The Effects of Supplementing with Constituents of Flaxseed during Exercise Training on Inflammation in Older Adults

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By

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Abstract

This thesis evaluated supplementation with two components of flaxseed during exercise training on inflammation in older adults.

Experiment 1: This experiment assessed secoisolariciresinol diglucoside (SDG) supplementation during aerobic exercise training on inflammation in older adults. **Methods:** One hundred subjects aged 50y or older were randomized to receive either SDG or placebo before completing a 6-month walking program. Fasting concentrations of interleukin-6 and tumor necrosis factor-α, glucose, triacylglycerol (TAG), high density lipoprotein (HDL), low density lipoprotein, and total cholesterol as well as leukocyte cell count were measured every two months while body composition, resting blood pressure, and a composite Z-score of six metabolic syndrome risk factors were assessed at baseline and 6 months. **Results:** Men on placebo increased metabolic syndrome composite Z-score ($p<0.05$). TAG increased ($p=0.017$) in men on placebo relative to men on SDG and men on SDG decreased ($p=0.045$) DBP relative to men on placebo. **Conclusions:** SDG had no effect on inflammation while it is effective in attenuating risk factors associated with metabolic syndrome in older males but not females.

Experiment 2: This experiment evaluated alpha-linolenic acid (ALA) supplementation during strength exercise training on inflammation in older adults. **Methods:** Fifty-one healthy older adults (65.4±0.8y) were randomized to receive ALA or a placebo before completing a 12 wk strength training program. Subjects were evaluated at baseline and 12 weeks for TNF-α and IL-6, muscle strength, body composition, and muscle thickness. **Results:** Males supplementing with ALA decreased IL-6 concentration ($p=0.003$). The female placebo and male ALA group had a significant increase in knee flexor thickness ($p<0.05$). Chest and leg press strength, lean tissue mass, and muscle thickness significantly increased, while percent fat and total body mass

decreased with training ($p<0.05$), with no difference between ALA and placebo. **Conclusions:** ALA lowers IL-6 in older men, but has minimal effect on muscle mass and strength during resistance training.

General Conclusion: A composite score of metabolic syndrome is attenuated in males supplementing with SDG. ALA reduces IL-6 in older men. Older men, but not older women, derive specific health benefits from increased consumption of components of flaxseed consumed during an exercise program.

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Dedication

This thesis is dedicated to my late grandparents: Lyla Myrtle (Aspinall) Taylor (August 15, 1927-June 16, 2000), Donald Thomas Taylor (June 8, 1925-June 13, 1996), Ellen Ruth (Langman) Cornish (July 27, 1917-February 2, 1999), and Lloyd George Cornish (April 22, 1917-March 10, 2002). Each of these wonderful people passed from this world to the next as a result of diseases related to inflammation. It is my hope and desire to continue researching how exercise and nutrition will reduce the inflammatory process associated with many types of disease so to reduce suffering and pain in old age; I want people to age successfully! I can only hope that the suffering and pain my grandparents experienced due to inflammatory disease can and will be alleviated or prevented in older adults as a result of my chosen career.

Deuteronomy 28 discusses the blessings and curses of God on the nation of Israel and says:

> "If you fully obey the LORD your God and carefully follow all his commands I give you today, the LORD your God will set you high above all the nations on earth … However, if you do not obey the LORD your God and do not carefully follow all his commands and decrees I am giving you today, all these curses will come upon you and overtake you: … The LORD will plague you with diseases until he has destroyed you from the land you are entering to possess. *The LORD will strike you with wasting disease, with fever and inflammation*, with scorching heat and drought, with blight and mildew, which will plague you until you perish."

I wonder how much of this is applicable to our society today. I am grateful that in the New Testament book of Revelation an answer to the problem is present:

> "And I heard a loud voice from the throne saying, 'Now the dwelling of God is with men, and he will live with them. They will be his people, and God himself will be with them and be their God. He will wipe every tear from their eyes. *There will be no more death or mourning or crying or pain, for the old order of things has passed away*.'"

May we all be able to persevere and ease the suffering, pain, and disease experienced on this earth until then.

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List of Abbreviations

AA - Arachidonic Acid

ALA - Alpha-linolenic Acid

AMDR - Acceptable Macronutrient Distribution Range

ANCOVA - Analysis of Covariance

ANOVA - Analysis of Variance

APC - Antigen Presenting Cell

ATP III - Adult Treament Panel III

BMI - Body Mass Index

CAM - Cell Adhesion Molecule

CD - Cluster of Differentiation

ConA - Concanavalin A

COPD - Chronic Obstructive Pulmonary Disease

COX - Cyclooxygenase

CRP - C-Reactive Protein

DHA - Decoshexaenoic Acid

DNA - Deoxyribonucleic Acid

DRI - Dietary Reference Intakes

ED - Enterodiol

EL - Enterolactone

ELISA - Enzyme Linked Immunosorbent Assay

EPA - Eicosapentaenoic Acid

GM-CSF - Granulocyte Monocte Colony Stimulating Factor

HDL - High Density Lipoprotein

HMG-CoA - 3-Hydroxy-3-Methylglutaryl -Coenzyme A

IFN - Interferon

Ig - Immunoglobulin

IL - Interleukin

iNOS - Inducible Nitric Oxide Synthase

LDL - Low Density Lipoprotein

LPS - Lipopolysaccharide

LT-α - Lymphotoxin Alpha

MANOVA - Multivariate Analysis of Variance

MHC - Major Histocompatibility Complex

mRNA - Messenger Ribonucleic Acid

MUFA - Monounsaturated Fatty Acid

NADPH - Nicotinamide Adenine Dinucelotide Phoshate (Reduced Form)

NFκB - Nuclear Factor kappa B

NK - Natural Killer

NO - nitric Oxide

O_2^- - Superoxide

OH^- - Hydroxyl

PMNL - Polymorphonuclear Leukocyte

PPAR - Perioxisome Proliferator Activated Receptor

RDA - Recommended Dietary Allowance

SDG - Secoisolariciresinol Diglucoside

sTNFR - Soluble Tumour Necrosis Factor Receptor

TAG - Triacylglycreol

TC - Total Cholesterol

TCR - T-Cell Receptor

TGF-β - Transforming Growth Factor beta

Th - T Helper

TLR - Toll Like Receptor

TNF-α - Tumour Necrosis Factor alpha

UV - Ultraviolet

VO_{2max} - Maximal Oxygen Consumption

WHO - World Health Organization

Chapter 1: Introduction and Review of Literature

1.1 General Introduction

Aging is associated with biological changes that may increase the risk of developing chronic disease. Of the various biological changes, decreased immune system function has been postulated to increase the risk of many diseases (Candore et al. 2006). Cytokines are peptide mediators of the immune system and influence local and systemic control of the immune system and other regulatory systems of the body by binding to cytokine receptors located on cell membranes. Aging results in a hyper-inflammatory state that is associated with an increased amount of pro-inflammatory circulating cytokines and a decreased production of anti-inflammatory cytokines (Gomez et al. 2005). The imbalance between pro- and anti-inflammatory cytokines is theorized to result in an increased susceptibility to infection and changes in tissue structure and function. Cardiovascular disease, auto-immune diseases, type-II diabetes, sarcopenia (loss of muscle mass), and osteoporosis are all considered diseases or conditions which may be linked by chronic dysregulated inflammatory processes (de Rekeneire et al. 2006; Figaro et al. 2006; Prelog 2006; Stoll and Bendszus 2006; Yun and Lee 2004). Although changes in the homeostatic balance between too much and not enough inflammation may be influenced by numerous environmental and genetic factors, eating a proper diet and regular physical activity seem to play an important role in reducing the amount of inflammation associated with aging (Nettleton et al. 2006; Nieman 2001; Shinkai et al. 1997; Yan et al. 2001a). Evidence suggests that older adults who have healthy diets and participate in regular physical activity have decreased markers of inflammation and thus, may be at a decreased risk for the development of chronic inflammatory disease associated with aging (Nicklas et al. 2004). Further, women tend to have a reduced risk of chronic disease compared to men until

menopause. At menopause, the risk of chronic disease increases in women to a level similar to and possibly greater than men. The differences noted in disease risk at menopause are largely attributable to changing hormonal concentrations which will also influence immune system function. There may be a difference in inflammatory responses in older women versus men, especially if women are post-menopausal (Kuller and Meilahn 1996; Maturana et al. 2007).

Physical activity is an important behavior that will reduce the risk and modify the outcome of chronic disease. Exercise immunology is a growing area of research that has revealed many benefits to immune system function with moderate exercise training. For example, natural killer (NK) cell activity is improved and T-cell function is maintained in older exercising adults, both of which may aid in defense against viral and bacterial pathogens (DiPenta et al. 2004; Ferrandez and De la Fuente 1996; Shinkai et al. 1997; Venjatraman and Fernandes 1997). Also, the homeostatic balance between pro- and anti-inflammatory cytokines is maintained in older adults who have participated in exercise training (Shephard and Shek 1995a).

Recently, *Linum usitatissimum* or flaxseed and its components have shown great promise as a nutraceutical (Bloedon and Szapary 2004; Hasler et al. 2000; Lee 2006; Nestel et al. 1997; Rajesha et al. 2006; Ranich et al. 2001; Velasquez et al. 2003). Flaxseed is composed of three main components: the fatty acid α-linolenic acid, the lignan secoisolaricresinol diglucoside, and fibre (Muir and Westcott 2003). Alpha linolenic acid (ALA) is considered an ω-3 fatty acid and is the precursor for the production of eicosapentaenoic acid (EPA) which has profound effects on inflammation by altering the production of pro-inflammatory eicosanoids and cytokines (Alexander 1998; James et al. 2000; Simopoulos 1999; Simopoulos 2002b). Also, the flaxseed lignan, secoisolaricresinol diglucoside (SDG), may exert anti-inflammatory effects via its action

as an anti-oxidant (Kitts et al. 1999; Prasad 1997b; Prasad 1999; Prasad 2000a; Prasad 2000b; Prasad 2000c; Prasad 2001; Prasad 2005b; Prasad et al. 2000). Increased consumption of SDG may down-regulate the production of reactive oxygen species resulting in a decreased amount of pro-inflammtory cytokines produced via the nuclear signaling pathway identified as nuclear factor kappa B (NFκB).

Flaxseed consumption added to a program of exercise will, theoretically, result in a reduction of markers of inflammation in older adults. Reducing the hyper-inflammatory state in older adults should result in an improvement of risk factors associated with cardiovascular disease and musculoskeletal health. This thesis was designed to evaluate the effect of supplementation with components of flaxseed during exercise training on inflammatory cytokines in older adults. In particular, the main objective was to evaluate whether the flaxseed lignan and the main fatty acid of flaxseed (alpha-linolenic acid) would reduce the hyper-inflammatory state often observed as a process in aging. To answer this question, two studies which involve interventions aimed at improving cardiovascular health (Experiment I) and musculoskeletal health (Experiment II) were utilized. For improving cardiovascular health, the literature supports the use of the lignan component of flaxseed as it is thought to act as an anti-oxidant/anti-inflammatory and improves lipid profiles. For musculoskeletal health, the literature supports the use of α-linolenic acid because it is the stronger anti-inflammatory component of flaxseed and theoretically would be more beneficial for reducing sarcopenia (i.e. muscle wasting). These flaxseed interventions will be combined with exercise programs deemed most beneficial for cardiovascular and musculoskeletal fitness so that Experiment I will involve flax lignan combined with a walking/jogging program (cardiovascular exercise), and Experiment II will involve flax oil (α-linolenic acid) combined with resistance training (musculoskeletal

exercise). The exercise components used in this research were applied to all study participants as a means to establish a "standard of care" that is most appropriate for reducing morbidity related to cardiovascular disease and musculoskeletal disease based on current available evidence. The main purpose of the studies was to evaluate the efficacy of supplementation with either the main lignan or the main fatty acid found in flaxseed on inflammatory cytokines in older exercising adults. The main hypotheses of the studies in this thesis were that each component of flaxseed would decrease markers of inflammation in older exercising adults. The following section will provide an overview of immune system function to aid in understanding how flaxseed and exercise may affect inflammation during aging. This will be followed by a review of the literature on the effects of exercise, secoisolariciresinol diglucoside, and alpha-linolenic acid on inflammation.

1.2 Overview of the Immune System

In humans, the immune system is a complex series of interactive humoral and cellular components which functions as a defense system against pathogenic attack and infectious agents that may cause disease. In general, the immune system protects the body from viral, bacterial, parasitic, and fungal infections by mounting attacks via the innate (or nonspecific) and/or the adaptive (or specific) components of the immune system (Mak and Saunders 2006). The immune system is also responsible for the inflammatory process which will heal damaged tissues (Mackinnon 1999). The innate immune system has cells present and ready to attack invading pathogens if the organism is to become infected at any time. The adaptive immune system requires time to develop via its recognition of antigen specificity and thus will only attack foreign cells that are specific to the antigen present from previous exposure (Eales 2003). This is

in contrast to innate immunity which will react equally to a variety of pathogens and thus lacks specificity. Also, the adaptive component of the immune system will develop memory cells, which function to recognize foreign organisms from previous exposure and mount a quick and rapid attack against the organism (Eales 2003). The innate immune system is composed of: physical barriers (skin, mucus), chemical barriers (pH of body fluids, complement, acute phase proteins), and cells (monocytes/macrophages, granulocytes, and natural killer cells). The adaptive branch of the immune system is composed of a humoral component, which includes memory cells and antibodies, and a cellular component which is composed of T cells (Mackinnon 1999).

The immune response happens in three stages with varying amounts of time between each stage (Mak and Saunders 2006). Phase I is the immune reaction that immediately happens, from 0-4 hours, and is termed the non-induced innate, non-specific response. This phase is regulated by the defenses of the body that include the skin, pH, and saliva proteases (Mak and Saunders 2006). The second phase is termed the induced innate, broadly specific response and usually occurs from 4-96 hours following a pathogenic attack. In this phase, the innate immune system is largely responsible for attacking a broad variety of pathogens by phagocytosis, activation of complement, and cytokine release. If a former pathogen is detected by memory cells of the adaptive immune system i.e. an antibody recognizes an antigen, the adaptive response will remove the pathogen from the host (Mak and Saunders 2006). Phase III of the immune response is termed the induced adaptive, highly specific response and usually takes over 96 hours to become fully functional after initial infection. During this phase, the pathogen must be processed by the innate system cells and presented to the T-cells to ensure a fully stimulated response. If the third phase is fully activated it will result in the production of T and B memory

cells which will aid in producing a more rapid response if the host is exposed to the same pathogen again (Mak and Saunders 2006).

