subjects were free of cardiovascular or respiratory disease and none smoked. A stress test was performed on visit 1 to exclude any participant with evidence of stress induced ischemia. Exclusion criteria involved participants with diagnosed uncontrolled hypertension, stroke, or myocardial infarction within the 6 months prior to the study. Participants were excluded if they had metabolic disease (diabetes mellitus), inflammatory diseases (rheumatoid arthritis and systemic lupus erythematosus), bleeding disorder, or were taking medications known to affect inflammation (aspirin), and any form of smoking. Participants who had suffered from common cold or influenza and bacterial or viral infection or upper respiratory tract infection 2 months preceding testing were also excluded. Participants who have already received a flu shot for the season, who were taking thyroid medication, or allergy medication on regular basis were excluded as well. Subjects were also excluded if they were taking over the counter pain/anti-inflammatory medication. All subjects were recruited from the local community and provided written informed consent prior to participation. This study was approved by the Institutional Review Board of the University of Illinois at Urbana-Champaign.

Study Design

Subjects were randomized to an acute exercise or no exercise (control) group. Before the measures, subjects did not consume caffeine, alcohol or exercise for 12 hours prior to testing. All the participants were asked to fast for at least 10 hours. All the measurements for every visit were performed between 6.30am to 9.30am to control for any diurnal variation. Participants rested in the supine position for a period of 10-min in a temperature-controlled room prior to testing.

Visit Details:

On Visit 1, all the included participants were asked to perform a maximal aerobic capacity test (VO2peak) in presence of a physican using a modified Balke Treadmill protocol. Speed was maintained at 3.0 mph throughout the test while the grade increases by 2% every 2 minutes. The starting grade on the treadmill was e 2%. Following the stress test the participants were randomly assigned to either the exercise group or the control group.

Participants received vaccine in the deltoid muscle (shoulder) of their non-dominant arm on the 2nd visit, Subjects randomized to the exercise group performed a 40 minute moderate intensity aerobic exercise bout at an intenisty of 55 – 65% of their maximum HR immediately preceeding the both the vaccination and sham injection. Those randomized to the non-exercise group did not perform any physical activity preceeding the vaccination and sham injection. The participant and the researchers were blinded to when they received the vaccine or sham injection.

On the 24 and 48 hours visits the partipants underwent blood draws.

Efficacy: All participants visited the lab 4 weeks after receiving the Influenza vaccine for the efficacy blood draw.

Anthropometrics: Height and weight was measured using a stadiometer (to the nearest 0.5 cm) and a beam balance platform scale, respectively. BMI was calculated as weight (kg) divided by height (m) squared.

Brachial BP: Following 10 minutes in the supine position, resting systolic and diastolic BP was measured in the supine position using an automated oscillometric cuff (HEM-907 XL, Omron Corporation, Japan). All BP measurements were made in duplicate and the average of the two values was recorded.

Maximal Aerobic Capacity and Stress Test:

Peak oxygen consumption ($VO_{2\ peak}$) was assessed using a modified Balke protocol until volitional fatigue in presence of a physician. Expired gases were analyzed using a Quark b^2 breath-by-breath metabolic system (Cosmed, Rome Italy). 12 lead EKG was recorded throughout the test. Following the test, all participants underwent 3 minutes of active recovery followed by 5 minutes of passive recovery.

Blood analysis: Following an overnight fast, blood samples were collected using a butterfly needle inserted into the antecubital vein. Samples were collected into 10 ml tubes containing EDTA (anticoagulant and chelating agent). Samples were separated by centrifugation 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 to assess systemic inflammation. High-sensitivity Separate Quantikine enzyme-linked immunosorbent assay (ELISA) kits (R&D systems, Minneapolis, MN & Abnova, Taipei City, Taiwan) were used to measure serum IL-6 and CRP respectively.

The Influenza anti-body titers were measured using a haemagglutination inhibition test. The Fluarix vaccine for 2010-11 contained 3 viral strains: H3N2, H1N1 and B-Florida and the Fluarix vaccine for 2011-12 contained the same viral strains. The serum samples were analyzed by the Clinical Virology Lab at Hackensack Medical Center, New Jersey, USA.

