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Effect of cardiovascular exercise training compared to strength flexibility training on inflammatory mediators in an elderly population

by

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A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE

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ABSTRACT

This longitudinal study was the first study to assess the effect of two different kinds of exercise on levels of proinflammatory cytokines in PBMC and serum. IL-1 β , TNF- α and IL-6 levels were measured using regular ELISA assay in PBMC; IL-6 was also measured in the serum using a high sensitivity ELISA kit. Physical activity measures and BMI was compared to assess the aerobic capacity, flexibility and effect of body weight on cytokine levels, with exercise.

There was a reduction seen in both the FLEX (strength and flexibility) and CARDIO (cardiovascular exercise) groups over ten months. There was a reduction in the IL-6 level in the CARDIO group, while there was an increase in the FLEX group. There were no changes in the TNF- α levels in either of the groups. No change in the serum cytokine levels in either group, a trend was seen in the CARDIO group. These results suggest that cardiovascular exercise may have a positive role in reducing the inflammatory effects of aging in an elderly population.

CHAPTER 1. INTRODUCTION

Cytokines are diverse and potent chemical messengers in the range of 5-20 kD secreted by the cells of the immune system. A number of cytokines are grouped as 'pro-inflammatory' as they accelerate inflammation and regulate inflammatory reactions either directly or by the induction of other inflammatory cytokines. Some of the major pro-inflammatory cytokines include Interleukin-1 alpha (IL-1 α), Interleukin-1 beta (IL-1 β), Interleukin-6 (IL-6) and Tumor necrosis factor-alpha (TNF- α).¹ They are released in response to stimuli such as signals from receptors (toll like receptors), or other cytokines and may initiate an inflammatory cascade. Other cytokines often considered to be anti-inflammatory include IL-1RA, IL-4 and IL-10 and may be involved in the resolution of an inflammatory response. In contrast, prolonged release of pro-inflammatory cytokines may result in tissue damage and continued inflammation.⁴⁵ The proinflammatory and anti-inflammatory cytokines maintain a balance until the homeostatic balance is disturbed.⁴⁵

Recent evidence suggests that several chronic diseases such as Type II diabetes, cardiovascular disease, arthritis, obesity and osteoporosis are associated with increased levels of pro-inflammatory cytokines.^{2,3} The 'inflammation hypothesis of aging'^{4,5} suggests that inflammation contributes to or potentially causes the aging process. As much as a four-fold increase in the serum levels of the pro-inflammatory cytokines may occur with aging.¹ Studies comparing younger and older age groups of people show increased levels of pro-inflammatory cytokines in the elderly akin to a state of low grade inflammation.¹

In contrast to the long-term low to moderate release of pro-inflammatory cytokines associated with chronic disease, a shorter increased release of proinflammatory cytokines is

associated with the acute phase response. As part of the inflammatory process, these locally released proinflammatory cytokines may stimulate the hepatocytes to release acute phase proteins such as C-reactive protein (CRP), transferrin and α -macroglobulin.⁶ Injection of TNF- α , IL-1 β and IL-6 in normal individuals has been shown to cause increased release of CRP and the acute phase proteins.⁶ The acute phase response occurs a short time after injury, infection or inflammation and may be induced by stimuli that are infectious (bacterial, fungal, viral), trauma, or stress related. The cardinal signs characterizing an inflammatory response are localized redness (rubor), swelling (tumor), heat (calor) and pain (dolor). The acute phase proteins are further involved in the activation of leucocytes, fibroblasts, endothelial and smooth muscle cells causing the release of inflammatory cytokines. With chronic and recurrent infection or injury, the acute phase proteins may cause complications such as tissue damage and further disease affecting the central nervous system and the cardiovascular system.⁷ Acute exercise results in increased circulating levels of proinflammatory cytokines.^{9,10} Exercise may be broadly classified according to the type of muscle activity. Concentric exercise describes a shortening of the muscle (the ends of the muscle come closer) as force is exerted, whereas eccentric muscle action involves force that pulls the origin and insertion apart. Acute exercise generally refers to a single session of exercise. In contrast, chronic exercise occurs over a period of time (typically weeks to months). Acute and chronic exercise appears to have differing effects on circulating levels of proinflammatory cytokines. Acute exercise typically does not cause an exaggerated increase in the levels of proinflammatory cytokines as seen in acute trauma or disease. Two previous studies^{29,46} have shown an increase in the plasma levels of IL-1 β , but other later studies show no increase,³⁰ or a minimal increase⁹ in the levels of plasma IL-1 β with a single exercise

session. Studies have also shown a one to two fold increase in the plasma^{9,10} levels of TNF- α with acute exercise; a study by Ullum et al. however showed no change in the plasma levels with a single session of eccentric exercise.³¹ The most consistent finding however is a marked increase in plasma IL-6 levels, immediately after acute exercise.^{9,10,11,31} The extent to which IL-6 increases with exercise seems to depend on the duration and the intensity of exercise. Higher exercise intensities result in greater increases in IL-6 levels.¹² Though the increase in the IL-6 levels are rapid and typically peak immediately after the exercise session, these levels return to pre-exercise levels 2-4 hours after the cessation of exercise.

In contrast to acute exercise that causes a temporary increase in levels of proinflammatory cytokines, chronic exercise training may reduce the levels of proinflammatory cytokines, although data is limited. Previous studies have shown an inverse association between levels of inflammation (as measured by serum and plasma levels of cytokines) and aerobic physical activity.^{13,14,15,16} One study evaluated the effect of exercise intervention on levels of pro-inflammatory cytokine produced by lipopolysaccharide (LPS)-stimulated peripheral blood mononuclear cells (PBMC). In this exercise intervention study by Smith et.al¹⁷ a 60% reduction in PBMC production of proinflammatory cytokines TNF- α , IL-1 β and IFN- γ (another proinflammatory cytokine) was found among middle age adults. If exercise can reduce the levels of pro-inflammatory cytokines in an older population, it is possible that the risk for developing multiple age-related diseases that are associated with elevated cytokines may be reduced. To our knowledge, no study has yet evaluated whether long-term exercise intervention may reduce the levels of pro-inflammatory cytokines in an older population (a population that most likely shows some signs of inflammatory aging). In addition, aerobic exercise has not been compared with strength/ flexibility exercises to

determine if one type of exercise has a greater impact on proinflammatory cytokines in older adults. The purpose of our study was to determine the effect of aerobic training compared to strength/flexibility training on the levels of serum cytokine and lipopolysaccharide (LPS)-induced cytokine release by peripheral blood mononuclear cells. We hypothesized that the levels of pro-inflammatory cytokines would decrease after aerobic exercise training to a greater extent than after strength/flexibility exercise intervention. The mechanisms for the change in proinflammatory cytokine levels are not examined in this study. Changes in body weight may also contribute to a change in pro-inflammatory cytokines and therefore, the analysis was designed to account for body weight.

CHAPTER 2. LITERATURE REVIEW

Proinflammatory Cytokines

Interleukin-1 (IL-1 β)

The human IL-1 β has been found close to IL-1 α on the long arm of chromosome 2,¹⁸ and is part of the Ig superfamily. It is a polypeptide and two genes for IL-1 (IL-1 α and IL-1 β) have been found. IL-1 β is secreted by activated antigen presenting cells i.e macrophages, dendritic cells and B cells. IL-1 β is pleiotropic in action (i.e multiple actions and effects) and plays a pivotal role in the body's response to infection, involved in both innate and adaptive immunity. Apart from co-stimulating the activation of T helper cells, IL-1 β promotes maturation and clonal expansion of B cells and may enhance NK cell activity.²⁰ T helper cells, B cells, NK cells are target cells for IL-1 β . IL-1 β functions to attract macrophages and neutrophils, causing an accumulation of phagocytic cells during an inflammatory reaction.