Cells of the immune system all originate in stem cells found in bone marrow and are differentiated into the myeloid cell line and the lymphoid cell line (Eales 2003). The myeloid cell line includes monocytes which differentiate into macrophages, Kupffer cells, and dendritic cells as well as granulocytes which differentiate into neutrophils, basophils, and eosinophils (Mak and Saunders 2006). Cells coming from the lymphoid cell line include T-cells, B-cells, and natural killer (NK) cells. T-cells will differentiate into T-helper, T-suppressor, and T-cytotoxic cells while B-cells will differentiate into plasma cells which are responsible for secreting antibodies (the immunoglobulins) (Eales 2003). T-cells are so named due to the fact that they must migrate to the thymus gland to mature and differentiate into the various sub-types of T-cells. Once in the thymus gland, the T-cells will differentiate into 2 distinct cell types with a specific protein marker termed the cluster of differentiation (CD)4^+ T helper cells and $CD8^+$ pre-cytotoxic cells (Mak and Saunders 2006). T-helper 1 (Th1) cells will aid pre-cytotoxic cells to differentiate into cytotoxic T-cells and T-helper 2 (Th2) cells aid B-cells in differentiating into plasma cells (Mak and Saunders 2006).

1.2.1 Myeloid Immune Cells

The general purpose of monocytes and macrophages in the immune system is to phagocytose foreign organisms and act as antigen-presenting cells (APCs) for the lymphoid cell line (Eales 2003). These cells are found predominantly in the circulation but will migrate to various types of tissue when infection, inflammation, or injury occurs. Monocytes and macrophages are involved in the production of cytokines including IL-1α, IL-1β, IL-1ra, IL-6,

and TNF-α, which help stimulate the adaptive immune response (Eales 2003). These cells produce proteases from lysosomes and produce oxygen free radicals as well as nitric oxide which are all toxic to microorganisms, particularly bacteria (Mackinnon 1999).

Granulocytes, another type of myeloid immune cell, are large immune cells that differentiate into three types of cells including neutrophils, basophils, and eosinophils. Neutrophils are phagocytic cells which release proteases, phospholipases, and generate oxygen radicals which all destroy microorganisms (Eales 2003). Eosinophils function by phagocytosis and function to destroy parasitic infections while basophils are mostly involved in allergic and inflammatory reactions (Mackinnon 1999).

1.2.2 Lymphoid Immune Cells

The lymphoid cells include T-cells, B-cells, and NK-cells. T and B-cells are part of the adaptive immune system while NK-cells function in the innate immune system. The T-cells are distinguished from other cells by the T cell receptor (TCR or CD3) located on the cell membrane (Eales 2003). The main function of T-cells is to control the adaptive immune response via various subsets of T-cells. Helper and inflammatory T-cells are identified by the cell surface protein CD4 and cytotoxic T-cells are identified by the CD8 cell surface protein (Eales 2003). All T-cells are able to distinguish between self and non-self by a protein complex termed the major histocompatibility complex (MHC), which aids T-cells in identifying foreign antigens on infectious cells, and then produces an immune response via T-cell activation (Eales 2003). Also, cytokines, such as IL-2, IL-3, IL-4, and IL-6, are released by T-cells which are responsible for regulating and coordinating further immune cell function. $CD8^+$ cells will seek out virally infected cells, which have viral proteins on the cell surface, and release chemicals inside the

infected cell to destroy DNA and prevent further replication (Mak and Saunders 2006). $CD4^+$ cells are further subdivided into helper T-cells and inflammatory T-cells. The helper T-cells activate B-cells to produce an antibody to a specific antigen and are responsible for killing extracellular immunogens (Mak and Saunders 2006). Intracellular pathogens are destroyed by inflammatory T-cells where they activate monocyte/macrophage phagocytic activity as well as antibacterial activity (Mak and Saunders 2006).

The B-cells of the lymphoid cell line are responsible for bearing antibody on the cell surface. The unique feature of B-cells is their ability to produce antibody from previous exposure to some pathogen and thus, maintain memory of previous exposure to antigens that invade the system (Mak and Saunders 2006). The B-cells, in combination with activation from T-helper cells, will produce a faster immune response to invading antigens if the system has been 'primed' by previous exposure to the same antigen (Eales 2003). This feature of the immune system is what the immunization process is based upon and has resulted in a reduction in infectious disease in the developed world (Mak and Saunders 2006).

Natural killer (NK) cells are part of the innate immune system and are identified by the cell surface markers $CD16^+$ and $CD56^+$ but lack the $CD3^-$ marker of T-cells (Eales 2003). The main function of NK cells, as their name would suggest, is to kill tumor cells and cells infected with viruses. They do so by discharging toxic enzymes into infected cells after binding with them (Eales 2003). NK cells are also mediators in antibody-dependent cell-mediated cytotoxicity, which means they act to aid in recognition of antibody coated cells that require destruction (Mak and Saunders 2006).

1.2.3 Soluble Mediators of the Immune System

Many mediators of immunity are found in blood and other body fluids. The different types of soluble mediators include: immunoglobulins, acute phase proteins, complement, and cytokines. Immunoglobulins (Ig) are glycoproteins that are produced by plasma cells in response to an immunogen and which produce antibodies (Eales 2003). The primary function of immunoglobulins is to bind to one specific antigen producing an antigen-antibody complex which will protect the host species from attack by the antigen by stimulating plasma cells to produce more antibodies to the specific antigen infecting the host (Mackinnon 1999). Acute phase proteins, such as C-reactive protein and serum amyloid-A, are released from the liver on stimulation by pro-inflammatory cytokines (Eales 2003). The purpose of acute phase proteins is to bind to a wide variety of microbes (i.e. acute phase proteins have the ability to recognize self from non-self) on their cell surfaces which will activate the complement cascade of events (Mak and Saunders 2006). Complement represents a series of 30 serum and membrane proteins that when activated will induce cell lysis, opsonization, clearance of antigen-antibody complexes, enhance antigen presentation, enhance B-cell activation, and generate by-products that signal a number of cellular events related to the immune and inflammatory response (Eales 2003; Mak and Saunders 2006). Each mediator has a specific function in the immune response but this review will focus on the effects cytokines have on immune system regulation, and specifically the inflammatory process.

1.2.3.1 Cytokines

Cytokines are peptide or glycoprotein molecules that influence a wide variety of cell functions but whose primary role is intercellular communication (Mak and Saunders 2006).

Cytokines, hormones and growth factors are similar in that they are all soluble and they need to be bound by receptors on cells to exert their influence (Eales 2003). The site of production, mode of operation, and range of influence are what distinguishes cytokines from hormones or growth factors. Cytokines exert regulatory function by controlling innate and adaptive immunity as well as the growth and differentiation of hematopoietic cells (Mak and Saunders 2006). Cytokines are involved in a number of cellular events related to immune function including: pro-inflammation, anti-inflammation, antiviral replication, growth of cells, differentiation of cells, cell-mediated immunity, humoral mediated immunity, Ig isotype switching, and chemotaxis (Mak and Saunders 2006). They exert their effects by binding to specific receptors on cell membranes to produce intracellular signaling that results in gene transcription or changes in the cell's activity (Mak and Saunders 2006). The cytokine network is complex and it is reasoned that the complexity exerts exacting control over the cells of the immune system which, if left unchecked, could produce extreme tissue damage or auto-immune disease (Mak and Saunders 2006). Currently, there are more than 100 cytokines identified; this review will focus on the cytokines that are involved in inflammation.

The interleukin (IL) cytokines are produced by leukocytes including T-cells, monocytes/macrophages, and NK cells. These cytokines are not structurally related but share function by influencing immune cell operation (Mackinnon 1999).

Interleukin-1 is present in two isoforms, IL-1α and IL-1β, but IL-1β is found in greater amounts than IL-1α (Mak and Saunders 2006). The prominent role of IL-1 is to increase inflammatory events in innate defense. At low concentrations, IL-1 will promote the expression of adhesion molecules and induce the production of other pro-inflammtory cytokines including IL-6, IL-8, and TNF-α. If the concentration of IL-1 is moderate, it will induce fever, the acute

phase response of the immune system, and may be cachexic. High concentrations of IL-1 may result in endotoxic or septic shock in which the circulatory and metabolic systems collapse possibly resulting in death (Mak and Saunders 2006). IL-1 also functions to induce hematopoietic growth factors and is involved in bone remodeling by increasing bone resorption by osteoclasts and decreasing bone deposit by osteoblasts (Mak and Saunders 2006).

Recent debate has suggested that interleukin-6 may act either as a pro or anti-inflammatory cytokine but general nomenclature still considers it a pro-inflammatory cytokine (Starkie et al. 2003). IL-6 exerts a strong influence on B-cell differentiation, especially at the end stages of B-cell production. It is produced by mononuclear phagocytes, fibroblasts, and vascular endothelial cells in response to stimulation by IL-1 and TNF-α, thus, it usually exerts it effects in conjunction with other inflammatory cytokines (Mak and Saunders 2006). Some activated T-cells will produce IL-6 and it has recently been demonstrated that skeletal muscle acts as an endocrine organ by releasing IL-6 which may influence adipose, hepatic, and arterial tissue (Febbraio and Pedersen 2002; Febbraio and Pedersen 2005; Mak and Saunders 2006).

Interleukin-8 is considered a chemokine, which is a cytokine that exerts chemoattraction of leukocytes, and not an interleukin (Mak and Saunders 2006). IL-8 is a mediator that selectively allows passage of leukocyte subsets between the blood and various types of tissue compartments as well as influences the actions of endothelial and nervous system cells (Mak and Saunders 2006). In general IL-8 acts as a powerful attractor for neutrophils and will promote the inflammatory response (Mak and Saunders 2006).

Interleukin-12 is a cytokine that plays a pivotal role in linking the activation of macrophages by bacteria to the activation of NK and Th1 outcome functions (Mak and Saunders 2006). Thus, IL-12 is a key cytokine that links innate and adaptive immunity because it

stimulates the production of interferon γ (IFNγ), a cytokine involved in suppressing viral replication, and is responsible for defense against a number of intracellular pathogens as well as activation of the adaptive immune response (Mak and Saunders 2006).

Interleukin-17 is produced by $CD4^+$ T-cells and induces the production of other pro-inflammatory cytokines including IL-6, IL-8, and granulocyte-colony stimulating factor (G-CSF) (Mak and Saunders 2006). High amounts of IL-17 will induce IL-6 reliant neutrophilia which may increase resistance to bacterial infections. Also, IL-17 up regulates the production of intercellular adhesion molecule (ICAM-1), a cell surface ligand, which plays a role in leukocyte adhesion and the inflammatory response (Mak and Saunders 2006).

There is an overlap of activities between IL-1, IL-12 and IL-18. Interleukin-18 stimulates the propagation of IFNγ and IL-2 receptor from Th1 cells, stimulates the release of IL-2 and granulocyte-monocyte colony stimulating factor (GM-CSF) from activated T-cells, and will promote natural cytotoxicity by release of IFNγ and TNF-α from NK cells (Mak and Saunders 2006).

In general, interleukin-22 is considered pro-inflammatory due to its ability to stimulate acute phase protein release from hepatocytes (Mak and Saunders 2006). IL-22 will increase the numbers of platelets and basophils as well as decrease the number of erythrocytes (Mak and Saunders 2006). Interleukin-24 is found in high levels in some tumors and promotes the induction of apoptosis in tumor cells. Also, IL-24 may be responsible for aiding wound healing by increasing fibroblast proliferation or supporting the inflammation associated with wound healing (Mak and Saunders 2006). Interleukin-27 promotes the Th1 response, increases the amount of pro-inflammatory cytokines, decreases the Th2 response, and has powerful anti-tumor effects (Mak and Saunders 2006).

Interferons (IFN) are so named due to the discovery that primary viral infection will induce interferon release from cells resulting in resistance to infection by a second virus thus, interfering with viral infection (Eales 2003). There are four types of interferons found in humans including: IFNα, IFNβ, IFNγ, and IFNω. All interferons share antiviral and antiproliferative properties but IFNγ alone has effects on the immune system cells and adaptive immunity (Mak and Saunders 2006). IFNγ is considered pro-inflammatory since it stimulates the secretion of IL-1β, IL-12, IL-18, and TNF-α from macrophages and thus, promotes the inflammatory response (Eales 2003). Also, IFNγ effectively eliminates intracellular-replicating pathogens by upregulating the expression of inducible nitric oxide synthase (iNOS) and reactive oxygen intermediates to eliminate pathogenic organisms (Mak and Saunders 2006).

Tumor necrosis factor (TNF), which is also known as TNF-α, is a very pleiotropic (i.e. has multiple systemic roles) cytokine that exerts effects on many tissue types (Mak and Saunders 2006). In low concentrations TNF-α functions to upregulate adhesion molecules on cell surfaces to stimulate the movement of leukocytes from circulating blood into surrounding tissue that may be under pathogenic attack or damaged due to injury or insult. TNF-α also promotes granulocytes to destroy invading microbes as well as stimulating the release of IL-1, IL-6, IFNγ, chemokines, and TNF-α itself. At moderate concentrations TNF-α will enter the blood and begin acting like a hormone where it affects cells of many tissue types throughout the body. At high concentrations, TNF-α may be lethal as it may stimulate endotoxic shock (Mak and Saunders 2006). In addition, TNF-α has been implicated in the proliferation of fibroblasts which may result in the infiltration of fibrous tissue and fibrous degeneration observed in chronic inflammation of various tissue types (Mak and Saunders 2006). TNF-α has immunoregulatory roles, similar to IL-1 and can promote both apoptosis and cell survival depending on the TNF-α

receptor that is engaged as well as the cellular milieu present on binding (Eales 2003). TNF-α is used to facilitate the response of B and T-cells to antigenic presentation, may induce tumor cell death by necrosis or apoptosis, may act in an antiviral fashion, and aids in the activation of the blood clotting cascade of events (Mak and Saunders 2006).

Lymphotoxin α (LTα), which was formerly known as TNF-β, is also considered a pro-inflammatory cytokine related to the TNF family of cytokines (Eales 2003). Its main role is to promote inflammation, kill tumor cells by apoptosis, stimulate neutrophil activity, and act as an antiviral. Thus, LTα has very similar roles to TNF-α but its potency is only a fraction of that of TNF-α (Mak and Saunders 2006).

Interleukin-4 is a very potent, pleiotropic cytokine that exerts it effects on most cell types. The role of IL-4 in inflammation is to inhibit the secretion of pro-inflammatory chemokines and cytokines (TNF-α and IL-1β) from macrophages as well as impair the ability of macrophage cells to produce reactive oxygen species (Eales 2003). Other roles of IL-4 include: differentiation and growth of Th2 subsets, which aid in the humoral response to combat extracellular pathogens, upregulation and formation of major histocompatibility complex II, regulation in allergic reactions, and stimulatimg the synthesis of IL-12 from dendritic cells and macrophages thus, acting in a negative feedback method (Mak and Saunders 2006).