Power analysis / Justification of sample size

Based on previous data showing higher antibody titers in individuals who performed moderate exercise prior to vaccination (31), we calculated an effect size of 1.25 resulting in an estimated N of 18 subjects will be needed for 80% power at the 5% level of significance to detect a difference in vaccine efficacy between exercise and control.

We then performed a power analysis to detect an interaction between exercise (exercise or no exercise) using an ANOVA model on the variables with smallest effect size. This analysis yielded an estimated a total sample size of 56 for this study. Nevertheless, because we are proposing a novel design with variables not studied previously in this context we aimed for a total sample size of 60 for this study.

Statistical Analysis:

All data are reported as means + SEM. A priori significance was set at $p < 0.05$. Normality of distribution was assessed. If the data were not normally distributed, outcome measures were logarithmically transformed. A 2 (time) by 2 (exercise group as between subject factor) Analysis of Variance with repeated measures (ANOVA) was performed for all the continuous variables. A 2 (time) by 2 (sex as between subject factor) Analysis of Variance with repeated measures (ANOVA) was performed for all the continuous variables. Because the baseline antibody titers for the H1N1 strain were lower in women in the exercise group, we performed a 2 (exercise vs

control) by 2 (men vs women) analysis of covariance on the H1N1 antibody titers at the 4 week time point, using baseline values as the covariate. Pearson's correlation coefficients were used to assess relationships between variables of interest. Data analysis was carried out using Statistical Package for the Social Sciences (SPSS, v 18, SPSS, Inc., Chicago, IL).

Results:

The participant characteristics are presented in Table 4.1. There were significant time effect in CRP and IL-6 (Figure 4.1) at 24 and 48 hours as compared to baseline after the Influenza vaccine. Both CRP and IL-6 increased significantly at 24 and 48 hours as compared to the baseline (Figure 4.1). There were no differences in the change of inflammatory markers between the exercise and the control group. There were no differences in the levels of antibody titers for H3N2 between the exercise groups as compared to the control group (Figure 4.2). There was a significant time by gender by exercise group interaction for H1N1 strain (Figure 4.3). The antibody titers increased significantly post 4 weeks in all the groups except women in the control group. There was a significant difference in the pre antibody titers for H1N1 in women. When covarying for baseline values of H1N1, there was still a significant interaction of exercise and sex, showing that the women in the exercise group increased their H1N1 antibody titers significantly more than the women in the control group. There were no differences in the number of sero-protected individuals between exercise and control group (Table 4.3). There was a strong correlation ($r = 0.7$) between IL-6 levels 24 hours post-vaccination and antibody titers post 4-weeks in the exercise group. The correlation was slightly weaker in the control group ($r = 0.5$).

Discussion:

To our knowledge, this is the first study to evaluate the effect of acute moderate aerobic exercise prior to Influenza vaccination on antibody response in older adults. In the present study we did not find any evidence of immune-enhancement as a result of acute moderate aerobic exercise in older men, but exercise provided an immune enhancement for the H1N1 strain in older women.

Studies have shown that acute stress can be immune-enhancing (9, 24). The immune-enhancement of an acute stressor is related the duration of the stressor, type of stressor and the temporal relationship between the stressor and the challenge (24). Close proximity of the acute stressor prior to the antigen exposure seems to boost the immune system (24). In the present study, the acute moderate exercise prior to the Influenza vaccination did not provide immune-enhancement for men, while there was a selective immune enhancement for the H1N1 strain in women. Interestingly, the overall sero-protection was unaltered by exercise in the present study.