In addition to the effects of IL-1 on T cells, B cells and NK cells, IL-1 β has other biological actions. For example, IL-1 β is known to be a potent pyrogen with regards to the central nervous system.²⁰ IL-1 β may also stimulate prostaglandin synthesis. Prostaglandins are produced and released by leucocytes at the site of injury; they are chemical compounds that activate the inflammatory response and induce symptoms of pain and fever. The biological properties of IL-1 β may mimic host responses to infection, inflammation, injury or an immunological challenge. The effects of IL-1 β have also been shown to mediate catabolic processes many days after an inflammatory stimulus. *In vivo* experiments have shown that similar to TNF- α and IL-6, IL-1 β has a role in protein catabolism. IL-1 β works

synergistically with other cytokines especially tumor necrosis factor in the inflammatory process.

Interleukin-6 (IL-6)

IL-6 is a glycoprotein and part of the IL-6 superfamily. It is found on the long arm of chromosome 7. Like IL-1 β , IL-6 is a pleiotropic cytokine with multiple functions. IL-6 receptors are found in B cells, T cells, osteocytes, partially committed bone marrow cells and tumor cells. IL-6 is secreted by monocytes, macrophages, bone marrow stromal cells and T helper 2 cells. Some of the target cells for IL-6 may be activated B cells, plasma cells, and stem cells.²⁰ IL-6 varies in its effects to inhibit growth of the cell, or promote differentiation of the cell. For example, IL-6 may act as a modulator of bone re-absorption, as a promoter of hematopoiesis, and in inducing plasma cell development.²¹ IL-6 promotes terminal differentiation of B cells into plasma cells, stimulates antibody production, drives differentiation of myeloid stem cells and helps in the synthesis of acute phase proteins. IL-6 has an important role as a co-stimulatory factor for mitogen or antigen induced T cell activation. Lipopolysaccharide (LPS) enhances production of IL-6 in monocytes and fibroblasts, while glucocorticoids inhibit it. In addition to its effects on the immune response, IL-6 is known to affect other tissues. IL-6 may induce the release of adrenocorticotrophic hormone (ACTH) production in the anterior pituitary and subsequent release of glucocorticoids.²¹ IL-6 produced by the contracting muscle may have a role in hepatic regulation of glucose, consequent to depletion caused by exercise,³³ acting directly on hepatocytes in the liver to release glycogen. IL-6 is also expressed by adipose tissue. In healthy men and women, increased levels of adipose tissue have been related to increased

levels of circulating IL-6.⁴⁷ Higher levels of IL-6 may also be expressed by the hypothalamic nuclei and its receptors, involved in body fat regulation.⁵⁶ Therefore, the increased level of circulating IL-6 from adipose tissue suggests that obesity resembles a low-grade state of inflammation. Multiple chronic disease conditions have now been associated with elevated serum IL-6 including diabetes, atherosclerosis, hypertension and metabolic syndrome. The elevated IL-6 levels in serum may reflect spillover from local tissue sites, but this remains to be established.

Tumor necrosis factor (TNF- α)

The gene for TNF- α is found on the short arm of chromosome 6 and contains five exons with a length of about 5 kb. It was first identified as an anti-tumor agent that caused cell death in various tumor types.²⁰ TNF- α is a potent proinflammatory cytokine and is part of the TNF super-family.²⁴ TNF- α is produced by mononuclear phagocytes, T-lymphocytes, Kupffer cells, glial cells, mast cells and endothelial cells. TNF- α also causes B and T lymphocyte proliferation. LPS, antibodies, oxygen radicals, parasites, and irradiation are some of the stimuli that trigger production of TNF- α .⁶¹ Receptors for TNF- α are present in all human cells except red blood cells. Production of TNF- α in the body is tightly regulated; systemic overproduction may cause inflammatory responses such as septic shock.⁶¹ Higher levels of TNF- α produced locally and persisting over time may cause tissue injury.⁶¹ In contrast, shorter term local low levels of TNF- α help in tissue repair and regeneration⁶¹. TNF also acts as an inflammatory mediator with regard to other proinflammatory cytokines. While

TNF- α induces production of IL-1 and IL-6, the effects of TNF- α on inflammation are intensified when other proinflammatory cytokines such as IL-1 and IL-6 are present.

Increased TNF- α levels may also have some impact on heart disease.⁶² Higher levels of TNF- α are related to hypertension, higher levels of circulating triglycerides and endothelial cell activation in the artery walls. The endothelial cell activation leads to expression of chemokines and adhesion molecules, attracting leukocytes to the site. Other tissues may produce TNF- α , resulting in systemic effects. For example, TNF- α is produced by adipose tissue and stimulates lipolysis. Tissue wasting associated with several disease states is generally considered to result from the actions of TNF- α ²⁴.

Disease and Proinflammatory Cytokines

Interleukin-1 (IL-1 β)

IL-1 is considered to be a major mediator of inflammation and is a key to many host defense responses. During normal homeostasis when the body is not infected, a balance exists between the levels of IL-1 and the receptor antagonist IL-1Ra. In conditions such as infection or disease there is an increase in the level of the proinflammatory cytokine IL-1 β ; higher levels of IL-1 β stimulate the production of IL-1Ra. Even though IL-1Ra is produced by the liver to balance the actions of IL-1, higher levels of IL-1 β are produced by the body in disease.²⁰ Higher levels of IL-1 β may be seen in autoimmune disorders, bacterial and viral infections; the increase in IL-1 β may be either due to over production of IL-1 β or inadequate levels of IL-1Ra.²⁰

IL-1 β may be elevated in autoimmune diseases such as osteoarthritis and rheumatoid arthritis. In rheumatoid arthritis, increased levels of IL-1 β are found in the synovium and

synovial fluid, causing breakdown of the cartilaginous matrix.²⁰ Higher levels of IL-1 β are seen in neurological disorders like Guillian-Barre' syndrome and multiple sclerosis affecting the peripheral nervous system and the central nervous system respectively.²⁰ Increased levels of IL-1 β may be found in conditions such as trauma, malignancy, tuberculosis, pneumonia cystic fibrosis, sepsis, sarcoidosis, psoriasis and dermatitis.²⁰

The proinflammatory cytokine IL-1 β may also play a role in atherosclerosis. IL-1 β induces expression of adhesion molecules on endothelial cell surfaces in the arteries. The leucocytes that accumulate in the lumen release cytokines further amplifying the inflammatory response. This leads to smooth muscle cell proliferation and formation of a fibrous cap in the lumen of the vessel. As a result, formation of plaques may occur in the blood vessels that may cause symptoms of stroke and heart disease.²⁰

Interleukin-6 (IL-6)

Higher levels of IL-6 are found in most inflammatory diseases. Like IL-1 β , increased levels of IL-6 have been associated with the inflammatory cascade of atherosclerosis. Cytokines and their receptors play an important role in the initiation and the perpetuation of the disease. Ikonomidis et.al²⁵ found high plasma levels of IL-6 were found in patients with stable angina; the levels of IL-6 were directly proportional to the severity of artery disease. Studies have also shown an increase in proinflammatory cytokines in obese individuals, smokers and in non-insulin dependent diabetes mellitus.² Higher levels of CRP and IL-6 are both known to be associated with Type II-Diabetes Mellitus (DM),² suggesting that Type-II DM involves inflammation. Work by Pradhan et.al²⁶ showed IL-6 to be a systemic inflammatory marker for risk of type-II DM. Spranger and Kroke et.al also concluded that

there was a three fold higher risk of developing DM with elevated levels of IL-6 and IL-1 β together than just IL-1 β alone.²⁷ The study showed that proinflammatory cytokines work as a network in stimulating the acute phase proteins, which further have a role in the inflammatory process seen in DM. In addition, hyperglycemia may be associated with an acute increase in levels of inflammatory cytokines such as IL-6 in hyperglycemia perhaps as a result of oxidative stress. Other studies have shown a link between the levels of pro-inflammatory cytokines in the blood and the levels in adipose tissue.²⁸ Higher levels of pro-inflammatory cytokine like IL-6 are seen in various neurodegenerative diseases like Parkinson's disease and Alzheimer's disease.⁶⁰ Elevated IL-6 is also seen along with other proinflammatory cytokines in various kinds of degenerative conditions like arthritis.²⁰ Elevated levels of IL-6 are typically found in many autoimmune diseases such as rheumatoid arthritis encephalomyelitis, cancer and Diabetes Mellitus and levels depend on the extent and severity of the disease.