Interleukin-10 has a regulatory function in both innate and adaptive immunity which demonstrates the cross-talk between both branches of the immune system (Eales 2003). IL-10 can act to slow down inflammation by targeting granulocytes and mast cells to inhibit the release of massive amounts of IL-1 and TNF-α to reduce the risk of endotoxic shock (Eales 2003). Also, IL-10 inhibits activation of NFκB, a transcription factor that upregulates the production of TNF-α, IL-1, IL-6, IL-8, and IL-12 therefore reducing the amount of pro-inflammatory cytokines

produced. IL-10 also operates to inhibit the respiratory burst and nitric oxide (NO) dependent killing of microbes associated with macrophage activity (Mak and Saunders 2006).

Anti-inflammatory effects are exerted by interleukin-11 due to the inhibition of pro-inflammatory cytokines and NO production by macrophages (Mak and Saunders 2006). IL-11 is similar to IL-6 and is redundant with some of its function. IL-11 will stimulate hematopoietic cells, growth of T and B-cells, and promote collagen deposits by fibroblasts (Mak and Saunders 2006). IL-13 is considered anti-inflammatory as it inhibits the production of TNF-α, IL-1β, and pro-inflammatory cytokines secreted by macrophages (Mak and Saunders 2006). However, IL-13 may upregulate the synthesis of IL-12 from dendritic cells and macrophages (Mak and Saunders 2006).

Transforming growth factor β (TGF-β) is a pleiotropic cytokine vital to cell growth and the adaptive immune response (Mak and Saunders 2006). It is considered anti-inflammatory due to the decreased production and effect of IL-1, IL-2, IL-6, IFNγ, and TNF-α exerted by TGF-β. Further, TGF-β will decrease the release of NO by macrophages consequently acting to decrease the inflammatory response (Mak and Saunders 2006).

The purpose of this project was to evaluate the effect of flaxseed supplementation interventions during exercise training on IL-6 and TNF-α in older adults. These two cytokines are considered pro-inflammatory, although IL-6 possesses anti-inflammatory functions as well. There is evidence that IL-6 and TNF-α are elevated by 2-4 fold in older adults which may increase the risk of various diseases associated with aging (Krabbe et al. 2004). These pro-inflammatory cytokines have been studied extensively in relation to the development of dysregulated inflammation. The evidence gathered from this project will aid in determining the

efficacy of supplementation with components of flaxseed while exercise training on the amelioration of diseases associated with increased amounts of IL-6 and TNF-α.

The following sections discuss the inflammatory response and how inflammation is related to the development of atherosclerosis and sarcopenia. The studies completed for this thesis project were designed to investigate how flaxseed supplementation may reduce inflammation associated with the development of these two diseases associated with the aging process.

1.2.4 Inflammatory Response

Inflammation is the term given to the immune response that results from tissue damage or pathogenic attack. It is a series of events that comprises the second and third phase of the immune response and thus, is an essential component of both adaptive and innate immunity (Eales 2003). The clinical signs of inflammation include redness, swelling, heat, and pain. The redness and heat accompanying inflammation is a direct result of the vasodilation of blood vessels in the involved area. The swelling that accompanies inflammation is largely a result of increased amount of leukocytes that are coming to the affected area (i.e. chemoattraction). The augmented number of leukocytes occurs in response to increased adhesion of white blood cells to endothelial blood cells and increased permeability of local blood vessels (Eales 2003). Pain is felt due the release of bradykinin, which is a plasma enzyme that stimulates pain receptors of the nervous system, induces vasodilation, smooth muscle contraction and increases permeability (Mak and Saunders 2006).

Leukocytes that migrate to a site of infection or injury will be attracted initially by chemotaxis, which is a process whereby injured cells release chemokines. The leukocytes are

attracted via a chemical gradient. Some chemokines that are released include: macrophage activating factor, macrophage inflammatory protein-1α , interleukin-8, and neutrophil activating protein (Eales 2003). Each of these chemokines will be released by cells from the myeloid and lymphoid cell lines as well as platelets and the endothelium of damaged tissue (Mak and Saunders 2006). During this part of the inflammatory response, the innate effector cells are largely responsible for phagocytosis of the infected or injured tissue. Cells of the innate system will release factors that will attract effector cells of the adaptive immune response and stimulate the process whereby antigen recognition results in a more rapid attack if infected in the future (Mak and Saunders 2006).

The inflammatory response is also mediated by the cytokines TNF-α and IL-6, which are released by activated macrophages in response to infection or injury (Mak and Saunders 2006). TNFα and IL-6 stimulate the liver to release acute-phase proteins that act in a manner similar to antibodies, by activating the complement cascade, but are non-specific and will bind to a vast array of bacterial pathogens. In the beginning stages of inflammation, the concentration of the acute-phase proteins will increase rapidly which results in an increased activation of complement (Eales 2003). One type of acute-phase protein is C-reactive protein (CRP) which when bound to cell walls of bacteria and fungi can activate the complement cascade of events resulting in the destruction of the agent (Mak and Saunders 2006).

Another group of inflammatory mediators are collectively known as eicosanoids and include prostaglandins, thromboxanes, and leukotrienes (Eales 2003). These lipid-based molecules are derived from the degradation of phospholipids in the membranes of cells in the myeloid cell line (Eales 2003). Degradation of the phospholipids results in the production of arachidonic acid which can be metabolized to the various eicosanoids by the enzymes

phospholipase A, cyclooxygenase and lipoxygenase (Eales 2003). In general, activation of arachidonic acid metabolism will result in an increased inflammatory environment by: inducing leukocyte chemotaxis and aggregation, proliferating T-cell formation, increasing the release of pro-inflammtory cytokines (such as TNF-α and IL-6), and increasing platelet aggregation (Alexander 1998; James et al. 2000; Mak and Saunders 2006).

During inflammation there is an upregulation of cell adhesion molecules (CAMs) on the activated endothelial cells which are responsible for increasing the leukocyte migration to a place of injury (Eales 2003). CAMs are responsible for binding leukocytes and allowing them entry from the blood into the tissues that are affected. The leukocytes will then follow the chemotactic signals to the site of injury and begin phagocytosing the infected cells. Eventually, if the pathogen is not destroyed by complement, bound to acute-phase proteins, or phagocytosed, macrophages will release TNF-α and IL-1 to attract T-cells to the area thereby activating the adaptive branch of the immune system (Mak and Saunders 2006). Thus, the interaction between the innate and adaptive system is again intermingled and the two systems should not be thought of as separate entities but fully functional and synergistic branches that aid in host defense.

The inflammatory process would not be complete without the repair of tissue damaged during the assault on the host. The increase in permeability of blood vessels allows enzymes of the fibrinolytic system to enter into the damaged area and begin the blood clotting cascade of events by building up a network of fibrin to aid in clotting and initiating wound healing (Eales 2003). Platelets and mast cells are also involved in the inflammatory healing process. Platelets are needed to complete wound healing, while mast cells release heparin, histamine, and 5-hydroxytryptamine to cause vasodilation and “speed up” the healing of the wound (Mak and

Saunders 2006). In general, a complete inflammatory response involves both the destruction of foreign pathogens and/or the repair of damaged tissue (Mak and Saunders 2006).

In older adults, it is hypothesized that chronic low-grade levels of inflammation result in a hyper-active inflammatory response whereby complete wound healing or defense against foreign pathogens is compromised due to elevated levels of pro-inflammatory cytokines and a decreased amount of anti-inflammatory cytokines (Bruunsgaard and Pedersen 2000; Kohut and Senchina 2004; Shinkai et al. 1997). The main purpose of this thesis was to evaluate supplementation with components of flaxseed during exercise training on cytokines related to inflammation in older adults.

1.3 Review of Literature

1.3.1 Aging and Inflammation

One theory explaining the development of chronic disease with age is that the amount of inflammation progressively increases. The terms "inflamm-aging" or "hyper-inflammation" have been used to describe the physical characteristics associated with immunosenescence (Franceschi et al. 2000). As individuals age, there is an increased amount of antigenic presentation, which alters the capability of the innate and adaptive immune system. The inability to combat antigenic challenge, due to changes to both innate and adaptive immunity during aging, may be responsible for increased incidence of infectious, auto-immune, and chronic diseases (Punzi et al. 1996; Roubenoff et al. 1998; Sindermann et al. 1993). One study evaluated the immune response to novel antigenic challenge and found that older sedentary men have less of a response on stimulation versus younger and older physically active men suggesting a depression of acquired immunity with age (Smith et al. 2004). Also, T-cell function is depressed

in aged sedentary males when compared to aged active males (Yan et al. 2001b). In this cross-sectional study there was an increase in helper T-cells with a corresponding decrease in cytotoxic/supressor T-cells which accounted for an increase in the $CD4^+$:$CD8^+$ ratio in older sedentary males when compared to younger controls and older exercising men. Further, NK cell function was reduced in older sedentary men when compared to older active men suggesting a preservation of both adaptive and innate immunity with moderate physical exercise into old age. In an animal model of sepsis, old rat myocardium responded with increased expression of components of the innate immune system (CD14 and nitric oxide synthase) and a reduced β-adrenergic response to stimulation when compared to young rat hearts (Rosas et al. 2001). This study suggests a role for altered innate immune system function in old age that may increase mortality during septic shock due to altered hemodynamic properties associated with normal heart function. The authors of this study suggested that increased cytokine concentrations may be the stimulus for the altered immue response and it has recently been demonstrated cross-sectionally that older adults maintain the ability to produce high amounts of cytokines during septic shock (Marik and Zaloga 2001). Evidence for the role cytokines play in the process of "inflamm-aging" is growing and indicates a increase in pro-inflammatory cytokines and a decrease in anti-inflammatory cytokines as part of the aging process (Shinkai et al. 1998). Also, due to alterations in T-cell, B-cell, and NK-cell concentrations with aging, there is a corresponding alteration in the inflammatory milieau present in older adults promoting an increased amount of inflammation (Rosas et al. 2001; Smith et al. 2004; Yan et al. 2001b; Zanni et al. 2003).

1.3.2 Cytokines and Aging

In general, there are some differences in the type of cytokines predominantly expressed between *in vitro* and *in vivo* studies in older adults; various studies indicate both an increased amount of pro-inflammatory or anti-inflammatory cytokines (Franceschi et al. 2000; Gomez et al. 2005; Krabbe et al. 2004). Possible explanations for the differences between *in vivo* and *in vitro* research are: additional immune cells or tissue cell types producing pro-inflammatory cytokines than those assessed *in vitro*; the variety of inclusion/exclusion criteria used in human aging studies (possibly masking underlying inflammatory events); or alteration of cell function *in vitro* as compared to *in vivo* due to a change in phenotype caused by differing environmental conditions (Gomez et al. 2005). Nonetheless, there is disparity between studies utilizing different methodologies as to the immune response with aging.

Comparative research of young and old individuals examining the effects of *in vitro* cytokine release from whole blood found a trend toward increased levels of IL-6 in older adults but no differences between the levels of IL-2 or IFNγ (Sindermann et al. 1993). The researchers also found a higher ratio of $CD4^+/CD8^+$ cells in the older adults which might explain the increased incidence of infection in older versus younger adults. Another cross-sectional study, which compared 742 elderly (average age 79 years) adults from the Framingham study to 21 younger (average age 39 years) adults, examined the production of cytokines from monocytes (Roubenoff et al. 1998). The elderly group had higher levels of IL-6 and IL-1ra than the young group. Also, there was a direct correlation between higher concentrations of C-reactive protein and IL-6 in the elderly but no similar relationship in the young group. Conversely, the results indicated no difference in TNF-α or IL-1β between the young and elderly subjects. The authors

indicated that there does seem to be dysfunction arising in inflammatory mediators with age but would not speculate on the function inflammation plays in the aging process.

In another study examining the effects of the acute phase response of young versus old subjects to endotoxin injection, the elderly had greater increases in TNF-α, soluble TNF receptors, and a prolonged increase in soluble TNF receptors when compared to the young participants (Krabbe et al. 2001). The elderly participants in the study also exhibited a prolonged fever response and had a more rapid increase in CRP than the younger control group. The conclusion of the research indicated aging is associated with an increased inflammatory response, a heightened acute phase response, and an extended fever reaction.

An *in vitro* study examining the cytokine profile of T-cells isolated from individuals of varying ages indicated a significant increase in the production of IFNγ, IL-2, and TNF-α in all 3 sub-type T-cells while IL-4, IL-6, and IL-10 were only increased in memory $CD8^+$ T-cells of older adults (Zanni et al. 2003). The authors concluded that the results give evidence for the "inflamm-aging" hypothesis. In contrast, IL-1 and TNF-α production from monocytes was not altered *in vitro* when a group of malnourished elderly nursing home patients, an age-matched control group, and a group of young healthy adults was compared (Bradley et al. 1990). This disparity between the two studies is likely due to the various cell types used in each investigation. Another study examining the effects of *in vitro* stimulation by phytohemagglutinin, a mitogen to lymphocytes, of human peripheral mononuclear cells from young (average age 27 years) and old (average age 80 years) found significant increases in the production of IL-1β, IL-6, and TNF-α in the old subjects (Fagiolo et al. 1993). The authors suggested that the increased production of these cytokines in response to stimulation may increase the risk of some pathology such as atherosclerosis, dementia, and osteoporosis.

In vitro research of lipopolysaccharide (LPS) stimulated monocytes shows lower amounts of TNF-α, IL-1β, and IL-6 production in older (> 65 years) versus younger (< 39 years) cell cultures (Bruunsgaard et al. 1999b; Gabriel et al. 2002; Gon et al. 1996; McLachlan et al. 1995). Other *in vitro* research indicates an increased release of IL-1β, IL-6, or TNF-α from polymorphonuclear (i.e. leukocytes with varied nuclei structure) cells or whole blood stimulated with LPS or phytohemagglutinin in cells donated by old versus young adults (Born et al. 1995; Fagiolo et al. 1993; Gabriel et al. 2002; McNerlan et al. 2002; O'Mahony et al. 1998; Riancho et al. 1994; Sandmand et al. 2003; Saurwein-Teissl et al. 2000). Furthermore, there is research indicating no difference between the release of pro-inflammatory cytokines from various types of leukocyte cultures between older and younger adults (Beharka et al. 2001; Gabriel et al. 2002; O'Mahony et al. 1998; Riancho et al. 1994; Roubenoff et al. 1998; Rudd and Banerjee 1989). A conclusion is difficult to draw given the evidence from various types of studies evaluating the cytokine changes with aging. Higher levels of circulating inflammatory mediators such as IL-6, TNF-α, and acute phase proteins have each been associated with increased morbidity and mortality in the elderly (Harris et al. 1999; Krabbe et al. 2004; Taaffe et al. 2000). Broadly, one hypothesis is that the systemic circulating levels of IL-6 may represent the level of inflammation in younger and healthy older populations whereas TNF-α levels would represent the level of inflammation in frail elderly populations (Krabbe et al. 2004). The authors postulate that TNF-α would not be released systemically by varying tissue types, such as adipocytes and endothelial cells, until a certain threshold in terms of age and disease activity was met. Once the threshold was met, the result would be a systemic increase in TNF-α concentration resulting in increased morbidity and mortality. In general, it is not known whether increased amounts of pro-inflammatory cytokines are a cause of the chronic diseases associated with aging or a

consequence of the events surrounding the pathological and/or physiological changes associated with the aging process (Petersen and Pedersen 2005). Nonetheless, quantifying soluble markers of inflammation, such as cytokines, is one strategy that, in the future, may aid clinicians in determining disease morbidity and mortality. In general, the consensus seems to be that aging is associated with decreased amounts of IL-2 and increasing amounts of TNF-α and IL-6 (Bruunsgaard and Pedersen 2000; Kohut and Senchina 2004; Shinkai et al. 1998).