There may be several possible explanations for this lack of enhanced sero-protection. It is possible that the intensity and mode of the acute exercise could affect the findings. Our choice of moderate aerobic exercise was partly based on previous data using cycle ergometry at 55% of predicted maximum workload where they reported that the antibody titers for A/Panama strain were higher in women for both mental and exercise group as compared to the control group (31). As our participant population was older adults we selected moderate aerobic exercise bout between 55% - 65% of heart rate maximum for 40 minutes on treadmill based on American College of Sports Medicine (ACSM) guidelines (1). This intensity of the exercise may not have been a strong enough stimulus to augment vaccine efficacy. However, intense and prolonged exercise is associated with a post-exercise “open window” for infection because of a high plasma cortisol level, decrease in the activity of NK cells, decrease in granulocyte oxidative burst

activity, decrease in mitogen-induced lymphocyte proliferation and a decrease in nasal mucociliary clearance (86, 88, 89, 137). Hence, using a higher intensity of exercise may be detrimental for the antibody response in the older adults.

Campbell et. al. have previously shown that there was no immuno-enhancement in young healthy individuals with a relatively healthy immune system (13). The authors suggested that the immune response to the influenza vaccine could be maximal and hence, there would be limited room for immunoenhancement by exercise. Similarly, although our study focused on older individuals, our inclusion criteria were set specifically for healthy individuals and hence this may explain the lack of immune-enhancement in men and lack of overall enhancement of sero-protection. However, considering the relatively large number of individuals who did not receive sero-protection in the present study, this may be an unlikely explanation.

It has been shown that acute exercise prior to an influenza vaccine produced greater antibody responses in young women compared to young men in the A-Panama strain (31). However, other exercise training studies (68, 69) have not reported any sex differences following vaccination. The results from the present study show that acute moderate intensity aerobic exercise has no differential effects between older men versus women on the antibody titer of H3N2 strain (Figure 4.2) of the Influenza vaccine. Interestingly, there was a significant exercise effect in women as compared to men for the H1N1 strain of the Influenza vaccine (Figure 4.3). However, we had to be careful in interpretation of these data as the females in the exercise group had significantly lower pre vaccination antibody titers. The post antibody titers were similar in the women in the exercise group compared to controls. This may suggest that the exercise effect was primarily driven by the low pre vaccination antibody titers values in the women in the exercise group.

However, the significant effect was still present after covarying for baseline values, suggesting exercise may indeed enhance H1N1 antibody titers in older women.

IL-6 has been noted as a potential candidate playing a key role in acute stress induced immune-enhancement. IL-6 is among the first cytokines to be released and elevated post-exercise, thus it may help regulate the immune response (31). As previously reported in humans, the antibody responders to a virus strain of *Francisella tularensis* had a higher level of IL-6 pre-immunization compared to non-responders. It may be speculated that the enhancement of antibody titer could be related to levels of IL-6. Hence, in the present study we evaluated the correlation between IL-6 levels and antibody titers. Even though we did not note any exercise effect for the IL-6 values 24 and 48 hours post exercise, we found that there was a strong correlation ($r = 0.7$) between IL-6 levels 24 hours post-vaccination and antibody titers post 4-weeks in the exercise group. The correlation was slightly weaker in the control group ($r = 0.5$). However, based on the results in the present study, we are unable to evaluate if IL-6 is the key mechanism for the immune-enhancement of the antibody titer. Unfortunately, due to a smaller sample size we were unable to perform a regression modeling for mediation analysis to implicate the role of IL-6.

Limitations: In the present study we did not perform a detailed immunological assessment, thus, we are unable to address key mechanisms and modulations of the immune system following acute moderate intensity aerobic exercise. We did not control for the pre-vaccination antibody titers. The present study included only healthy older adults thus our data may not be representative of the population of older adults at large.

Conclusions: The present study suggests that acute moderate aerobic exercise may not be immune-stimulatory in healthy older men, but may provide selective immune enhancement in older women. The association between IL-6 and post 4 weeks antibody titers may be indicative

of potential mechanism but can only be speculated in the present study. Sero-protection was unaltered by prior exercise but re-enforce the fact that there is a large number of older individuals who remain unprotected even after obtaining the vaccine.