Tumor necrosis factor- α (TNF- α)

Elevated levels of TNF- α are found in many disease conditions. Elevated levels of TNF- α are associated with atherosclerosis. In a study comparing symptoms of disease in 181 elderly subjects by Bruunsgaard et al, higher levels of TNF- α were related to a higher risk of atherosclerosis and heart disease.²⁸ Elevated levels of TNF- α have been seen in heart diseases such as left ventricular failure, cardiomyopathy and pulmonary edema.²⁰ In addition to the potential role in heart disease, TNF- α also has a role in the regulation of adipose tissue. TNF- α stimulates leptin production by adipose tissue. Leptin has a role in maintaining the normal

homeostasis by controlling stores of fat. Leptin also causes reduced food intake, increased caloric expenditure and decreased body weight – (often observed as clinical signs of old age).⁵⁹ Therefore, it has been suggested that TNF- α may be a link between fat stores regulation and its effects on disease especially in the elderly population.⁵⁹

TNF- α is thought to have a major role in autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus (SLE).²⁰ Increased levels of TNF- α are seen in the synovium of affected joints in rheumatoid arthritis.²⁰ Therapies that aim at neutralizing the levels of TNF- α in the joints have shown some success in the treatment process. TNF- α has an important role in the pathology and treatment of cancer. Muscle wasting seen in cancer patients is thought to be due to TNF- α overactivity;²³ in lower doses TNF- α is also used as a local intratumoral drug. High levels of TNF- α are also seen in nervous inflammatory conditions like stroke, meningitis, Alzheimer's disease and multiple sclerosis.²³ In addition to the effects in multiple chronic diseases, increased circulating levels of TNF- α have been found during infectious diseases states such as malaria and leishmaniasis.²³

In summary, there are many studies showing elevated levels of IL-1 β , IL-6 and TNF- α during infectious disease conditions. Often levels return to normal after infection is resolved. In contrast, increased level of IL- β , IL-6 and TNF- α have been found in multiple chronic diseases (diabetes, hypertension, heart disease etc). In chronic disease states, pro-inflammatory cytokines may remain elevated.

Acute Exercise and Proinflammatory Cytokines

There have been many studies that have looked at the effect of a single exercise session on pro-inflammatory cytokines. These studies have generally shown a short-term increase in proinflammatory cytokine levels with acute exercise. However, these studies do not specifically address the question of whether exercise training can reduce the 'slightly elevated' level of proinflammatory cytokines associated with chronic disease.

Interleukin-1 (IL-1 β)

Study by Evans et.al compared the effect of a high intensity single session of eccentric exercise on trained and untrained men (45 minutes cycle ergometry at 250 W).³⁰ There was a 40% increase seen in the plasma IL-1 levels 3 hours after the exercise in all five untrained subjects. With the exception of one subject, all other three trained subjects showed a reduction in plasma IL-1 levels 3 hours after exercise (the resting levels before exercise were higher in the trained group). Therefore one session of strenuous exercise may cause a decrease in the levels of IL-1 in endurance-trained men as compared to untrained men. The study by Ostrowski et.al on ten young (27.5 years) male trained marathon runners showed a significant increase in plasma IL-1 β levels immediately after a 3.5 hour race⁹. Another study by Ostrowski et.al on 16 male marathon runners (30.5 years) found a significant increase too, in plasma IL-1 β levels immediately after the 2 hour race¹⁰. Taken together, these studies tend to suggest that exercise potentially resulting in muscle damage (eccentric exercise or marathon running) may result in increased plasma IL-1 β for some period of time post-exercise.

Though studies have shown an increase in the level of IL-1 β with acute exercise, other studies have found no increase or minimal increase. However, there is debate about the role of concentric exercise (as opposed to eccentric exercise) on cytokine production. A study by Ullum et.al³¹ on 17 young moderately trained individuals with concentric bicycle ergometry exercises (at 75% Vo_2 max), showed no change in plasma levels of IL-1 β , but the levels of IL-1 β were below detection limits. PBMC mRNA for IL-1 β also did not change in response to exercise. It is also possible that the type of exercise is important in determining the extent to which IL-1 β may be altered. Muscle damaging types of exercise (eccentric exercise or marathon running) may result in increased release of IL-1 β , whereas exercise that does not result in muscle damage may have a different effect or no effect on IL-1 β .

Interleukin-6 (IL-6)

Acute exercise causes a major transient change in IL-6 levels. Various studies^{9,32} have confirmed a direct increase in the IL-6 levels associated with different intensities of acute exercise, there was a increased IL-6 level seen with increased duration and intensity of exercise. Two studies by Ostrowski et. al. noted a large increase in IL-6 production immediately after a high intensity marathon race.^{9,10} In one study¹⁰ a marked increase (128 fold) in the plasma levels of IL-6 in 16 young trained athletes after a marathon was seen. Similarly in the other study on 10 trained marathon runners by Ostrowski et al⁹, the IL-6 levels increased in the serum 75 fold (and IL-6 mRNA increased in muscle in 5 or 8 runners). The acute increase in the IL-6 levels after the marathon was attributed to eccentric nature of muscle activity that dominates a marathon race⁴. The study by Starkie et al³⁶ comparing plasma levels of IL-6 in five trained runners after a marathon showed the highest levels

immediately after the race. The levels were higher than pre-race values as late as 24 hours post exercise. In another study, by Toft et.al³² comparing the effects of acute exercise on young and elderly subjects, there was an increase in the plasma levels of IL-6 with lower extremity eccentric exercise for 60 minutes in both the groups. The exercise-induced increase was less in the elderly compared to the younger subjects.

Ullum et.al,³¹ investigated the effect of acute concentric exercise on plasma levels of IL-6. The subjects exercised for 60 minutes at 75% of Vo_2 max, of bicycle ergometry (182-276 W). There was almost an 80% increase in the plasma level of IL-6 in the trained athletes (levels of other proinflammatory cytokines remained unchanged). The IL-6 levels returned to preexisting levels 1-hour post.

Therefore the levels of IL-6 in the body increase drastically with acute exercise. The levels are higher in older adults and they increase less in trained versus non-trained individuals. The study by Febbraio and Pedersen⁶⁵ showed that the muscle is the main source of increase levels of IL-6 with acute exercise.

Tumor necrosis factor- α (TNF- α)

Acute exercise causes a marked increase in the levels of TNF- α . Work by Dufaux and Order³³ on eight moderately trained young men showed an initial increase in the levels of plasma 120 minutes after an endurance run. The higher levels of TNF- α were found 24 hours after the 2.5-hour exercise session. Ostrowski et.al⁹ in the study on 10 male athletes found a 2.3 fold increase (compared to pre-race levels) in the plasma TNF- α levels at the end of the first hour after the marathon. In the other Ostrowski et.al study¹⁰ on trained marathon runners, the plasma TNF- α levels were 2.5 times higher immediately after the marathon but

reduced to twice the pre-race value two hours post. The Starkie study³⁶ showed a two-fold increase in plasma levels of TNF- α immediately after the marathon; the values reduced with time, but stayed higher than pre-race values 24 hours post.

In the Ullum study,³¹ 60 minutes of concentric bicycle ergometry exercise at 75% VO₂-max showed no change in the plasma TNF- α levels after the exercise. Pedersen et. al attributed the comparative lower levels of TNF- α after acute exercise to the surged release of IL-6.³⁵ Though IL-6 is considered a pro-inflammatory cytokine it also has some anti-inflammatory properties. Muscle damage during acute exercise causes release of IL-6 in the muscle. This increased release of IL-6 has an inhibitory effect on TNF- α levels. Therefore, it is possible that muscle-damaging types of exercise have the greatest impact of TNF- α , similar to the findings with respect to IL-1 β ³⁴.