1.3.3 Cytokines in Diseases of Aging

Chronic low grade inflammation has been implicated in a number of disease processes and is associated with the aging process (Petersen and Pedersen 2005). The purpose of the following two sections is to draw a link between the inflammatory processes and the pathogenesis of atherosclerosis, metabolic syndrome, and sarcopenia. The studies in this thesis determined the effects of supplementation with components of flaxseed during exercise training on pro-inflammatory cytokines, with a focus on prevention of metabolic syndrome and sarcopenia in older individuals. The theoretical link between altered inflammatory processes and the development of these two diseases is outlined below.

1.3.3.1 Atherosclerosis/Metabolic Syndrome and Inflammation

Atherosclerosis is considered a chronic inflammatory disease of the arteries (Stoll and Bendszus 2006; Tedgui and Mallat 2006). For most of the 20th century, the lipid hypothesis of atherosclerosis was widely accepted which suggested that smooth muscle cells proliferate in response to high blood lipid levels causing arterial narrowing. It was later suggested that atherosclerosis involves a response to injury whereby monocytes and lymphocytes adhere to

endothelial cells to induce a chronic inflammatory reaction (Raines and Ross 1993; Ross 1979; Ross 1986; Ross 1987; Ross 1999; Ross and Glomset 1976a; Ross and Glomset 1976b). Indeed, current evidence indicates that the concentration of CRP is a better predictor of cardiovascular disease than low-density lipoprotein (LDL) cholesterol (Ridker et al. 2002). It is acknowledged that high concentrations of blood lipids, especially LDL, have a clear role in the development of atherosclerosis, as atherosclerosis usually does not occur in those with normal blood cholesterol concentrations; however, oxidized LDL seems to be the initial activator of the inflammatory process in vascular cells (Stamler et al. 1986; Tedgui and Mallat 2006). Once macrophages and lymphocytes are recruited, due to oxidized LDL activation, they will secrete numerous cytokines, including TNF-α and IL-6, which stimulate inflammation resulting in increased atherosclerotic lesions (Tedgui and Mallat 2006).

Metabolic syndrome is defined as a group of risk factors (elevations in each of central adiposity, serum triacylglycerol, serum glucose, blood pressure, inflammation, and lowered high density lipoproteins) that increase the risk of developing atherosclerosis and may lead to insulin resistance and cardiovascular disease (Wassink et al. 2007). Recently, higher concentrations of inflammatory proteins and cytokines have been linked to the development of metabolic syndrome (Wassink et al. 2007). Increased adipose tissue deposits will increase the amount of TNF-α and decrease the concentration of adiponectin, an anti-inflammatory adipokine, released into circulation which may stimulate the development of insulin resistance (Petersen and Pedersen 2005; Wassink et al. 2007). Once the inflammatory mediators are up-regulated systemically, there is an increased risk of pathology related to metabolic syndrome (Trayhurn and Wood 2004). Increasing obesity results in higher amounts of inflammatory mediators and it seems metabolic syndrome diagnosis, with concomitant higher concentrations of pro-

inflammatory cytokines, may further perpetuate the risk of developing cardiovascular disease (Lago et al. 2007; Wu et al. 2007). Although not conclusive, there is strong evidence suggesting a role for cytokines in the development of metabolic syndrome and atherosclerosis leading to clinical end-points of cardiovascular disease (Trayhurn and Wood 2004).

Higher concentrations of TNF-α were observed in a group of centenarians when compared to younger counterparts and the higher levels were associated with atherosclerosis (Bruunsgaard et al. 1999a). This suggests that underlying alterations in inflammatory processes precludes the development of clinical end-points of the atherosclerotic process. TNF-α concentrations in apparently healthy men (mean age 50 years) were associated with the development of early atherosclerotic risk factors in terms of metabolic and cellular disturbances (Skoog et al. 2002) and higher concentrations of TNF- α and IL-6 are associated with a poorer result in terms of the clinical outcome of individuals suffering strokes (Castellanos et al. 2002; Leira et al. 2006). The role TNF-α has in the development of atherosclerosis has recently been questioned as TNF receptor (TNFR) levels were associated with carotid plaque thickness but TNF-α was not (Castellanos et al. 2002). This suggests that the cytokine receptor density and affinity may be more important in the development of atherosclerosis than the amount of cytokine being produced. However, the concentration of IL-6 is also related to mortality from coronary artery disease and is higher in those individuals with uncontrolled angina when compared to those with controlled angina (Biasucci et al. 1996; Harris et al. 1999). Interventions aimed at those individuals with high levels of IL-6 on admission for treatment, due to coronary artery disease, dramatically reduced mortality in a one-year follow up (Lindmark et al. 2001). Thus, utilizing interventions aimed at reducing pro-inflammatory cytokines in a preventative

manner may be an effective strategy for decreasing the pathological changes associated with the inflammatory atherosclerotic process.

The first study proposed in this thesis will use the lignan component of flaxseed (combined with a walking exercise program) because the lignan is the component that has the most potential for acting as an anti-oxidant and favorably changing the blood lipid profile. If LDL levels can be reduced by supplementation with flaxseed lignan, this may lead to a smaller LDL-stimulated inflammatory response and lower levels of the inflammatory cytokines. Also, if the lignan or its metabolites are able to scavenge reactive oxygen species *in vivo*, there would likely be a down-regulation of inflammatory cytokine production from NFκB. Further, cardiovascular exercise training is considered the most appropriate type of exercise for altering the blood lipid profile by raising high density lipoprotein cholesterol and lowering triacyglycerol (Paterick and Fletcher 2001). Thus, the combination of these two interventions should produce favorable results in ameliorating the increased amount of inflammation observed in older adults who may be at risk of developing metabolic syndrome and atherosclerotic pathologies.

1.3.3.2 Sarcopenia and Inflammation

Sarcopenia refers to the age related loss of muscle mass and strength and the derivation of the word comes from Greek meaning 'poverty of flesh' (Borst 2004). Sarcopenia is not considered a disease but a condition of the normal aging process. Recently, it has been determined that the costs associated with health-care due to sarcopenia are 1.5% of the total health care costs for one year in the United States (Janssen et al. 2004). It is estimated that this cost will continue to rise as the population ages. Chronic low grade inflammation is related to decreasing muscle mass and strength with age (Borst 2004; Crepaldi and Maggi 2005; Roubenoff 2001).

High levels of IL-6 (>5 $pg \cdot mL^{-1}$) and C-reactive protein (>6.1 $\mu g \cdot mL^{-1}$) in older adults (mean age 76.4 years) increase the risk of muscle strength loss by 2-3 times that of older adults without high amounts of these inflammatory mediators (Schaap et al. 2006). In addition to lower strength, IL-6 has been associated with a decrease in muscle mass, and fiber number in older adults (Bautmans et al. 2005; Deschenes 2004; Ferrucci et al. 2002; Payette et al. 2003; Roubenoff 2003; Roubenoff et al. 2003). Thus, it is apparent that IL-6 plays a role in the relationship between muscle mass and strength loss due to aging.

Modest concentrations of IL-6, mimicking the amount of IL-6 observed post-exercise and in older adults with chronic low-grade inflammation, infused into the tibialis anterior muscle of rats demonstrated a catabolic effect on the muscle as it significantly lowered the mass of the tibialis anterior muscle compared to saline infused animals (Haddad et al. 2005). The growth hormone - insulin like growth factor-1 (GH-IGF-1) axis and IL-6 may have similar signaling mechanisms related to the Janus kinase-signal transducer activator of transcription (JAK/STAT) pathway (De Benedetti et al. 1997; Lieskovska et al. 2002). It is theorized that IL-6 stimulates suppressors of cytokine signaling (SOCS) expression via JAK activation and that SOCS activation will decrease the GH-IGF-1signaling associated with their respective receptors ultimately resulting in a down-regulation of anabolic stimulus for muscle hypertrophy. Evidence in animals has suggested that SOCS activation from IL-6 infusion resulted in a change in the balance of STAT signaling proteins that favored catabolism in muscle (Haddad et al. 2005). Also, a recent human study with recombinant IL-6 infusion, at a concentration of 140 $pg \cdot mL^{-1}$, demonstrated a greater decrease in muscle protein synthesis than muscle protein breakdown resulting in an increased net muscle protein breakdown (van Hall et al. 2008). The authors found that rhIL-6 infused skeletal muscle significantly increased the release of amino acids with a corresponding decrease in arterial amino

acid concentration suggesting other tissues were utilizing the amino acid acid release from the skeletal muscle (van Hall et al. 2008). This may be one mechanism whereby moderate concentrations of IL-6 result in loss of skeletal muscle amino acids eventually resulting in loss of myocytes and eventually muscle function.

TNF-α is considered a catabolic signaling agent and increasing levels with age result in a decreased amount of muscle mass and strength (Ladner et al. 2003). Aged skeletal muscle displays a higher amount of TNF-α and mRNA for TNF-α than young skeletal muscle (Greiwe et al. 2001). Apoptosis, or programmed cell death, has been implicated as a possible contributor to the etiology of sarcopenia (Leeuwenburgh 2003; Phillips and Leeuwenburgh 2005; Roubenoff 2001). It is postulated that as people age increases in TNF-α will inundate apoptotic death receptors in aged skeletal muscle fibers. TNF-α will up-regulate the NFκB signaling pathway leading to increased production of pro-inflammatory cytokines and the progressive deterioration of myocytes via apoptosis. Over time, this will result in a decrease in total muscle mass and a corresponding loss of strength (Ladner et al. 2003; Phillips and Leeuwenburgh 2005). It is unclear whether strength training can reduce the concentration of TNF-α. In one study, very old adults were unable to reduce TNF-α with resistance training but muscle strength was still improved after 12 weeks (Bruunsgaard et al. 2004) and in another study of identical length resistance training reduced the amount of skeletal muscle expressing TNF-α (Greiwe et al. 2001). Interventions aimed at reducing chronic low-levels of inflammation may be beneficial for improving muscle strength and muscle size in older adults.

High concentrations of TNF-α will signal the extrinsic pathway for apoptotic signaling in aging muscle by binding to the tumor necrosis factor receptor 1 associated death domain on the cell membrane. This will signal an activation of procaspase-8 which will then cleave resulting in caspase-8 activation of caspase-3. The activated caspase-3 results in proteolytic activity which

eventually culminates in cellular collapse. TNF-α may also contribute to apoptosis by increasing the intracellular signaling of B-cell leukemia/lymphoma-2 associate-X protein which will signal the release of cytochrome c from the mitochondria. Cytochrome c will signal proapoptoic events by binding 2'-deoxyadenosine 5'-triphosphate and apoptosis protease activiating factor-1which forms an apoptosome that cleaves caspase-9 increasing caspase-3, and the corresponding proteolytic activity associated with this protein, eventually resulting in apoptosis (Alway and Siu 2008; Leeuwenburgh 2003). TNF-α has also been shown to inhibit protein synthesis induced by insulin like growth factor-1 in myoblast cell cultures suggesting another mechanism whereby protein degradation of skeletal muscle may occur in old age (Broussard et al. 2003).

Another possible cause of myocyte loss in old age is myocyte death induced by other factors such as poor nutrition and alteration in immune system function related to adaptive immunity. These factors stimulating myocyte death could promote an inflammatory response thus signaling chemokines to attract neutrophils and monocytes/macrophages to the area which would release inflammatory cytokines in the area of myocyte death, thus promoting an inflammatory environment to clear the cellular debris resulting from the myocyte death. Recent evidence in old rats subjected to endotoxemia and nutritional deficiencies found that even though the old rats had low-grade inflammation as a result of endotoxin and nutrient deficiency, there was not a corresponding increase in muscle loss (Mayot et al. 2007a; Mayot et al. 2007b). These results suggest that myocyte apoptosis as a result of increased inflammatory cytokines may not be the only possible mechanism whereby skeletal muscle is decreased in old age. It is probable that many factors contribute to myocyte loss with old age and inflammation may be either a causative factor or consequential to increased inflammation as a result of an inflammatory response to necrosis of old tissue.

The purpose of the second study of this thesis was to evaluate the effects of flaxseed oil, which is high in alpha-linolenic acid, on IL-6 and TNF-α concentrations during progressive resistance training in older adults. Alpha-linolenic acid (ALA) is considered the most potent anti-inflammatory component of flaxseed and will likely exhibit the greatest potential for reducing the amount of muscle wasting by reducing concentrations of TNF-α and IL-6. Also, resistance training has been shown to be the most effective method of exercise training to reverse the loss of muscle mass and strength associated with the aging process (Roubenoff 2003).

1.3.4 Exercise and Inflammation

It is theorized that chronic exercise training has beneficial effects on immune system function as people age (Malm 2004). Exercise results in: a reduced stress response via modulation of sympathetic nervous system activity and reduced catecholamine release; assists sleep, which is when NK cell activity is highest; reduces cross-linkage formation, which reduces damage to the ability of DNA to replicate; maintains T-cell function; restores a homeostatic balance between pro- and anti-inflammatory mediators; and lessens the damaging effects of free radicals (Shinkai et al. 1997). Although the theory seems plausible, there is debate as to whether or not the beneficial effect of exercise will translate into improved immune system function or that exercise is anti-inflammatory in older individuals.

Epidemiological data indicates that individuals who are physically active have a reduced amount of inflammation (Colbert et al. 2004; King et al. 2003; Panagiotakos et al. 2005; Pitsavos et al. 2005; Reuben et al. 2003). A large cross-sectional study of older adults (n = 3,075; age = 70-79 years) determined that those who perform ≥ 180 min·wk^{-1} of physical activity have lower amounts of inflammatory markers (i.e. CRP, IL-6, and TNF-α) when compared to their non-

active counterparts (Colbert et al. 2004). Another cross-sectional study compared young active, young inactive, old active, and old inactive adults for *in vitro* stimulated release of IL-1β, IL-6, TNF-α and CRP analyzed from plasma (McFarlin et al. 2006). Inactive subjects had higher amounts of IL-6 and TNF-α, by 24% and 21% respectively, than the active groups. Also, IL-1β was 14% higher in the old-inactive group versus the young-active group. Overall, physically active subjects had 60% lower CRP than their inactive counterparts while the older subjects had a 53% higher CRP than the younger participants. It was concluded that being physically active has anti-inflammatory effects which may be beneficial for lowering chronic inflammation observed with aging. The two studies in this thesis involve cardiovascular ("aerobic") and musculoskeletal training; therefore, both types of training and their influence on inflammatory cytokines will be reviewed.