Table 4.1: Descriptive variables for exercise and control groups

	Exercise (n=28)	Control (n=27)
Age (years)	66 ± 0.93	67 ± 0.77
Height (cms)	168.54 ± 1.68	165.61 ± 2.28
Weight (kg)	79.96 ± 2.78	71.52 ± 3.49
BMI (kg/m^2)	28.07 ± 0.83	25.94 ± 0.98
VO2peak	25.90 ± 1.20	25.14 ± 1.29

Note: Values are mean ± SEM. BMI, Body Mass Index; IMT, Intima-media Thickness. There were no statistically significant differences between groups.

Table 4.2: Comparison between exercise and control groups for the influenza vaccine strains of H1N1 and H3N2

	Exercise (n= 26)		Control (n=28)	
	Baseline	4 Weeks	Baseline	4 Weeks
H1N1	1.17 ± 0.39	3.16 ± 0.41*	2.44 ± 0.37	3.95 ± 0.39*
H3N2	3.36 ± 0.38	5.38 ± 0.28*	3.56 ± 0.38	5.54 ± 0.27*

Note: Values are mean ± SEM. * differs significantly from baseline ($p<0.05$).

Table 4.3: Number of individuals sero -protected expressed as a percentage for the strains of H1N1, H3N2 and B-Florida

	Exercise (n=26)	Control (n=28)	Total (n=54)
H1N1	17.9	25	20.3
H3N2	51.9	46.4	48.2
B - Florida	46.7	29.4	37.5

Note: Values are mean percentages. There were no statistically significant differences between groups.

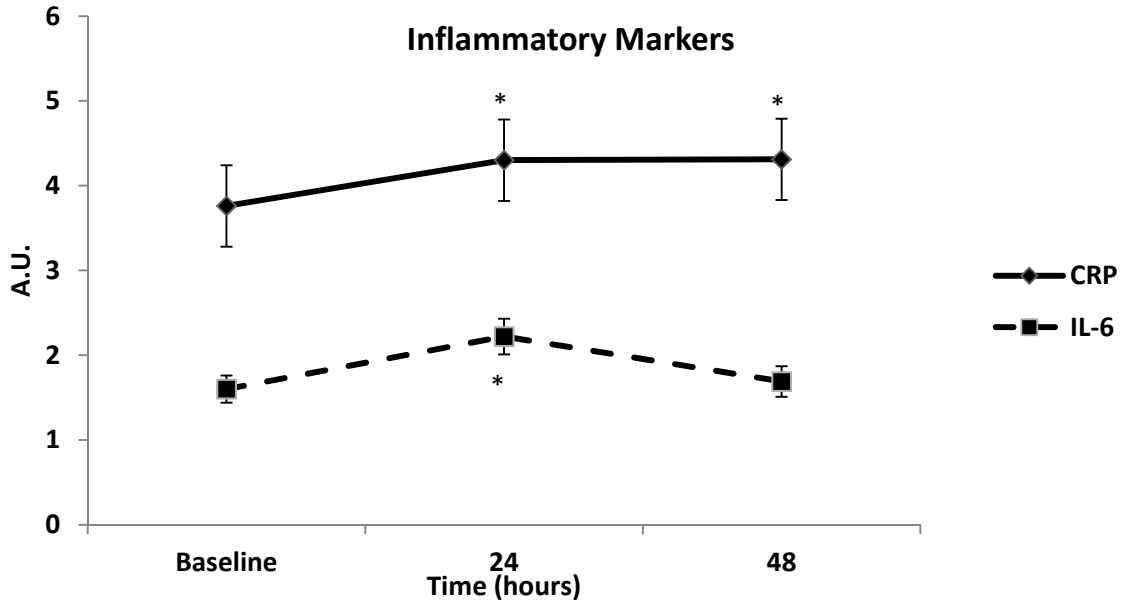

Figure 4.1: Effect of influenza vaccination on CRP and IL-6. Subejcts were measured at baseline (before vaccination) and 24 and 48 hours following vaccination. The * denotes a significant change from baseline ($p<.05$)