The cytokine response to exercise appears to be dependent upon the duration and the type of exercise. Of the six studies looking at the effect of acute exercise on proinflammatory cytokines, all six showed an increase in the levels of IL-6 after exercise, and three of the four showed increased levels of IL-1 β . Of the four studies on TNF- α , two showed no increase with acute exercise (refer table 1). Therefore the effect of acute exercise on cytokine levels is variable due to undetermined factors.

Table 1. Previous studies on effects of acute exercise on proinflammatory cytokine level.

Study	Duration	Subjects /protocol	Cytokine	Results
Ostrowski et al ⁹	Single marathon	10 male runners (24-37 years old)	Plasma IL-6, TNF- α , IL-1 β , IL-10	Increase in plasma levels: 128 fold - IL-6, 2.1 fold - IL-1 β , 2.3 fold - TNF- α and 27 fold - IL-10
Ostrowski et.al ¹⁰	Single marathon	16 trained marathon runners (30 years)	Plasma IL-6, mRNA for IL-6 and IL-1 β	90 fold increase in plasma levels of IL-6, almost 3 fold increase in IL1RA levels.
Evans et al ³⁰	45 minutes cycle ergometry (250W)	4 male trained runners 5 untrained men	Plasma IL-1, creatine kinase	47% increase in plasma levels of IL-1 and 56% increase in the CK levels in the untrained.
Ullum et al ³¹	60 min. bicycle ergometry (75% V _O ₂ max)	17 trained men (23-39 years)	Plasma: IL-6, IL-1 α , IL-1 β and TNF- α	70% increase in plasma levels of IL-6, no change in IL-1 α , IL-1 β and TNF- α levels
Toft et al ³²	60 min. cycle ergometry	10 elderly (67-75 years) and 10 young subjects (20-27 years)	Plasma IL-6 and TNF- α	No effect of exercise on TNF- α levels; eight fold increase in IL-6 in the younger adults, only two fold increase in the elderly.
Starkie et al ³⁶	Single marathon	5 male trained runners	Plasma IL-6 and TNF- α	40% increase in plasma levels of TNF- α , IL-6 levels increased 200%.

Chronic Exercise and Proinflammatory Cytokines

There have not been many studies investigating the role of long-term (chronic) exercise on cytokine levels. The long-term studies can be broadly classified as intervention studies (divided here as aerobic or resistance training) and epidemiological studies. Epidemiological studies are classified as those studies without a direct exercise intervention. Chronic studies are categorized as having duration of at least 12-weeks of intervention.

Interventional studies

Cardiovascular exercise

Smith et al¹⁷ investigated the effects of 6 months exercise of training in 43 middle-

aged people. The subjects performed weight training, walking, cycling, stretching, aerobics, rowing and climbing exercises as part of this study, exercising 2.5 hours per week. Almost 35% of the subjects lost weight (average weight loss was 3.3%) at the end of 6 months of supervised physical activity. There was a reduction in the proinflammatory cytokine levels produced by PHA stimulated peripheral blood mononuclear cells. The study showed almost a 59% decrease in IL-1 β and TNF- α levels.

The three-year multidisciplinary by Esposito et al study³⁷ assessed the role of physical activity and a Mediterranean diet in reducing proinflammatory cytokine levels in 120 obese middle-aged premenopausal women. As part of the two-year intervention, the subjects were advised about diet and physical activity. The activity levels were charted using a questionnaire. There was a reduction seen in serum CRP and IL-6 levels at the end that correlated with reduced BMI levels. Although physical activity played an important role, the reduction in IL-6 and CRP was attributed to more than one factor in the study.

Baum et.al³⁷ found an increase in the amount of IL-1 β and IL-6 produced by PBMC after exercise training for 12 weeks. Eight healthy adults ran 30 km, 3 times/week, whereas 7 control subjects did not exercise. There was an increase in IL-1 β and IL-6 levels in PBMC after 12 weeks, found in the subjects that ran as compared to controls .

Reynolds et. al⁴³ examined the effect of aerobic exercise on six months of aerobic exercise 14 elderly hypertensive women. The women performed aerobic exercise for 6 months, consisting of treadmill walking, stair-climbing and stationary cycling. At the end of six months, the exercises were performed for 40 minutes each day, three days per week. The intensity was at 75-80 % HRR. There was a decrease in body fat levels, but no changes in plasma TNF- α levels, after exercise.

Resistance/endurance training

The Greiwe study³⁸ evaluated the effect of a 12-week resistance training program on muscle TNF- α level in 12 elderly subjects over 75 years. The experimental subjects exercised using machines, while the control group did light callisthenic exercises. After 12 weeks there was a reduction in the levels of TNF- α in the muscle tissue (measured at rest).

Rall et al⁴¹ compared the effects of progressive resisted exercises on 3 groups of subjects; elderly, young and patients of (rheumatoid arthritis) (RA). The elderly subjects were randomly divided into either the strength or non-strength group. The RA group, the elderly strength group and the young subjects exercised at an intensity of 1 repetition maximum (RM). One RM is the maximum amount of weight that can be lifted by a subject once through the full range). on trunk and lower extremity machines; totaling about 8 repetitions per sets, 3 sets per session performed twice weekly at approximately 45 minutes per session. The elderly non-strength group did not exercise and served as controls. The production of IL-1 β , IL-6 and TNF- α by PBMC were measured and no change in PBMC cytokine levels after 12-weeks of exercise were found.

Mattusch et.al, measured the CRP levels in 12 moderately trained young runners (25-40 years) over a 9 month period.⁵³ The subjects exercised 3-4 times per week for a duration of 50 minutes. Although there was an increase in the intensity of exercise over time (31 km/week to 53 km/week over nine months), there was a decrease in the CRP levels in plasma for 10 out of 12 runners measured, at the end of the training session.

Taken together, the limited data from intervention trials appears to be inconclusive. In general, studies employing weight training as the primary type of exercise did not observe effect on serum cytokines or cytokine production by PBMC's. However, weight training

exercise did appear to alter TNF- α production in the muscle tissue. With respect to studies using aerobic exercise as the primary type of activity, it appears that serum cytokines or PBMC production of cytokines is reduced if the duration of the training program was longer term (greater than 6 months). Weight loss may also be a factor in these longer term aerobic intervention studies.

Epidemiological studies

There have not been many epidemiological studies that assessed the effects of physical activity. The study by Shinkai et. al.⁴⁰ compared the plasma levels of proinflammatory cytokines IL-1 β and IFN- γ in PBMC. There was no difference in the levels of these cytokines in between the habitual elderly runners and the sedentary controls. The study by Pischon et al¹³ on 405 healthy men and 454 healthy women showed an inverse relationship between physical activity and inflammatory markers. There was a decrease in the plasma levels of TNF- α and IL-6 with unsupervised physical activity; this association was greater in subjects with lesser body fat. Pradhan et al²⁵ examined the plasma cytokine levels of IL-6 in 188 patients of DM and 362 disease-free controls. Higher levels of IL-6 and CRP were found in subjects that had DM. There was a direct relationship between disease levels and IL-6 levels. They also exercised less and had greater body mass indices. 43% of the subjects rarely exercised, 26 % exercised once a week, 25 % exercised 1-3 times a week and about 5 % exercised greater than 4 days a week. A cross sectional study by Colbert et. al¹⁶ examined the association between physical activity and inflammatory markers. 3075 healthy men and women (between 70 and 79 years) completed a questionnaire about physical activity (gardening, housework, walking, weight training etc.) over the previous 12 months.

CRP and IL-6 (serum) and TNF- α (plasma) levels were measured. There was an 18% reduction in IL-6 levels and an 8% reduction in plasma TNF- α levels with >180 min. per week. The CRP levels were reduced by 17% with the same exercise over the same time period. There was an inverse relationship between inflammatory markers and exercise levels. When BMI was accounted for, this effect was still consistent across different body types in the study.