Relatively few prospective trials have been completed on the effects of cardiovascular training on the inflammatory response in healthy older adults. The majority have evaluated the beneficial effects of cardiovascular training on inflammatory markers in adults at risk of cardiovascular disease (Adamopoulos et al. 2001; Conraads et al. 2002; Gielen et al. 2003; Goldhammer et al. 2005; Larsen et al. 2001; Rauramaa et al. 2004; Smith et al. 1999; Tisi et al. 1997). All of these studies have shown that inflammation, analyzed by acute phase proteins and pro-inflammatory cytokines, is reduced by cardiovascular exercise training. Also, a trial performed on healthy adults, who were recruited to train with endurance exercise for 9 months, found a significantly lower amount of CRP in the trained versus untrained control condition (Mattusch et al. 2000). Further, a retrospective trial found that healthy older men who were physically active had a lower degree of inflammatory markers than those men who were inactive (Jankord and Jemiolo 2004). These results indicate that chronic cardiovascular exercise training

is anti-inflammatory in nature for diseased populations and perhaps for healthy populations. More research on the anti-inflammatory nature of cardiovascular exercise is warranted, especially from a preventative viewpoint.

Experiment I in this thesis employed cardiovascular training to establish a standard of care that would be appropriate for reducing the risk of atherosclerosis and metabolic syndrome, which includes inflammation as one of a cluster of risk factors. The cluster of risk factors associated with metabolic syndrome, as classified by WHO or ATP III guidelines, indicates that the risk of cardiovascular disease is increased in older adults diagnosed with metabolic syndrome (Scuteri et al. 2005) and that metabolic syndrome is more common in old versus young adults (Meigs 2002). Longitudinal data has indicated that 10 years of cardiovascular exercise training (30-45 min·session^{-1}, 3 d·wk^{-1}, 75-85% of VO_{2max}) improved the cardiovascular fitness level and decreased metabolic syndrome risk factors in older adults (Petrella et al. 2005) and 6 years of low to moderate intensity aerobic exercise for 45-60 minutes, 5 times per week slowed the progression of atherosclerosis in men (Rauramaa et al. 2004). To date, one exercise trial has indicated that a combination of cardiovascular and resistance exercise training is effective at lowering metabolic syndrome risk in older adults (Stewart et al. 2005a). However, another trial determined that cardiovascular exercise training and resistance exercise training had similar effects in enhancing glucose metabolism but cardiovascular training was the only effective method of improving insulin activation of glycogen synthesis in older men which could decrease risk of metabolic syndrome (Ferrara et al. 2006). Furthermore, 2 days per week of moderate intensity aerobic exercise for 6 months significantly decreased cell adhesion molecules and the atherogenic lipid profile in older, overweight, type II diabetics (Zoppini et al. 2006) while epidemiological evidence suggests that 30 minutes of moderate intensity aerobic exercise lowers

the risk of type II diabetes by 30-50% (Bassuk and Manson 2005). For Experiment I it was deemed that cardiovascular exercise training would be the most appropriate recommendation to give to older individuals as this mode of exercise is effective at decreasing metabolic syndrome risk (Meigs 2002; Pitsavos et al. 2005) and reducing the risk of atherosclerosis (Rauramaa et al. 2004; Smith et al. 1999) most likely by modifying the inflammatory milieau, insulin sensitivity, and the blood lipid profile thus, reducing cardiovascular disease development (Ford 2002; Goldhammer et al. 2005).

Resistance training studies are mixed on whether they reduce inflammation. Evaluation of the effects of 12 weeks of resistance training in older and younger women indicated that there was no effect on IL-1β, TNF-α, IL-6, or IL-2 levels (Rall et al. 1996). Ten weeks of resistance training in older women had no effect on cytokine mRNA expression; however, IL-6 and TNF-α release from LPS-stimulated blood monocytes were significantly lower in resistance trained women than controls (Flynn et al. 2003). These two studies should be interpreted with caution as the first study contained women with rheumatoid arthritis which has known effects on cytokine proliferation and the second study utilized some pharmacological agents in different groups which may influence the cytokine response. Additional studies indicated 12 weeks of resistance training in a very old population of nursing home residents (age 85-96 y) had no effect on circulating IL-6, TNF-α or soluble TNF receptors (Bruunsgaard et al. 2004), but 6 weeks of intensive training in older adults (average age 68 y) tended to decrease circulating concentrations of IL-6 and increase IL-10 and TGF-β (Bautmans et al. 2005). The effect of resistance training on inflammatory markers requires further study before specific conclusions can be made.

Acute exercise (i.e. muscle contraction) is associated with a substantial increase and release of IL-6 and a minor increase in TNF-α content in skeletal muscle which would seem a

paradox for promoting muscular growth or metabolic adaptations to improve exercise ability (Steensberg et al. 2002). Both of these cytokines induce the acute phase inflammatory response and TNF-α is known to be a catabolic cytokine (Bautmans et al. 2005). The tissue site of origin of IL-6 may be an important regulator of its physiological action and a local release of IL-6 from muscle may have some beneficial effects (Fischer 2006). The purpose of IL-6 release from skeletal muscle seems to be for metabolic regulation. When glycogen content of muscle is low or exercise intensity or duration is increased, higher amounts of IL-6 are released from muscle (Chan et al. 2004; Helge et al. 2003). IL-6 release from skeletal muscle appears to promote a catabolic environment by increasing fat oxidation, lipolysis, glucose output, and energy expenditure from adipose and liver tissue so the increased substrate release may be utilized by contracting skeletal muscle to maintain exercise (Febbraio and Pedersen 2002). IL-6 may also stimulate some anti-inflammatory mediators. For example, IL-6 stimulates the release of IL-1 receptor antagonist (IL-1ra), a cytokine that will attenuate the pro-inflammatory effects of IL-1β. IL-6 also stimulates the release of IL-10, a potent anti-inflammatory cytokine, as well as soluble TNF receptors which would attenuate the effects of TNF-α (Steensberg et al. 2003; Tilg et al. 1994). Cortisol, a known anti-inflammatory hormone (Barnes 1998) is released when recombinant IL-6 is infused (Steensberg et al. 2003). IL-6 release from contracting skeletal muscle is maintained into old age which may be one mechanism whereby exercise is anti-inflammatory in older adults (Pedersen et al. 2004).

Toll-like receptors (TLR) are trans-membrane signaling receptors that respond to lipopolysaccharide (LPS), a component of gram-negative bacteria, and stimulate an innate inflammatory reaction by releasing pro-inflammatory cytokines and proteins (Gleeson et al. 2006). Those who are physically active or participate in resistance exercise training have shown

down-regulation of a specific TLR called TLR4 (Flynn and McFarlin 2006; Flynn et al. 2003). Individuals that have high expression of TLR4 also have higher LPS-stimulated production of pro-inflammatory cytokines (Flynn et al. 2003; McFarlin et al. 2004). Therefore, a down-regulation of TLR4 is a possible mechanism whereby exercise training reduces the concentration of inflammatory cytokines such as IL-6 and TNF-α (Gleeson et al. 2006; Stewart et al. 2005b).

The underlying theme of this thesis was to provide evidence for the anti-inflammatory effects of flaxseed components during standardized exercise training. Many conditions are associated with the hyper-inflammatory state often observed in older adults, including sarcopenia. Experiment II is designed to utilize progressive resistance training as the mode of exercise and standard of care for older adults in an attempt to reduce the risk of sarcopenia. Sarcopenia has been defined as a loss of muscle mass equal to or greater than 2 standard deviations below the young reference mean and its development is associated with physical disability and other chronic diseases (Castillo et al. 2003). Various methods have been employed to reverse sarcopenia in older adults, including testosterone and human growth hormone, but high intensity progressive resistance training remains the most effective method of decreasing the risk of sarcopenia, increasing lean tissue mass, and increasing strength in the elderly (Binder et al. 2005; Borst 2004; Charette et al. 1991; Fiatarone et al. 1994; Frontera et al. 1990; Hakkinen et al. 2002; Ivey et al. 2000; Lemmer et al. 2000; Roubenoff 2000; Yarasheski et al. 1999). As discussed above, resistance training may be effective for lowering inflammatory markers but by having each subject in the study complete the same resistance training program, this thesis establishes a standard of care that would reduce the incidence of sarcopenia and thereby evaluate if an anti-inflammatory nutritional supplement (ALA) will improve skeletal muscle mass to a greater extent than resistance training alone. Experiment II utilized a different

mode of exercise than Experiment I as secondary hypotheses of each study were that the exercise mode utilized would be most appropriate as a clinical prescription for the condition of interest (sarcopenia for Experiment II and metabolic syndrome/atherosclerosis for Experiment I).

From the above review it is difficult to speculate on the effects exercise has on the entire cytokine network during the aging process. Studies to date indicate that exercise training is either anti-inflammatory or has no effect on inflammation. The research done on the response of cytokines to physical activity or exercise in older adults is limited and it is probable that the type, duration, intensity, and volume of the exercise task all influence the response of cytokines. One study has indicated that prolonged exercise (2 hours) significantly increased IL-6 and TNF-α when compared to either 5 minutes of high intensity exercise (90% VO_{2max}) or circuit training (Brenner et al. 1999). Also, light volume aerobic exercise training (70-85% heart rate maximum, 3 $d \cdot wk^{-1}$ for 12 weeks) was suggested to be optimal for immune system function when compared to moderate volume training (same intensity but 4-5 $d \cdot wk^{-1}$) (Shore et al. 1999). It is important to note that eccentric exercise tasks (i.e. when muscle is forced to lengthen, such as when walking or running downhill) will result in localized skeletal muscle inflammation in response to the stimuli of the exercise task (Hamada et al. 2005; Toft et al. 2002). Older adults' ability to respond to an eccentric exercise task may be compromised due to changes in the cytokine milieu after the exercise task but recent evidence suggests that an inflammatory event is not necessary for anabolism in older adults completing eccentric muscle exercise (LaStayo et al. 2007). The type and intensity of exercise influences the cytokine response and very intense forms of exercise (i.e. eccentric loading or long duration high intensity cardiovascular exercise) may not result in the same cytokine response as moderate exercise intensity. Thus, although evidence from cross-sectional, retrospective trials seems to increasingly favor the anti-inflammatory effects of

physical activity, it seems necessary to perform more prospective randomized controlled trials on the cytokine response to chronic exercise training to determine the effectiveness of exercise for ameliorating the increase in low-grade chronic inflammation associated with aging. Exercise prevents many diseases; a reduction in inflammation is likely one mechanism whereby exercise training maintains health into old age (Salvioli et al. 2006).

1.3.5 Flaxseed and Inflammation

The anti-inflammatory potential of many food compounds may lead to novel approaches in preventative and therapeutic care of pathological conditions related to inflammation (Murakami and Ohigashi 2006). Components of flaxseed, specifically the oil and lignan, hold potential for reducing inflammation when consumed in higher amounts. The following section reviews the anti-inflammatory potential of these two components of flaxseed.

1.3.5.1 Secoisolariciresinol Diglucoside

Lignans are a group of polyphenolic compounds in plants that share structural similarities with estrogen and thus have been classified as phytoestrogens. Bacterial flora in the colon are able to metabolize various plant lignans, including flaxseed lignans, to the mammalian lignans enterodiol and enterolactone (Borriello et al. 1985; Heinonen et al. 2001; Wang et al. 2000). The flaxseed lignan, secoisolariciresinol diglucoside (SDG), has received research interest due to its potential to reduce the risk of chronic health conditions including cancer, cardiovascular disease, diabetes, and osteoporosis (Thompson and Ward 2002). There is no established Dietary Reference Intake for SDG and currently in Canada it is termed a natural product and is considered a phytochemical. SDG is first metabolized to secoisolariciresinol (SECO), which is

its aglycone, and then further metabolized to the mammalian lignans enterodiol and enterolactone. SECO shares structural similarity with nordihydroguaiaretic acid, which is a antioxidant, and various lignans have been shown to bind to sex hormone binding globulin in *in vitro* studies (Schottner et al. 1997; Schottner et al. 1998). The possible mechanisms whereby SDG may exert its health effects are: 1) acting as an anticancer agent in hormone dependent cancers such as breast and prostate cancer (Lin et al. 2002; Rickard et al. 1999; Thompson et al. 1996) or colon cancer (Jenab and Thompson 1996; Sung et al. 1998); 2) modifying lipid metabolism to reduce the risk factors associated with cardiovascular disease (Hallund et al. 2006a; Hallund et al. 2006b; Prasad 1999); 3) exerting an estrogenic-like effect which may have positive effects on bone metabolism (Ward et al. 2001a; Ward et al. 2001b); or 4) acting as an antioxidant which may have beneficial effects for a number of disease processes related to inflammation (Kitts et al. 1999; Prasad 1997b; Prasad 2000a; Prasad 2000c). Of the various effects SDG is proposed to exert on health, this review will focus on its ability to reduce inflammation especially in relation to cardiovascular disease.

In response to inflammation, activated immune cells respond to injury or microbe invasion by phagocytosis which involves the respiratory burst. The respiratory burst is a time of increased oxygen consumption whereby the oxidation of molecules foreign to the organism occurs. During this time, NADPH-dependent oxidases catalyze the conversion of oxygen to the superoxide (O_2^-) anion and produce a number of reactive oxygen and nitrogen intermediates that result in the degradation of the foreign material (Mak and Saunders 2006). One of the reactive oxygen intermediates formed in this process is the hydroxyl radical (OH^-) which breaks hydrogen bonds and is associated with damage to nucleic acids. *In vitro* analysis of the OH^- scavenging ability of SDG was determined by HPLC when hydrogen peroxide was subjected to

photolysis by UV light (Prasad 1997b). This study also utilized another *in vitro* measure in liver homogenate to evaluate if lipid peroxidation was reduced in the presence of SDG. The results indicated that OH^- radical was scavenged by SDG in the first model and assessment of malondialdehyde, a measure of lipid peroxidation, was reduced by SDG in the second model. One other study has confirmed the antioxidant effect of SDG, and its metabolites enterolactone and enterodiol *in vitro* (Kitts et al. 1999). It was concluded in both studies that SDG had antioxidant properties which may be the mechanism whereby it exerts anti-cancer effects.