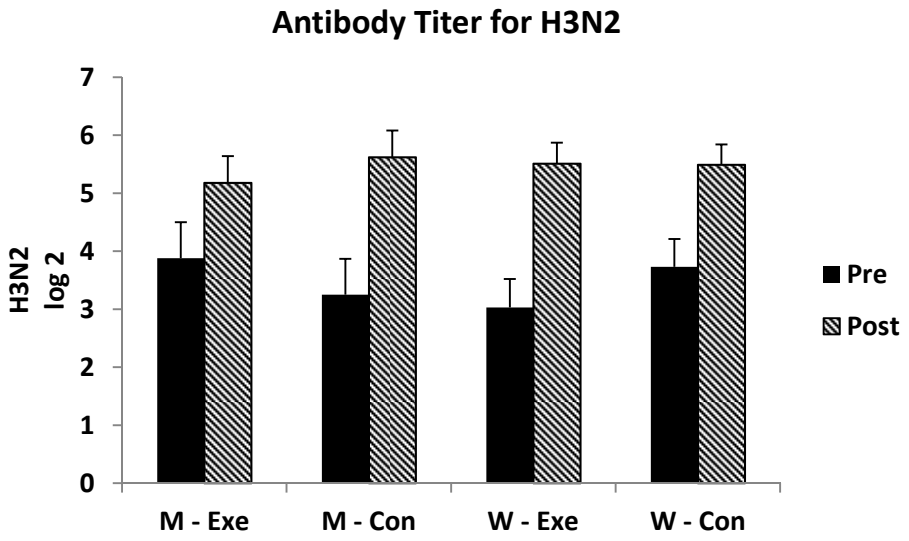

Figure 4.2: Comparison of antibody titer for H1N1 between men and women and exercise and control groups. M – Exe, men in exercise group (n=10); M – Con, men in control group (n=10); W – Exe, women in exercise group (n=16); W – Con, women in control group (n=17). There were no significant differences between men and women, nor between exercise and control conditions.

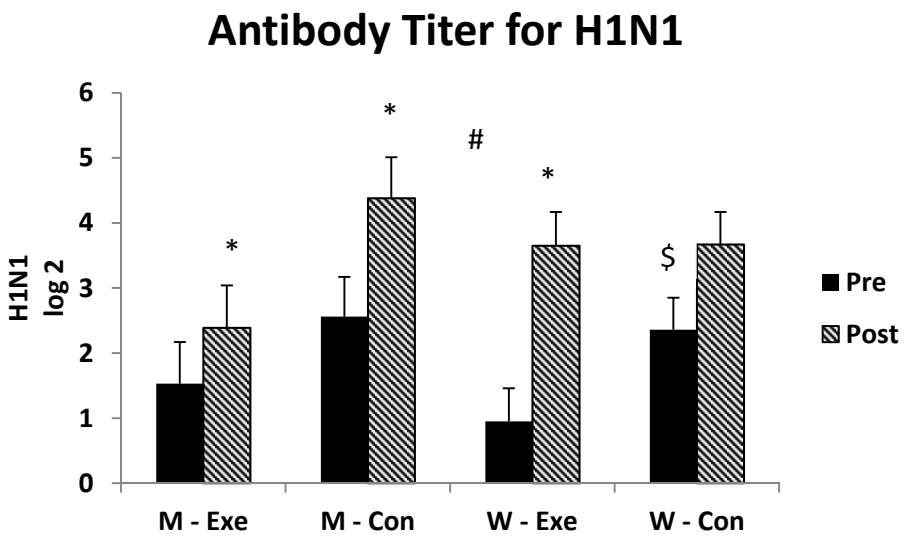

Figure 4.3: Comparison of antibody titer for H1N1 between men and women and exercise and control groups. M – Exe, men in exercise group (n=10); M – Con, men in control group (n=11); W – Exe, women in exercise group (n=16); W – Con, women in control group (n=17). # denotes a significant time by sex by exercise status interaction ($p<0.05$). * denotes significantly different from pre within group ($p<0.05$). $ denotes significantly different from women in exercise group ($p<0.05$).

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