There have not been any studies greater than 6 months duration that have assessed the effect of aerobic exercise, and proinflammatory cytokines in older adults. It is important to determine whether age-associated elevations in serum cytokine levels may be reduced by an exercise intervention. The information may have clinical relevance and may provide additional insight into the mechanisms by which exercise reduced the risk of multiple chronic diseases.

Table 2. Previous studies on effects of chronic exercise (≥ 12 weeks) on proinflammatory cytokine levels.

Study	Duration	Subjects/protocol	Cytokine	Results
Smith et al ¹⁷	6 months exercise (2.5 hours per week, 70 min/ session)	52 men and 18 men (mean: 48.5 years).	PBMC IL-1 α , TNF- α , IFN γ , IL-4, IL-10, serum CRP	Reduction in PBMC IL-1 α (3%), TNF- α (28%) IFN γ (44%)
Esposito et al ³⁸	2 years, diet and physical activity, Questionnaire	120 premenopausal women	Serum IL-6, IL-18, CRP	35% reduction in IL-6 and 25% reduction CRP levels
Greiwe et al ³⁹	12-week resistance training (50-90 min/ day, 3 times a week)	12 elderly and 12 young untrained elderly adults, 5 elderly controls	TNF- α in muscle	28% reduction in TNF- α levels
Baum et al ⁴⁰	12-weeks of endurance exercise (3-5 hours/week, 3 times a week)	4 men and 4 women (mean age: 42 years)	Plasma IL-1 β , IL-2, IL-6 and IFN γ	42% increase in levels of IL-1 β and 11% increase in IL-6 levels
Rall et al ⁴²	12 weeks of PRE. 3 sets per session twice/ week	8 subjects with RA, 8 young healthy and 8 elderly healthy	Plasma IL-1 α , IL-6 and TNF- α	PBMC cytokine levels did not change
Reynolds et al ⁴⁴	6 months 3 days per week	14 older hypertensive women, 75% of V_{O_2} max	Plasma TNF- α	No change in levels

Table 3. Previous epidemiological studies (no exercise intervention) and the effect on proinflammatory cytokine levels.

Study	Type of study	Subjects/protocol	Cytokine	Results
Pischoon et al ¹³	Questionnaire	405 men and 454 women	IL-6, TNF- α and CRP	Inverse relationship seen with physical activity
Pradhan et al ²⁶	Questionnaire	188 women (DM) and 362 (disease free control)	IL-6 and CRP	Increased levels of IL-6 and CRP.
Shinkai et al ⁴⁰	Questionnaire	17 elderly, 16 younger runners and 19 elderly controls	Plasma IL-1 β , IL-2, IFN γ , IL-4	No change in IL-1 β or IFN- γ levels.

CHAPTER 3. HYPOTHESIS AND METHODS

Hypothesis

I hypothesized that the levels of IL-1 β , TNF- α , IL-6 produced by LPS-stimulated peripheral blood mononuclear cells and IL-6 in serum would decrease with long-term aerobic exercise training to a greater extent than with a strength/flexibility exercise training program.

Methods

Subjects

Subjects were healthy individuals over 64 years of age. The study was approved by the Institutional Review Board (IRB) of Iowa State University. All subjects completed a medical history form and participants with conditions that may interfere with the immune response were excluded (e.g autoimmune disorders, cancer within the previous five years etc.). Any subject taking medication that may have altered the immune response such as oral corticosteroids and non-steroidal anti-inflammatory drugs were also excluded. Subjects (n=50) were randomly assigned to an aerobic exercise or a flexibility / strength group (25 participants per group). A log was maintained for attendance and adherence to the exercise protocol for both groups.

Initially there were 12 females and 13 males in the CARDIO group and 10 females and 15 males in the FLEX group; by the end of the intervention there were 20 subjects in the CARDIO and 22 subjects in the FLEX group. A study timeline is shown in Figure 1.

Pre and post testing

The baseline testing included a physician supervised maximal exercise treadmill test, the Standard Senior Fitness tests (Rikli and Jones 2001) and a diet survey to chart nutritional habits or deficiency (Block 98 Food Survey). Skin-fold measures were taken before and at the end of the study at ten sites; the results from the Jackson Pollock and Durnin Wornesky equations were averaged to calculate body fat.⁶³ Body Mass Index (BMI) was also measured using the following equation –

$$\text{BMI} = \frac{(\text{Weight in pounds})}{(\text{Height in inches})^2} \times 703$$

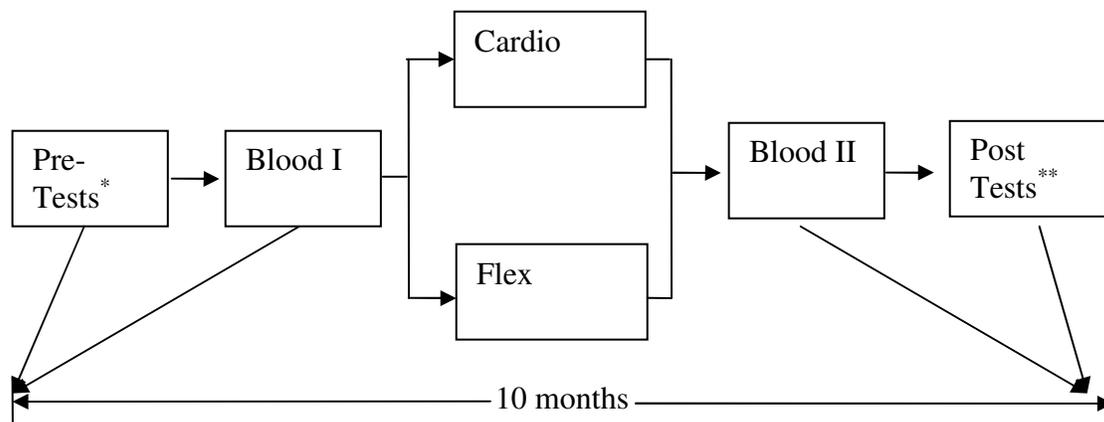


Figure 1. Timeline for study

Apart from the initial medical history taking, the Pre-Tests* included:

1. Physician supervised graded maximal exercise test
2. Fitness testing – Senior Fitness Test (Rikli/Jones)
3. Body Fat/weight measurements

The pre-tests were followed by blood draws I (after Blood I the subjects began their participation in either the CARDIO or FLEX groups). At the end of the 10-month exercise period, blood draw II was performed. This was followed by Post Tests** which again included

1. Fitness testing - Senior Fitness Test (Rikli/Jones)
2. Body fat/weight measurements

Exercise intervention**Cardiovascular (CARDIO) group**

The CARDIO group initially exercised at 45-55% of the heart rate reserve (HRR) and then gradually progressed to 75% of HRR. The group met three times a week, performing exercise in the target heart rate zone for 25-30 minutes every session, over a 10-month intervention period. The initial warm-up consisted of 10 minutes of stretches and walking and the cool-down at the end consisted of stretches, walking, and some toning exercises. The exercise attendance, target heart rate and adherence to protocol were monitored for all participants.

Flexibility and strengthening (FLEX) group

The FLEX group performed flexibility, balance and toning exercises for the first 6 months. During the last four months of the intervention, light strength training exercises were added to the routine, averaging one set of 12-15 repetitions per workout (at an estimated load ranging from 50-80% of 1 RM). The exercise sessions began and ended with a warm-up and ended with a cool-down session of 10 minutes; the exercise lasted 25-30 minutes each session (not including warm up and cool-down). The stretching and strengthening exercises included all major upper and lower extremity muscle groups such as pectorals, latissimus dorsi, trapezius, quadriceps, hamstrings and gluteal stretches. Free exercises using a wobble board and therabands were used. The machines used for strengthening were the military press, Latissimus pull down, seated chest press, upper back, arm curl, triceps, lower back, abdominals, leg curl and leg extension.