Another *in vitro* study of the effects of SDG and its metabolites SECO, enterodiol (ED), and enterolactone (EL) was performed to analyze the scavenging activity of each substance on zymosan-activated polymorphonuclear (PMNL) leukocytes (Prasad 2000a). The results indicated that all metabolites were able to scavenge reactive oxygen species but ED and SECO displayed the greatest scavenging ability by reducing PMNL chemiluminescence by 94.2% and 91.2% respectively. The author speculated that the antioxidant ability of SDG is a function of the scavenging ability of its metabolites and may be effective in inhibiting and treating various inflammatory diseases including cardiovascular disease and auto-immune disease. Recent *in vitro* examination of the antioxidant properties of SDG and SECO at concentrations likely achievable *in vivo*, within the gastrointestinal tract, indicates they are effective in a dose dependent-manner while ED and EL did not show as much scavenging efficacy (Hosseinian et al. 2007; Hu et al. 2007). The direct scavenging ability of SDG and SECO at concentrations achievable *in vivo* in other tissue types is unknown at this time.

In an animal model representative of Type I diabetes, confirmation of the above *in vitro* result was obtained *in vivo* (Prasad 2000c). Rats were divided into three groups: a control group not prone to developing diabetes, a group that was untreated and prone to developing type I

diabetes, and a group treated with SDG who were prone to developing type I diabetes. The incidence of diabetes development in the SDG group was 21.4% whereas the non-supplemented group's incidence of developing diabetes was 72.7%. Also, results indicated that serum malondialdehyde levels were lower when comparing the SDG treated versus untreated animals. Pancreatic chemiluminescence was reduced in the SDG treated group when compared to the untreated group. The author concluded that type I diabetes may be mediated by reactive oxygen species and SDG supplementation may be effective in lowering the incidence of this auto-immune disease. Results from the same laboratory in relation to the development of type II diabetes, in an animal model, also indicate that SDG is effective in reducing the incidence of this disease by reducing oxidative stress (Prasad 2001). Further, a study in type II diabetic humans indicated improved glycemic control but no difference in glucose, insulin, or blood lipids after 12 weeks of SDG supplementation (Pan et al. 2007).

The effects of a flaxseed lignan complex containing SDG on the development of atherosclerosis in rabbits fed a high cholesterol diet produced conflicting results (Prasad 2005b). In this study, female rabbits were separated into four groups: a control group, a group fed lignan complex, a group fed a high cholesterol diet, and a group fed a high cholesterol diet as well as lignan complex. Antioxidant reserve was increased in the SDG group on the high cholesterol diet whereas it was decreased in the SDG only group. Also, aortic malondialdehyde concentration was lower in the cholesterol/lignan group compared to the cholesterol only group but the lignan complex increased aortic malondialdehyde in the lignan supplemented group when compared to the control group. Serum malondialdehyde concentration was 35.7% less after 2 months in the cholesterol/lignan group as compared to the cholesterol diet group. Prasad (Prasad 2005b) stated that a reduction of oxidative stress was seen in this animal model which reduced

the extent of atherosclerosis. These results suggest SDG has beneficial effects when animals are fed a high cholesterol diet but may have pro-oxidant effects in those consuming a normal diet which could be an indicator of future health detriments if supplementation was continued.

Supplementation with flaxseed (7.5 $g \cdot kg^{-1}$) over 8 weeks reduced polymorphonuclear leukocytes (PMNL) production of oxygen free radicals in rabbits who were either on normal or high-cholesterol diets (Prasad 1997a). It was not known whether the α-linolenic acid or the SDG found in flaxseed exerted the decrease in free radical production. Other studies out of the same laboratory indicated that SDG was the effective component; flaxseed with low α-linolenic acid content reduced atherosclerotic plaque formation in animals on a high cholesterol diet (Prasad et al. 1998) and direct SDG supplementation increased antioxidant capacity and decreased lipid peroxidation in animals on a high cholesterol diet (Prasad 1999).

SDG was shown to prevent the development of streptozotocin-induced diabetes in a rat model (Prasad et al. 2000). In this study rats were divided into four groups: a control group, a group supplementing with SDG for 24 days, a group injected with streptozotocin, and a group supplementing with SDG before and after streptozotocin injection. The rats supplemented with SDG and injected with streptozotocin, that did not develop diabetes, showed a link with decreases in the amount of serum and pancreatic malondialdehyde and an increase in pancreatic antioxidant reserve. Further analysis revealed that leukocyte chemiluminescence was decreased in the SDG-streptozotocin group when compared to the streptozotocin group, which suggests SDG may scavenge reactive oxygen species produced during inflammatory reactions. The authors speculated that a reduction of hydrogen peroxide by SDG scavenging could decrease the activation of the complement cascade of events and possibly delay the development of diabetes.

A cross-over human study investigating the effect of defatted flaxseed on indicators of oxidative stress found contrasting results (Jenkins et al. 1999). Serum protein thiol and carbonyl groups were measured to assess oxidative stress. Protein carbonyl concentrations did not differ between the control and flaxseed supplement conditions but protein thiol concentrations were significantly lower at the end of the flaxseed trial in comparison to the control trial. Increased oxidative stress with defatted flaxseed consumption may or may not be harmful depending on perspective. If the increased oxidative stress results in the degradation of lipids and proteins necessary for tissue function it could be considered harmful but, if the increase was due to an improvement in innate immune function against foreign pathogens it could be beneficial (Jenkins et al. 1999).

In contrast to the above animal research, the first human study to evaluate the effects of supplementation with a flaxseed lignan complex found no changes in oxidative stress or measures of inflammation in 22 healthy post-menopausal women who supplemented for 6 weeks (Hallund et al. 2006a; Hallund et al. 2006b). In this study the researchers employed a cross-over design where women consumed a muffin containing 500 $mg \cdot d^{-1}$ of lignan complex for 6 weeks, followed by a 6 week wash-out period, and then a 6 week consumption of a placebo muffin. Serum lipoprotein resistance to oxidation, Trolox-equivalent antioxidant capacity, and ferric reducing ability of plasma did not differ significantly between the placebo and lignan complex intervention suggesting no effect of lignan complex on oxidative status (Hallund et al. 2006a). Further results revealed no effect on plasma markers of NADPH oxidase and endothelin-1, which indicates endothelial relaxation and constriction respectively, as well as asymmetric dimethylarginine, which is an endogenous inhibitor of endothelial derived nitric oxide synthase and is implicated in endothelial dysfunction (Hallund et al. 2006b). Also, Pan et al. (Pan et al.

2007) found improved glycemic control with 12 weeks of SDG supplementation in type II diabetic patients but no improvement in blood lipids, glucose control or insulin sensitivity. Furthermore, 8 weeks of flaxseed lignan complex (600 $mg \cdot d^{-1}$) supplementation significantly reduced total cholesterol and glucose concentrations in a group of hypercholesterolaemic subjects (Zhang et al. 2007).

There is the possibility of a sex difference when supplementing older adults with flaxseed lignan. Women who are post-menopause will have a decreased production of estrogen and possibly a down-regulation of estrogen receptors whereas this change would not be as dramatic in men (Castelo-Branco and Rostro 2006). Although speculative, the flaxseed lignan or its metabolites may not bind as effectively in the women versus the men due to a lack of receptors. This may result in less of a potential health benefit in women versus men. Recent work in post-menopausal women has indicated no effect of SDG supplementation for 6 weeks on blood lipids or antioxidant status (Hallund et al. 2006a). The first study in this thesis addressed this issue by supplementing men and women with SDG for 6 months. Experiment I was designed to evaluate the sex differences in the dependent variables. It was also designed to evaluate if supplementing with SDG for a longer time period (6 months versus 6 weeks) would have an effect on the dependent variables measured.

There is a possibility of age-specific effects on lignan metabolism. Older individuals have a decrease in bacterial flora which may affect the conversion of SDG to SECO and the mammalian lignans enterodiol and enterolactone (He et al. 2001; Leibovici 1995). It has been theorized, from *in vitro* models, that the conversion of SDG to the mammalian lignans may be the mechanism driving the beneficial lipid lowering effects of SDG as enterolactone may act similarly to estrogen by stimulating hepatic and macrophage cells to increase uptake of LDL

(Owen and Abbey 2004; Owen et al. 2004). If the conversion of SDG to the mammalian lignans is compromised due to reduced bacterial flora in old age then there may not be the same purported health effects in older adults. Also, recent evidence has found that prebiotic and probiotic ingestion will positively influence bacterial flora (Bartosch et al. 2005), possibly enhancing immune function and reducing inflammation in older adults (Schiffrin et al. 2007). A synergistic effect of probiotics and a high soy diet on the bioavailability of active isoflavones (daidzein, genistein) in older adults was not observed (Larkin et al. 2007). It is not known if prebiotic or probiotic ingestion will influence the gut metabolism of flaxseed lignans. The design of Experiement I did not directly test this relationship but it is worth mentioning the possibility of an age and nutritional effect in this review.

In general, research completed so far on the anti-oxidant or anti-inflammatory effect of flaxseed lignan in humans is in its initial stages. There is potential for this phytochemical to aid in health promotion given the evidence from animal models, but the paucity of information surrounding the health effects of SDG supplementation in humans definitely requires a more thorough investigation. Theoretically, if SDG or its metabolites act as anti-oxidants, and decrease the amount of reactive oxygen species generated due to their scavenging ability, SDG supplementation may be an effective means to down-regulate NFκB's production of pro-inflammatory cytokines such as TNF-α and IL-6 and may be an effective nutritional strategy to combat the hyper-inflammation associated with the aging process. The purpose of the first study of this thesis was to evaluate the efficacy of supplementation with a flaxseed lignan complex during cardiovascular training on inflammatory status and blood lipid profiles in older adults at an increased risk of developing cardiovascular disease. It was theorized that increased oxidative stress, from oxidized LDL, and increased inflammation, due to more circulating pro-

inflammatory cytokines, will intensify the risk of metabolic syndrome and cardiovascular disease due to atherosclerosis in older adults. Experiment I hypothesized that standardized exercise designed to decrease blood lipid profiles (walking 6 $d \cdot wk^{-1}$, 30-60 $min \cdot d^{-1}$) and a nutritional intervention designed to decrease oxidative stress and inflammation (flaxseed lignan complex supplementation) would decrease the inflammatory process and reduce the development of metabolic syndrome.

1.3.5.2 Alpha-Linolenic Acid

The purpose of this section is to summarize the effects of supplementing with α-linolenic acid from flaxseed on immune function with a focus on cytokine production in humans. Alpha-linolenic acid (18:3ω-3; ALA) is an 18 carbon fatty acid with the last of the carbon-to-carbon double bonds located 3 carbon atoms before the methyl end. It is considered an essential ω-3 polyunsaturated fatty acid and the parent to longer carbon chain ω-3 fatty acids. Flaxseed oil is composed of between 45-60% ALA (Cunnane 2003). Currently, the Dietary Reference Intake has an established adequate intake of ALA which is 1.6 and 1.1 $g \cdot d^{-1}$ for men and women respectively with no upper limit set. One metabolic function of ALA in humans is incorporation into the structure of cell membranes. Also, ALA functions as a precursor to other ω-3 fatty acids (eicosapentaenoic acid and docosahexaenoic acid) via carbon chain elongation and further desaturation (Burdge 2004). Supplementation with ω-3 fatty acids has been used to decrease the amount of inflammation associated with various disease processes (Simopoulos 1999; Simopoulos 2002b). It is theorized that a high ratio of ω-6 to ω-3 fatty acids in the normal western diet increases risk for the development of many chronic diseases (Simopoulos 2002a). The main type of ω-6 fatty acid, derived from linoleic acid, is arachidonic acid (AA) which is the

precursor for various inflammatory eicosanoids and cytokines (Darlington and Stone 2001). Most research utilizing supplementation with ω-3 fatty acids shows a change in the ratio of ω-6:ω-3 fatty acids with numerous biologic effects. Decreasing the ratio of ω-6:ω-3 fatty acids will result in a decreased amount of inflammatory eicosanoids and cytokines because the ω-3 fatty acid, eicosapentaenoic acid (EPA), competes with AA metabolism along the same enzymatic pathways (cyclooxygenase and 5-lipoxygenase) (James et al. 2000). ALA is the precursor or "parent" fatty acid to EPA (Cunnane 2003). The specific change that occurs to mediate the inflammatory response is a change in the lipid bilayer of most human cells to a decreased amount of ω-6 fatty acids and an increased amount of ω-3 fatty acids (Alexander 1998). Increasing consumption of ALA will increase EPA concentration in men and women but the conversion is lower in men than women (Burdge 2004; Burdge et al. 2003; Burdge and Wootton 2003).

A dietary intervention, which had men (ages 21-37 years) eat all their meals at a nutritional test center for 126 days, evaluated the effects of a diet high (21% of the total fatty acids) and low (1% of the total fatty acids) in ALA on various parameters of both cellular and humoral immunity (Kelley et al. 1991). There were no changes in the number of circulating T-cells, T-cell subsets, or B-cells with the intervention. *In vitro* analysis of mitogen stimulated peripheral blood mononuclear cells revealed a suppression of T-cell proliferation with no change in the secretion of IL-2 or IL-2r when the ALA group was compared to the control group. Further results of B-cell function indicated no change in serum concentrations of immunoglobulins or complement components. The results suggest a suppression of T-cell function but no effect on B-cell function or cytokines in young men supplementing with large amount of ALA.

Research in a population diagnosed with rheumatoid arthritis, to evaluate the effects of ALA supplementation on markers of inflammation, revealed mixed results (Nordstrom et al. 1995). In this study patients were split into a group receiving 30 grams of flaxseed oil (~9.6 grams ALA) or 30 grams of safflower oil for 3 months. Serum ALA concentration as well as bleeding time was significantly increased in the patients consuming flaxseed while C-reactive protein, erythrocyte sedimentation rate and clinical data of disease activity were not significantly different between the two groups post-intervention. The interpretation of this study is difficult due to the fact that all patients were taking various pharmaceutical agents known to affect immune system function, to control their disease activity.

An evaluation of a 4 week flaxseed oil based diet on TNF-α and IL-1β was performed in healthy male subjects (Caughey et al. 1996). In this design, subjects were randomized to a flaxseed oil group who consumed an average of 13.7 $g \cdot d^{-1}$ of ALA or a sunflower oil group who consumed an average of 1.1 $g \cdot d^{-1}$ ALA. *In vitro* analysis of stimulated mononuclear cell production of cytokines before and after the supplementation period revealed a significant decrease of 30% and 31% in TNF-α and IL-1β respectively. Also, the high ALA group had decreased amount of inflammatory eicosanoids and increased amounts of ALA and EPA in mononuclear cell membranes when compared to the sunflower oil group. Another study from the same laboratory analyzed the changes in *in vitro* cytokine and eicosanoid production in a group of young men (average age 37 years) eating a diet high in ALA (~9.2 $g \cdot d^{-1}$) over 4 weeks (Mantzioris et al. 2000). The results indicated a 20% significant decrease in IL-1β after 2 and 4 weeks compared to baseline while TNF-α significantly decreased by 40% after 2 weeks but was not significantly different than baseline at 4 weeks. Eicosanoid production was decreased at 2

and 4 weeks when compared to baseline. This suggests that diets high in plant-based ω-3 fatty acids can effectively inhibit TNF-α and IL-1β production.

A larger (n = 48) randomized controlled trial has evaluated the effects of supplementation with moderate levels of different types of fatty acids on immune system function in healthy male and female participants over a 12 week period (Thies et al. 2001b). The purpose of the research was to evaluate any detrimental effects to immune system function in those following current dietary recommendations to increase consumption of ω-3 fatty acids. One of the groups in this study consumed an extra 2.0 $g \cdot d^{-1}$ of ALA. There was no effect of ALA supplementation on NK cell activity although a decrease in NK cell function was found in a group supplementing with EPA (Thies et al. 2001b). There were no significant effects of time, treatment or the interaction on TNF-α and IL-6 production in any of the groups (Thies et al. 2001a). The study also evaluated the effects that ALA supplementation had on the production of IL-2 and IFN-γ from *in vitro* stimulated lymphocytes and found no effect on these two cytokines (Thies et al. 2001c). At low levels of supplementation there seems to be no adverse effects of ALA although a criticism could be made, depending on perspective, that the low amount of ALA given may not be enough to produce a biological effect.

Another randomized, double blind, placebo controlled trial (n = 150) evaluated the immune function of participants ingesting a low or high ALA and EPA/DHA supplement in a broad group of adults (age range 25-72 years) over a 6 month time period (Kew et al. 2003). In this research, there was a group consuming a low amount of ALA (4.5 $g \cdot d^{-1}$) and a high amount of ALA (9.5 $g \cdot d^{-1}$). Cytokine response to LPS and Con A stimulation in cultured peripheral blood mononuclear cells resulted in no change in TNF-α, Il-1β, IL-6, IL-2, IL-4, and IFN-γ between groups at baseline or after the dietary intervention. Although there were no changes in

cytokine production between the groups, there was a significant difference in the composition of fatty acids incorporated into mononuclear cell phospholipids which reflected the dietary interventions. Thus, it seems that increased consumption of ALA may not affect cytokine production but will change the fatty acid composition of immune cell phospholipids. Additional study confirmed that ALA supplementation (3.5 $g \cdot d^{-1}$) for 12 weeks had no effect on TNF-α, IL-1β, or IL-6 (Wallace et al. 2003). In contrast, the results indicated that supplementation with medium and high amounts of fish oil (EPA and DHA) resulted in a decreased production of IL-6.

A double blind trial randomized hypercholesterolaemic men and women (mean age 55 years) to an ALA group (5.9 $g \cdot d^{-1}$) or a linoleic acid group (Bemelmans et al. 2004). The study analyzed the effects of ALA intake on C-reactive protein, IL-6 and IL-10 over a 2 year time period and found that CRP was reduced in the ALA group when compared to the linoleic acid group but no differences were found for IL-6 or IL-10. The study is limited in its interpretation because very few blood samples were within the detection limits of the ELISA kits used to analyze IL-6 and IL-10. Another study, in dyslipidaemic male patients (n = 76, mean age 51 years), found that supplementation with 15 $mL \cdot d^{-1}$ of ALA versus 15 $mL \cdot d^{-1}$ of linoleic acid over 3 months resulted in a significant decrease in CRP, serum amyloid A, and IL-6 (Rallidis et al. 2003).

A four day clinical intervention analyzed the effects of a low (0.5% of total energy intake) and high (5.0% total energy intake) amount of ALA on soluble markers of IL-6 receptor (sIL-6R) and TNF-α (sTNFR1, sTNFR2) in nine normal weight and seven overweight males (Nelson and Hickey 2004). There were no significant changes in sTNFR1 and sTNFR2 between the control and ALA diet although a 12% decrease in sTNFR2 was noted in the overweight group. The levels of sIL6R decreased significantly by 11% and 12% in the normal weight and

overweight males, respectively, who were on the ALA diet when compared to the control group. The results may have important clinical implications in reducing sub-clinical inflammation, especially in overweight/obese individuals.

A diet with moderate amounts of ALA may reduce inflammatory cytokine production in chronic obstructive pulmonary disease (Matsuyama et al. 2005). In this study, patients were randomized to either a ω-3 group (n = 32; 1.4% of diet ALA) or a ω-6 group (n = 32; 0.18% of diet ALA) and were evaluated for leukotriene B_4 (LTB_4), TNF-α, and IL-8 levels in the sputum and serum over a 2 year time period. The levels of serum LTB_4 decreased in the ω-3 group at 15 months when compared to the ω-6 group but no changes were seen in levels of TNF-α or IL-8. Sputum levels of LTB_4, TNF-α, and IL-8 were all decreased in the ω-3 group when compared to the ω-6 group. The authors recommended nutritional support with ω-3 fatty acids as a strategy in treatment of COPD.

The effects of ALA supplementation on inflammatory markers may vary between males and females. Burdge and colleagues (Burdge 2004; Burdge 2006; Burdge and Calder 2005; Burdge et al. 2003; Burdge et al. 2002; Burdge and Wootton 2002; Burdge and Wootton 2003) have investigated the metabolism of ALA in men and women and found that the conversion of ALA to EPA is higher in young women than in young men and that this relationship is mediated by estrogen. One purpose of Experiment II was to evaluate sex differences in the anti-inflammatory effects of ALA supplementation. The sample included post-menopausal women, who may convert less ALA to EPA as estrogen concentration is reduced, thus potentially reducing the anti-inflammatory effect of ALA supplementation. Therefore, Experiment II included an evaluation of differences between males and females in the dependent variables measured.

The research performed by Burdge et al. (Burdge 2004; Burdge 2006; Burdge and Calder 2005; Burdge et al. 2003; Burdge et al. 2002; Burdge and Wootton 2002; Burdge and Wootton 2003) on ALA conversion to longer chain fatty acids was done in young healthy males and females. In Experiment II, we recruited old healthy males and females. It is not known if age influences the conversion of ALA to EPA or if older adults will experience the same anti-inflammatory benefits as younger adults from consuming ALA as this hypothesis has not been directly tested. The available data seems to indicate that ALA is anti-inflammatory across age ranges (21-72 years) but the majority of this data was in patients with blood lipid abnormalities (Bemelmans et al. 2004; Kew et al. 2003; Rallidis et al. 2003; Thies et al. 2001b; Zhao et al. 2007; Zhao et al. 2004). The design of Experiment II will not test differences across age-ranges in terms of inflammatory cytokines but it is suggested that a difference may exist in the effectiveness of ALA to reduce inflammation in older versus younger adults.

In summary, the evidence for the effectiveness of ALA to decrease markers of inflammation seems conflicting but may reflect the differences in study design, methodology, and populations analyzed. Very few studies have evaluated the effects of ALA supplementation on the inflammatory process in older adults. There is more information in regards to the health effects of ω-3 fatty acids derived from fish oils than of those derived from plant-based sources such as flaxseed. As ALA is seen as the "parent" fatty acid to EPA, and EPA has known effects on the inflammatory process, it is sensible to evaluate the effects of supplementation with ALA on health and the inflammatory process. It seems evident that ALA supplementation does have an effect on the inflammatory response and immune function but more information in regards to its role in ameliorating the hyper-inflammatory response in aging is warranted.

1.3.6 Nutritional Considerations

Habitual diet may have a profound effect on the immune system and inflammation, especially in the elderly (Fernandes 2008). The experiments in this thesis attempted to control for nutrition by evaluating the typical self-reported dietary intake of subjects both before and after the interventions with a food frequency questionnaire. The Dietary Reference Intakes are comprised of reference values for specific macronutrients and micronutrients established by the National Academy of Sciences in collaboration with Health Canada. Specific dietary recommendations are made for individual nutrients, based on sex and age, in an attempt to optimize the health and reduce the risk of deficiency disease of almost all healthy individuals (Trumbo et al. 2002). A specific lack of certain nutrients can have a profound effect on immune system function. The elderly may be at increased risk of malnourishment from specific nutrient deficiencies but this seems to be more prevalent in older adults with a lower socio-economic status who are in poor health (Payette and Shatenstein 2005) while Canadian adults may not be eating the recommended servings of specific food groups (Starkey et al. 2001). In this regard, a short description of some nutrients that have an important function in immune regulation will now be addressed.

Protein ingestion plays an essential role in the regulation of the immune system. Unavailability of essential amino acids will decrease protein synthesis of immunoglobulins, complement, and cytokines as well as affect the proliferation and development of immune cells. The current recommended daily allowance (RDA) for protein is 0.8 g protein·kg·d^{-1} with an Acceptable Macronutrient Distribution Range (AMDR) of between 10-35% of the total energy intake per day (Trumbo et al. 2002). Seminal research on healthy adults given 0.1, 1.0, or 2.0 g protein·kg·d^{-1} demonstrated impaired antibody action in the group consuming the lowest amount

of protein (Hodges et al. 1962). A recent review of the subject has concluded that protein energy malnutrition is a major factor in reducing immune function in elderly, undernourished individuals as they are already at risk of decreased immune function due to age alone (Lesourd 2004). In particular, protein energy malnutrition significantly increases the inflammatory processes in older adults caused by loss of homeostasis of macrophage function and decreased T-cell function (Lesourd 2004).

The AMDR for fat intake in healthy adults is between 20-35% of total energy intake (Trumbo et al. 2002). The consumption of adequate amounts of fat is also essential to proper immune system functioning. In particular, the consumption of essential fatty acids (ω-6 and ω-3) has a strong relationship in modifying inflammatory processes. The RDA established for ω-6 fatty acids is 11 $g \cdot d^{-1}$ and 14 $g \cdot d^{-1}$ for women and men older than 50 years respectively and for ω-3 fatty acids is 1.1 $g \cdot d^{-1}$ and 1.6 $g \cdot d^{-1}$ for women and men over 50 years respectively (Trumbo et al. 2002). In general, the consumption of high amounts of ω-6 fatty acids is associated with a pro-inflammatory environment while the consumption of high amounts of ω-3 fatty acids is associated with an anti-inflammatory environment (Simopoulos 1999; Simopoulos 2002b). Also, consumption of mono-unsaturated fats, such as oleic acid, is associated with less inflammation and improved health into old age (Perez-Jimenez et al. 2005). High amounts of saturated fat consumption is associated with an increased amount of inflammation (Giugliano et al. 2006; Han et al. 2002; Lennie et al. 2005; Lopez-Garcia et al. 2005; Mozaffarian et al. 2004). Fats are essential to the immune system and inflammation as changing dietary fat intake will result in hormonal, metabolic, prostaglandin, and cell signaling changes, mostly mediated by the compositional change of cellular membranes with varying fat ingestion, that will either improve or wane immunological function.

The AMDR for carbohydrate ingestion is between 40-65% of total energy intake with a minimum intake of 130 $g \cdot d^{-1}$ (Trumbo et al. 2002). Low amounts of carbohydrate consumption may have an effect on the immune system by lowering blood glucose concentration which could stimulate a hypothalamic-pituitary-adrenal response causing an increased amount of adrenocorticotrophic hormone, cortisol, growth hormone, and a decrease in insulin. Increased amounts of stress hormones are known to increase immune stress (Flint et al. 2007; Lundberg 2005) but recent evidence suggests that a low carbohydrate diet may reduce inflammation (Forsythe et al. 2008) while high kilocalorie, nutrient poor carbohydrate consumption will increase inflammation (O'Keefe et al. 2008). It is likely that food choice in relation to carbohydrate is vital in regulating immune system function. Food choice in relation to carbohydrate consumption was not analyzed as part of this thesis but it is acknowledged that the type of carbohydrate consumed may have an effect on inflammatory markers.

Certain micronutrient deficiencies will affect immune system function and inflammation, especially in older adults who are immuno-compromised (High 1999; Lesourd 1997; Miquel 2001; Wintergerst et al. 2007). Vitamin A deficiency has a profound effect on infection by decreasing serum concentrations of IgG antibody (Bendich 1992; Bloem et al. 1990; Coutsoudis et al. 1992; Hussey and Klein 1990). B-vitamin deficiency results in lymphoid atrophy and vitamin B_6, folate, and pantothenate deficiency are associated with reduced immune function and decreased production of antibodies (Cramer et al. 1921; Rall and Meydani 1993). Vitamin B_6 is utilized in the development and function of T-cells which controls antibody production by B-cells. Older adults deficient in vitamin B_6 have decreased lymphocyte numbers and mitogen stimulated lymphocyte response (Ha et al. 1984; Meydani et al. 1991; Miller and Kerkvliet 1990). Sufficient intake of vitamin C stimulates proliferation of T-cells, prevents a suppression

of neutrophil activity, and directly protects phagocytes against auto-oxidation (Anderson 1982; Anderson and Lukey 1987; Evans et al. 1982; Frei et al. 1989; Frei et al. 1990; Herbaczynska-Cedro et al. 1994). Adequate vitamin D, or more specifically its active metabolite 1,25-dihyroxy vitamin D_3, is associated with a down-regulation of inflammatory markers and may have an anti-proliferative effect on immune cells; however, inadequate amounts of vitamin D are associated with increased incidence of auto-immune diseases (Chen et al. 2007; Lips 2006; Staud 2005). Vitamin E deficiency is quite rare but would have a great effect on immune function by decreasing T-cell function; conversely, vitamin E supplementation may have a beneficial effect on immune function in older adults (Meydani and Beharka 2001; Serafini 2000).

The major elements that may have the most extreme effect on immune function include zinc and iron (Chandra 1997). Iron overload is associated with several chronic inflammatory diseases and coronary heart disease risk increases with high iron intake most likely due to increased production of reactive oxygen species (Ascherio et al. 1994; Cardier et al. 1997; Shephard and Shek 1995b; van Asbeck et al. 1984). Alternatively, iron deficiency is known to suppress lymphocyte proliferative response to mitogen stimulation, decrease the release of IL-1 from macrophages, decrease NK-cell cytotoxic activity, and impair phagocytic activity of neutrophils (Chandra 1991; Cunningham-Rundles 1982; Helyar and Sherman 1987; Scrimshaw and SanGiovanni 1997; Sherman 1992). Thus, iron overload or deficiency harms the immune system. Zinc deficiency affects immune function by reducing thymulin effectiveness thus resulting in lymphoid atrophy, decreasing the production of the anti-inflammatory cytokine IL-2, impairing the proliferative response of lymphocytes to mitogen stimulation, as well as reducing NK-cell cytotoxic activity (Chandra 1997; Cunningham-Rundles 1982; Prasad 1993; Sherman

1992). High doses of zinc supplementation may be detrimental to immune function by impairing T-cell and polymorphonuclear cell activity (Chandra 1984).