Laboratory methods

Blood was collected at two points in the experiment - before and after the 10-month intervention period. Blood collection was performed at least 24 hours after exercise; the blood draws were scheduled so that the acute effect of exercise did not affect the sample drawn. PBMC were isolated by centrifugation over Ficoll-Paque Plus (Amershan Pharmacia Biotech, Piscataway, NJ) and adjusted to 4×10^6 cells/ml in AIM-V medium (Life Technologies, Grand Island, NY). ELISA kits (BD Bioscience Pharmacia) were used to measure the cytokines following the protocol supplied by the company. Supernatant was collected from peripheral blood mononuclear cells (PBMC) after 24 hours of incubation with 100 ng/ml lipopolysaccharide (LPS) at 37° C, 5% CO₂ in a humidified atmosphere and frozen at -80°C until analysis of IL-1 β , TNF- α and IL-6. IL-6 was measured both in the serum and PBMC. Serum was collected and frozen at -80° C for assay by high sensitivity ELISA.

Briefly, the wells of 96 well microtiter plates were coated with capture antibody and incubated overnight. After three washes, the wells were coated with assay diluent (blocking buffer). Supernatant samples were added along with the standards. After a two hour incubation, the detection antibody was added to each well. Lastly, substrate solution was added and after 30 minutes of incubation the absorbance was read at 655 nm using a microplate reader (Biorad).

Statistics

A mixed Analysis of Variance (ANOVA) was tested for effects of group

(cardiovascular or strength/flex groups) and time (pre versus post) was used to compare the differences in IL-1 β , TNF- α and IL-6. Body weight was included as a covariate in the model.

CHAPTER 4. RESULTS

Weight Change as a Covariate

The change in body weight was entered in all models and was not a significant factor in any of the models with respect to the change in cytokine levels. The data shown in the figures 2, 3, 4 and 5 reflect the results of the model when weight was included as a covariate. Overall there was a trend ($p = 0.10$) towards a reduction in body weight over time. (Table 4) There was a significant effect of time with respect to BMI such that both groups decreased at the post intervention time point ($t = 0.006$) (Table 4).

Physical Activity Measures

A significant improvement was observed in both treatment groups for the ‘chairstand’ and the ‘sit and reach’ activity (Table 4). The chairstand is a measure of lower extremity strength and endurance. Similarly, the sit and reach, is a measure of the flexibility of the subject. With respect to the 6-minute walk test as a measure of aerobic fitness, the cardio group performed better than the flexibility group over time (significant treatment by time interaction). There was no significant difference seen between groups with respect to the other physical activity variables (Table 4).

Cytokines in PBMC Cultures

There was a reduction seen in the levels of IL-1 β produced by LPS stimulated PBMC in both the groups after the ten-month intervention (significant main effect of time; Figure 2, $p < 0.05$).

With respect to IL-6 produced by LPS-stimulated PBMC, there was a reduction in the in the CARDIO group, and an increase in the FLEX group as suggested by the significant time by treatment interaction ($p = 0.037$; Figure 3). Unlike IL-6 and IL-1 β , TNF- α production by PBMC was not altered by the intervention (no significant treatment, time or treatment by time effects; Figure 4, $P < 0.05$)

Serum Cytokine

The only cytokine measured in the serum was IL-6. Serum levels of IL-6 were also not significantly changed by the intervention (no main effect of treatment, time, or treatment by time interaction; Figure 5). However, there was a trend towards reduced IL-6 in the CARDIO group ($p = 0.11$).

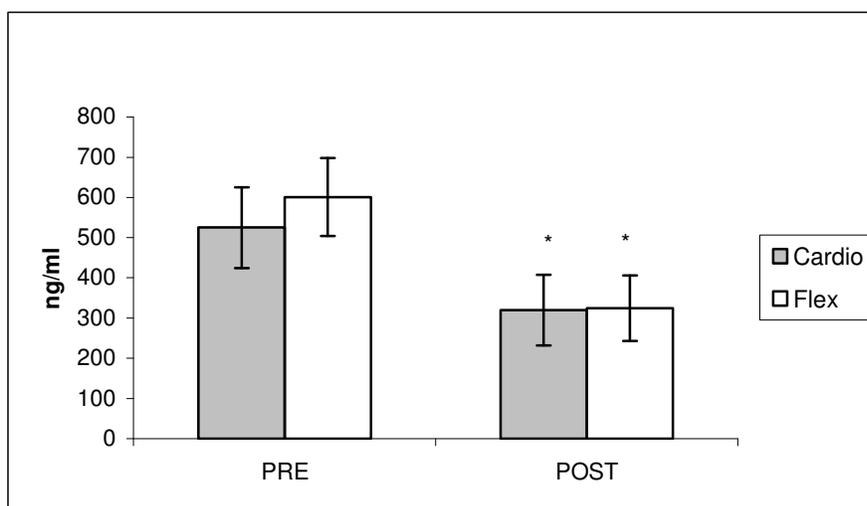


Figure 2. IL-1 β concentration pre and post exercise (mean \pm S.E).

LPS-stimulated IL-1 β by PBMC from subjects in the CARDIO group as compared to the FLEX group prior to the intervention (PRE) and following the intervention (POST). A significant main effect of time ($P < 0.05$) was observed such that IL-1 β decreased in both groups following the intervention. (N= 25/group)

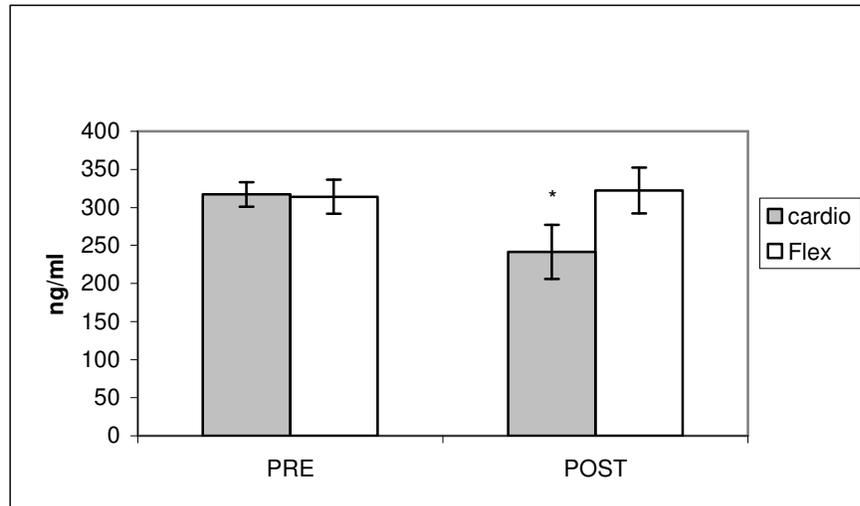


Figure 3. IL-6 concentration pre and post exercise (mean \pm S.E).

LPS-stimulated IL-6 by PBMC from subjects in the aerobic exercise group as compared to the flex group prior to the intervention (PRE) and following the intervention (POST). A significant treatment by time was observed ($p < 0.05$) such that IL-6 was lower in the CARDIO group, but not the FLEX group following the intervention. (N = 25/group)

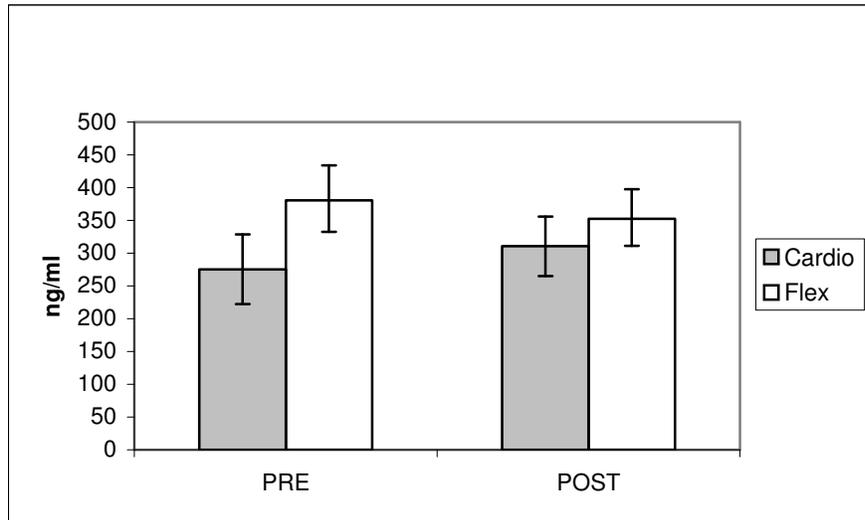


Figure 4. TNF- α concentration pre and post exercise (mean \pm S.E). LPS-stimulated TNF- α by PBMC from subjects in the cardio group as compared to the flex group prior to the intervention (PRE) and following the intervention (POST), No significant changes were observed (N = 25 / group)

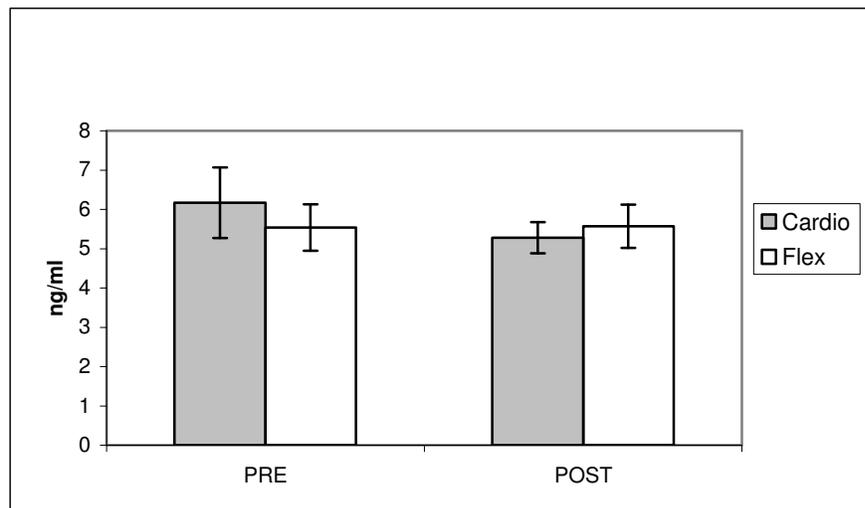


Figure 5. IL-6 concentrations in the serum pre and post exercise (mean \pm S.E). No significant changes were found. Treatment by time interaction, $p=0.11$

Table 4. Physical activity measures for the subjects in the CARDIO and FLEX group.

Physical Activity variables	Mean PRE (Std. deviation)		Mean POST (Std. deviation)	
	CARDIO	FLEX	CARDIO	FLEX
CHAIRSTAND	16.65 (4.9)	16.88 (3.8)	18.3* (7.6)	20.16* (5.0)
Lt. ARM CURLS	22.31 (4.1)	19.1 (8.5)	22.36 (4.7)	21.84 (4.0)
6 MIN. WALK	674.36 (124.7)	686.68 (87.4)	768.73** (159.7)	738.39 (161)
STEPS	102.44 (34.9)	104.29 (32.1)	108.83 (19.0)	112.05 (26.5)
SIT AND REACH	-0.16 (5.3)	-0.95 (5.0)	2.56* (4.9)	0.69* (5.0)
BACK SCRATCH	-3.02 (3.7)	-4.02 (3.6)	-2.46 (5.2)	-3.83 (4.2)
UP AND GO	4.92 (0.88)	5.05 (1.1)	4.41 (0.73)	4.54 (1.2)
BODY WEIGHT	164.7 (32.9)	173.7 (35.8)	161.8 (31.9)	172.5 (35.7)
BMI	28.4 (3.2)	26.8(4.7)	28.1 (3.7)	25.8 (3.8)

* $p < 0.01$ vs. PRE

** Treatment by time interaction such that CARDIO increased at POST compared to PRE, but FLEX did not change.

CHAPTER 5. DISCUSSION

In this study, I found a statistically significant association between chronic exercise training and reduction in cytokine levels. To our knowledge, this is the first study that has examined the effects of a long term (10-month) exercise intervention on pro-inflammatory cytokine levels among older adults. We observed that aerobic exercise decreased the level of both IL-6 and IL-1 β produced by PBMC whereas resistance training altered the level of only one cytokine, IL-1 β . Although the serum cytokine levels of IL-6 were not statistically different between groups after the intervention, there was a trend towards reduced serum IL-6 in subjects performing the aerobic exercise. Taken together, these findings suggest that cardiovascular exercise may be a better exercise modality than strength and flexibility training in altering the cytokine levels.

Proinflammatory Cytokines and Aerobic Exercise

Intervention studies

There have not been any other long-term interventional studies that have compared aerobic training and resistance training with respect to their effects on proinflammatory cytokine levels in the elderly. Our results are comparable to other interventional studies that evaluated chronic aerobic exercise and proinflammatory cytokine levels in adults of younger age groups. Another study with younger subjects (mean age 48 years) observed a significant reduction in mitogen-stimulated PBMC production of IL-1 α , TNF α , and IFN γ after a 6 month exercise intervention.¹⁷ Although I did not assess IL-1 α , we did find a significant decline in mitogen-induced production of IL-1 β in both exercise groups and a reduction of

another proinflammatory cytokine, IL-6 (only in subjects performing aerobic exercise). A more recent study with coronary heart disease patients also observed that 12 weeks of aerobic exercise reduced plasma concentration of IL-1, IL-6, and CRP.⁶⁴ This study lends further support to the concept that exercise has anti-inflammatory actions. Although our study only observed a trend towards reduced serum IL-6, it is possible that the magnitude of the decrease was greater in the recent study by Goldhammer et al., because their subject pool included only coronary heart disease patients (that are likely to have higher levels of proinflammatory cytokines at the onset of the study). One other study in older females (mean age = 62) failed to find a significant effect of an aerobic exercise intervention on serum TNF- α , similar to our results with respect to mitogen-induced TNF- α ⁴³. Although epidemiology studies tend to suggest that TNF- α is decreased with increasing activity, it is possible that the effect is small and a greater number of subjects or a longer period of exercise intervention is required to detect a significant difference.

In a separate intervention trial of obese women, a 2 year diet/exercise program resulted in decreased serum levels of IL-6, and CRP³⁷. Significant weight loss occurred in this study, but significant weight loss did not occur in our subjects. It is possible that the trend we observed with respect to reduced IL-6 might have been statistically significant if weight loss had occurred. The authors of the weight loss study measured adiponectin and showed an inverse relationship between IL-6 and adiponectin, suggesting that the two may influence each other. Unfortunately, we did not measure adiponectin, but in future studies it would be important to determine whether a long-term exercise program without significant weight loss would alter adiponectin. Adiponectin is a protein that is produced by adipose tissue, and a separate study by Engeli et.al⁴⁶ also showed an inverse relation between

adiponectin levels and IL-6 in obese women. Esposito et.al., differed from our study in that (1) the subjects were obese (2) the subjects followed a diet plan (3) greater number of subjects (4) longer duration of intervention and (5) significant weight loss by subjects. Given these differences in study design, the combination of exercise, diet, and weight loss may have a greater impact on serum IL-6 than exercise alone.

One study with younger adults actually observed an increase in production of IL-1 β in mitogen stimulate whole blood cultures following a 12 week aerobic exercise intervention (Baum)³⁹. It is not clear why an increase was found in this study, since most other studies tend to find a decrease of inflammatory cytokine production. Whole blood cultures were used instead of isolate PBMC and it is possible that something present in the whole blood after exercise training influenced the production of IL-1. Also, the subjects performed moderate to vigorous exercise 3-5 hours per week (substantially more than that performed in most other exercise intervention trials). It is possible that higher intensity, longer duration exercise training has a different effect on proinflammatory cytokine production. Perhaps tissue damage occurred as a result of the training program which may have resulted in increased production of inflammatory mediators. Future studies are needed to tease out the different effects of moderate exercise as compared to strenuous exercise on inflammatory mediators.

Interestingly, another exercise study by Greiwe, et al.,³⁸ found a reduction in TNF- α in muscle tissue following a 3 month resistance exercise program in older adults. Although we did not observe a significant decrease in TNF- α production by LPS-stimulated PBMC, it is possible that if we had examined TNF- α in the muscle, we may have found a change. Perhaps exercise-induced changes in TNF- α are tissue specific, and a longer duration of

exercise may be necessary to detect a change in PBMC expression of TNF- α as compared to TNF- α expression in muscle tissue. Another potential explanation may be related to the intensity of the strength training performed. The Greiwe study included supervised resistance exercises were performed (3 times a week for 3 months; ~ 90 minutes) at an intensity up to 100% 1 RM, an intensity much greater than our study. Also, the participants in the intervention group were much older (82 ± 1) than subjects in our study. This age difference may have caused a greater difference in TNF- α values (Aging is associated with greater decrease in muscle mass that may correlate with a higher TNF- α level). In future studies, it may be helpful to examine any potential correlation between TNF- α expression in muscle tissue and TNF- α in PBMC. Finally, one other study specifically examined the effect of strength training exercise on cytokine levels in both disease (Rheumatoid Arthritis, RA) and healthy individuals, but failed to find an effect of exercise on cytokine level.⁴¹ This study was much shorter in duration (only 12 weeks) and more importantly, serum levels of the cytokines were not detectable likely due to the fact that high-sensitivity kits were not available when this study took place.

Epidemiological studies

Most of the results of the epidemiological studies are consistent with the findings from our study. In the Pischon et al¹³ study of over 850 healthy men and women of all ages, there was an inverse relationship between physical activity and proinflammatory cytokine (plasma IL-6, CRP and soluble TNF-receptor (sTNF-R) level. In our study, there was a reduction in the IL-6 values with cardiovascular exercise, a trend towards reduced serum IL-6, and a reduction in IL-1 β in both the cardiovascular and flexibility/strength groups,

supporting the inverse association found between physical activity and serum cytokines in the study by Pischon et al. Similarly, in a study of 3,075 individuals in an age group more similar to that of our study (aged 70-79), found that higher levels of exercise were associated with decreased levels of serum CRP or plasma IL-6 and TNF- α . In both of these epidemiological studies, adjusting for body fatness or mass attenuated the effect of physical activity.

Our results differed from the results of the Shinkai et al study.⁴⁰ The Shinkai et al study compared the effect of regular running on proinflammatory cytokine levels.⁴⁰ There was no difference in the IL-1 β levels (in PBMC) between the elderly regular runners and the older sedentary controls. Though the physical activity was unsupervised, the experimental subjects had exercised 2-3 days/week for an average of 10 years before the study. One of the reasons for the apparent similarity in IL-1 β between the two groups may be because it was not a longitudinal study. As with other cross-sectional comparisons, though they help compare across populations, they do not prove causation.

Body weight

By measuring the body weight, we determined the extent to which changes in weight might contribute to the changes in IL-6 levels. Though IL-1 β , IL-6 and TNF- α are produced by adipose tissue, only IL-6 is released into the bloodstream.⁵¹ Weight loss leads to reduction in the fat stores and therefore, IL-6 serum levels decrease. I observed a trend towards weight loss in both groups in our study at the post time point compared to the pre time point; although this was not statistically significant. Also, when body weight was included as a covariate in the model, the effect of exercise remained (independent of body weight).

The molecules leptin and adiponectin may have a role in exercise related alterations in proinflammatory cytokines.⁴⁵ These molecules are produced by adipose tissue.⁴⁵ Higher levels of leptin are found in obese individuals, and leptin levels decrease with weight loss. Leptin also signals the hypothalamus with fat level and nutritional states, which further modulates food intake and energy expenditure. Leptin has inflammatory properties and stimulates (and is stimulated by) proinflammatory cytokine production. The molecule adiponectin, is also secreted by adipose tissue, but is inversely proportional to adipose tissue levels. Higher body weight is associated with lower levels of adiponectin. Adiponectin inhibits TNF- α production and is considered to have anti-inflammatory properties, by inhibiting proinflammatory cytokine production.

The leptin-adiponectin ratio may have a role in altering body weight measures. Higher or unchanged levels of adiponectin are related with weight loss due to exercise.⁵⁵ Weight loss due to exercise also decreases leptin levels in obese individuals.⁵⁵ Study by Ross et al⁵⁵ shows that exercise without weight loss may decrease visceral fat in obese men, which produces leptin. Therefore exercise may alter plasma leptin and adiponectin levels even without changes in body weight (but potentially through reductions in visceral fat). An exercise-induced modulation in the leptin-adiponectin ratio independent of changes in body weight may explain the trend towards a reduction in the plasma IL-6 levels with cardiovascular exercise observed in our study. In future exercise intervention studies, it would be worthwhile to examine leptin and adiponectin to determine the extent to which alterations in the levels of these hormones may influence cytokine levels (with and without weight loss).

Antioxidants

Regular exercise helps increase the antioxidant defenses reducing stress associated with tissue injury and exercise.⁵⁸ There is increased consumption of oxygen by the tissues with acute exercise; increased tissue oxygen consumption is related to increased production of free oxygen radicals. Antioxidants produced naturally by the body help reduce free oxygen radical induced damage. However higher levels of free oxygen radicals produced due to the oxidative stress of exercise may alter the normal Previous studies have shown the role of for antioxidants in attenuating the release of proinflammatory cytokine.^{49,58,66} Work by Powers et al⁴² showed the beneficial effect of exercise in increasing antioxidant levels, potentially lowering the inflammatory response. A study by M. De La Fuente⁵⁸ showed higher levels of antioxidant molecules like vitamin C and E in peripheral blood neutrophils as compared to sedentary controls, after graded exercise. Study by Laughlin et. al and Powers et.al., show sustained exercise cause greater adaptive response and resistance to oxidative damage, leading to greater oxidative capacity in the tissue. Therefore, it is possible that exercise via increases in anti-oxidant defenses leads to reduction of inflammatory factors.

Aging is associated with an increase in the resting free oxygen radical levels. Aging is also associated with reduced immune functions. A study by Vassilakopoulos et.al⁴⁹ showed that concentric exercise helped improve antioxidant levels more than eccentric and mixed exercise⁶⁵, in turn reducing proinflammatory cytokine levels. The subjects exercised for 45 min. at 70% VO₂ max. The cytokines were measured in plasma and were lower after antioxidant ingestion. Therefore, similar to younger adults, moderate chronic exercise may have a role in improving antioxidant response, leading to reduced pro-inflammatory cytokines in elderly individuals.

Summary

Long-term cardiovascular exercise or flexibility/strength appears to reduce the levels of LPS-induced PBMC production of IL-1 β . In contrast, only cardiovascular exercise appears to have an effect on serum IL-6 (trend) and PBMC production of IL-6. The 10-month exercise did not alter the levels of proinflammatory cytokine TNF- α in any of the exercise groups. Our results suggest that cardiovascular exercise may have a greater anti-inflammatory effect as compared to flexibility/strength exercise in older adults. Our findings also suggest that long term exercise (nearly one year) may also retard the inflammatory effects of aging. As aging may be associated with chronic diseases that increase proinflammatory cytokine levels; it is possible that an improved antioxidant response resulting from cardiovascular exercise has a protective effect with respect to reduction of inflammatory cytokines. However, the actual mechanism by which exercise results in a reduction of inflammatory mediators remains to be identified. A significant reduction of inflammatory cytokine level by exercise may therefore lead to a decreased risk of multiple “inflammatory” chronic disease states.

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