In summary, diet does influence immune function as deficiencies in macronutrients and micronutrients result in deleterious changes to immunity, especially in older adults, while supplementation may aid immune system operation. The studies presented in this thesis attempted to control for the nutritional influence on inflammation by having subjects record their typical dietary intake with food frequency questionnaires both before and after the intervention time period. This was done to control for possible changes in the subjects' typical diets that may influence immunological parameters, thus, influencing cytokine production (the primary dependent variables in both studies).

1.4 Statement of the Problem

Dysregulation of inflammation is considered a contributor to the metabolic syndrome and atherosclerotic process leading to cardiovascular disease in aging individuals (Wassink et al. 2007). Supplementation with the flaxseed lignan SDG ameliorates the atherosclerotic process and decreases the amount of oxidative stress in *in vitro* and in *in vivo* animal models (Prasad 1997b; Prasad 1999; Prasad 2000a; Prasad 2000c; Prasad 2001; Prasad 2005b). One human study has indicated no beneficial effect in reducing oxidative stress in post-menopausal women supplemented with a flax lignan complex containing SDG over a 6 week period of time (Hallund et al. 2006a) while another in type II diabetics resulted in improved glycemic control but no other health benefit (Pan et al. 2007). Recent evidence from hypercholesterolaemic subjects indicates SDG supplementation for 8 weeks effectively lowered TC, LDL and fasting glucose versus placebo (Zhang et al. 2007). No study has evaluated the effect of SDG supplementation during cardiovascular exercise training on inflammation or blood lipid profiles in healthy older males and females. One study has evaluated flaxseed lignan complex supplementation in post-menopausal women (Hallund et al. 2006a) and another in type II diabetics with neither study indicating any differences between the placebo and lignan groups in relation to blood lipid profiles (Pan et al. 2007). Also, a recent study in hypercholesterolaemic subjects demonstrated efficacy with higher dose flaxseed lignan supplementation in reducing total cholesterol and glucose (Zhang et al. 2007).

Supplementation with ALA decreased pro-inflammatory cytokines and eicosanoids in some studies (Babu et al. 2003; Bemelmans et al. 2004; Caughey et al. 1996; James et al. 2000; Rallidis et al. 2003; Wallace et al. 2003). Theoretically, this could reduce the amount of skeletal muscle wasting associated with aging by reducing the pro-inflammatory cytokine TNF-α which

may act as a signaling mechanism for skeletal muscle apoptosis (Leeuwenburgh 2003; Phillips and Leeuwenburgh 2005; Roubenoff 2003; Schaap et al. 2006). No research has evaluated the effect of supplementation with ALA during a strength training exercise program on inflammatory cytokines in older adults.

The purpose of this thesis was to evaluate the effects of supplementation with components of flaxseed on inflammatory cytokine concentrations of older healthy adults completing exercise training programs prescribed for the secondary conditions of interest (metabolic syndrome and sarcopenia). The research was designed to determine whether supplementation with either secoisolariciresinol diglucoside or α-linolenic acid from flaxseed and added to standard exercise prescriptions effectively lowers the concentrations of IL-6 and TNF-α in the blood of older humans. As individuals age concentrations of pro-inflammatory cytokines increase. There are no previous studies evaluating the effects of the above components of flaxseed during exercise on the blood concentration of IL-6 and TNF-α. This thesis includes two studies to elucidate the effects of SDG and ALA supplementation on the inflammatory response in healthy older humans. One study was an intervention with SDG supplementation during cardiovascular exercise training while the other was an intervention with ALA supplementation during strength exercise training.

1.5 Experiment I Hypotheses

The primary hypothesis of Experiment I was:

1. TNF-α and IL-6 concentrations will be reduced in elderly female and male SDG supplemented groups compared to elderly female and male placebo groups completing a 6 month cardiovascular exercise training program.

The secondary hypotheses of Experiment I were:

1. A composite score of metabolic syndrome will be reduced in elderly female and male SDG supplemented groups compared to elderly female and male placebo groups completing a 6 month cardiovascular exercise training program.
2. Triglyceride, total cholesterol, low-density lipoprotein cholesterol, and total cholesterol: high-density lipoprotein cholesterol ratio will be reduced in elderly female and male SDG supplemented groups compared to elderly female and male placebo groups completing a 6 month cardiovascular exercise training program.
3. There will be a significantly greater decrease in TNF-α, IL-6, triglyceride, total cholesterol, and low-density lipoprotein cholesterol concentrations as well as total cholesterol: high-density lipoprotein cholesterol ratio concentrations in the elderly male SDG supplemented group compared to the elderly female SDG supplemented group.

1.6 Experiment II Hypotheses

The primary hypothesis of Experiment II was:

1. TNF-α and IL-6 concentrations will be reduced in elderly female and male ALA supplemented groups compared to elderly female and male placebo groups completing a 12 week resistance training exercise program.

The secondary hypotheses of Experiment II were:

1. One-repetition maximum chest press and leg press strength will increase more in elderly female and male ALA supplemented groups compared to the elderly female and male placebo groups completing a 12 week resistance training exercise program and,

2. Lean tissue mass and muscle thickness will increase more in elderly female and male ALA supplemented groups compared to the elderly female and male placebo groups completing a 12 week resistance training exercise program.
3. There will be a significantly greater decrease in the TNF-α and IL-6 concentrations in the elderly male ALA group compared to the elderly female ALA group.

Chapter 2: Experiment I

2.1 Introduction

Chronic low grade inflammation has been implicated as a component of the metabolic syndrome and the development of atherosclerosis which are associated with the aging process (Paoletti et al. 2006; Rana et al. 2007). Metabolic syndrome is characterized by risk factors (i.e. elevations in each of central adiposity, serum triacylglycerol, serum glucose, blood pressure, and inflammation, and lowered high density lipoproteins) which in combination lead to increasing risk of developing insulin resistance, atherosclerosis and cardiovascular disease (Rana et al. 2007). Lifestyle interventions including dietary modification and aerobic exercise can reverse metabolic syndrome risk factors (Roberts et al. 2006). Interventions using components of flaxseed have demonstrated health benefits in relation to cardiovascular disease risk factors (Jenkins et al. 1999; Mozaffarian 2005; Psota et al. 2006).

The flaxseed lignan secoisolariciresinol diglucoside (SDG) is a phytoestrogen which exerts weak estrogenic or antiestrogenic effects in mammalian tissue, depending on the estrogen receptor (Dixon 2004). Evidence from *in vitro* and animal studies indicate that SDG has lipid lowering and anti-inflammatory potential (Hosseinian et al. 2007; Prasad 1999; Prasad 2000a; Prasad 2005b). Currently, there is a paucity of information surrounding the potential of the lignan component of flaxseed to act in a similar manner in humans. One study in healthy postmenopausal women found no effect of supplementation with a flaxseed lignan complex on lipid profiles, antioxidant capacity, and endothelial function (Hallund et al. 2006a; Hallund et al. 2006b). SDG supplementation had no effect on blood lipids, glucose, or insulin although it did improve glycemic control as measured by HbA1c in type II diabetic patients (Pan et al. 2007). Also, 8 weeks of SDG supplementation in hypercholesterolaemic subjects lowered total

cholesterol, LDL cholesterol and glucose concentrations (Zhang et al. 2007). There has been no assessment of supplementation with SDG on the prevention of alteration to lipid profiles that would pose a health risk in healthy older men or inflammatory cytokines or leukocytes in healthy older adults. The primary purpose of this study was to assess the efficacy of supplementation with flaxseed lignan complex on the inflammatory cytokines interleukin-6 and tumor necrosis factor-α as well as leukocyte cell number in healthy older men and women completing an exercise program designed to improve blood lipid profile. A secondary purpose was to evaluate the effects of flaxseed lignan complex on a composite score of metabolic syndrome which included measures of inflammatory cytokines. The hypothesis was that older adults completing a standardized cardiovascular exercise program and supplementing with the flaxseed lignan polymer would decrease serum concentration of IL-6 and TNF-α as well as reduce risk factors associated with the development of metabolic syndrome more so when compared to those taking a placebo.

2.2 Subjects and Methods

2.2.1 Subjects

One hundred men and postmenopausal women were recruited from the general population by newspaper advertisement from a single centre (all subjects were residents of Saskatoon, Saskatchewan, Canada and vicinity and were recruited through the University of Saskatchewan). For study inclusion, subjects were required to be at least 50 years of age. This age was chosen as epidemiological evidence indicates that those over 50 years of age are at increased risk of developing cardiovascular disease due to abnormal blood lipid profiles (Reeder et al. 1997). Subjects were excluded from the study if they 1) were taking pharmaceutical agents

that lower cholesterol levels; 2) had type I or II diabetes; 3) were smokers; 4) were diagnosed with inflammatory bowel disease; 5) were taking hormone replacement therapy; 6) ingested flaxseed supplements (flaxseed, flax oil, flax lignan, or flax fibre) within the previous 2 months; or 7) were involved in vigorous exercise training ($\geq$3 times$\cdot$wk^{-1}). A sample size of n = 39 per treatment group was calculated by using previous research (Huang et al. 2005) demonstrating a decrease in serum TNF-α from 36.3±14.0 pg$\cdot$mL^{-1} to 27.2±10.3 pg$\cdot$mL^{-1} with phytoestrogen (soy isoflavone) supplementation in post-menopausal women at 80% power and an alpha level of 0.05. (Statistica version 7, StatSoft Inc., Tulsa, Oklahoma). A baseline TNF-α concentration of 36.3±14.0 pg$\cdot$mL^{-1} was entered as the population mean and standard deviation. The post-intervention TNF-α concentration of 27.2 pg$\cdot$mL^{-1} was entered as the expected mean in the intervention group after supplementation. Sample size recruitment was increased to n = 50 per group due to an expected drop-out rate of 25%. Ethical approval for the study was received from the Biomedical Research Ethics Board at the University of Saskatchewan and a certificate of approval (Bio# 04-169) was issued (see appendix A). Also, all subjects were informed of the intent of the study and signed a consent form before beginning participation in the study (see appendix B).

2.2.2 Study Design

This study was double-blind, randomized, and placebo-controlled, with subjects randomized to supplement with a flax lignan complex (lignan group) or a placebo (placebo group) for 6 months. The trial was conducted as intent-to-treat and missing data points from individual cases for blood work were accounted for by carrying over the last data point if the subject had at least the baseline and 2 month data point assessed. Subjects were recruited in a

staggered fashion in four blocks. The first block of subjects was recruited in March 2005 and had follow up testing in October 2005. The second block of subjects was recruited in April 2005 and had follow up testing in November 2005. The third block of subjects was recruited in June 2005 and had follow up testing in January 2006. The final block of subjects was recruited in August 2005 and had follow up testing in March 2006. The randomized allocation sequence, with subjects stratified by sex (male/female), was computer generated by a research assistant who had no other involvement in the study. A random number generator (Microsoft Excel) was used to randomize individuals to either the flaxseed lignan or the placebo treatment group. This was done in blocks of 2, so if one subject was randomized to flaxseed lignan complex, the next person was randomized to placebo and vice-versa. Subjects were enrolled into the study by the principal investigator and assigned to groups by a research assistant who had no other involvement with the study. Subjects were considered part of the study once they had been interviewed and initially assessed on all dependent variables at baseline. Participants, those administering the intervention, and those evaluating the outcomes were blinded to group assignment.

2.2.3 Exercise Protocol

All subjects completed the same exercise protocol which involved cardiovascular training (walking) between 30-60 $min \cdot d^{-1}$, 6 $d \cdot wk^{-1}$ (Monday-Saturday) during the 6 month supplement intervention. The program initially prescribed 30 $min \cdot d^{-1}$ and progressed by 4 minutes per week until 60 minutes of walking was done per day (see appendix C). This volume of exercise is appropriate for raising high density lipoprotein cholesterol (HDL) levels and lowering triacylglycerol (TAG) levels (Durstine et al. 2002). The exercise program was semi-supervised;

that is, subjects had the option of attending a daily supervised walking session with a Certified Exercise Physiologist (Canadian Society for Exercise Physiology) or completing the walking program on their own. Subjects were instructed to complete the walking program at an intensity corresponding to 50-65% of their predicted maximal heart rate (220-age). Subjects were instructed on how to find a radial or carotid pulse and how to correctly count the number of beats in a 15 second span of time. Subjects were then instructed to multiply their 15 second count by a factor of 4 to calculate heart rate beats per minute. They were also instructed to take a pulse count approximately 10 minutes into their exercise session and towards the end of their exercise session. If intensity was found to be too low or too high, participants were instructed to modify their walking speed accordingly. If subjects desired to exercise at a higher intensity they were allowed to do so as long as they were able to complete the same duration of exercise. All subjects were given data sheets to record the total exercise time with each walking session for compliance monitoring. The data sheets were collected from the participants at the end of the 6 month intervention to monitor study compliance (see appendix C).

2.2.4 Supplementation

The subjects consumed the supplement orally in tablet form. Three tablets were consumed each day (one tablet at each meal) during the 6 month intervention. Each tablet weighed 1350 mg. The tablet containing the active flaxseed lignan complex provided 550 mg flax extract composed of 32.9% SDG, 13.9% cinnamic acids, 11.8% protein, 10.0% 3-hydroxy-3-methyl glutaric acid, 3.5% fat, 3.3% moisture, and 1.0% ash (Archer Daniels Midland Co., Decatur, IL). In place of the flaxseed lignan complex the placebo tablet contained 550 mg of 90% maltodextrin and 10% caramel color. The subjects randomized to the flaxseed lignan

complex group consumed ~ 543 mg·d^{-1} of SDG. As no human studies had been performed before this study, the dose of flaxseed lignan complex was speculative in nature but based on a personal communication with a flaxseed expert (Neil Wescott, personal communication) involved in the isolation of the lignan complex from flaxseed. Recent evidence from a human study has found a dose of 600 mg·d^{-1} of SDG to effectively lower total cholesterol, LDL cholesterol, and glucose concentrations in hypercholesterolaemic subjects (Zhang et al. 2007). Compliance was monitored by asking subjects to return any unused supplements to the primary researcher at the end of the study.

2.2.5 Measurements

Blood assays

Overnight 12 hour fasting blood samples were obtained at four time-points throughout the study (baseline, 2 mo, 4 mo, and 6 mo). All blood samples were obtained by a certified phlebotomist at the Royal University Hospital, University of Saskatchewan test laboratory. A blood sample (~10 mL) was drawn into Vacutainer serum separator tubes for the analysis of glucose, total cholesterol (TC), high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL), triacylglycerol (TAG), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α). A second blood sample (~10 mL) was drawn into Vacutainer tubes containing ethylendiaminetetraacetic acid (EDTA) for the analysis of hematopoietic parameters (leukocyte counts, lymphocytes, granulocytes, and monocytes). Both blood samples were centrifuged at 3200 rpm for 10 minutes at 20°C. A LX20 Beckman Coulter analyzer (Beckman Coulter Canada Inc., Mississauga, ON) was used to analyze glucose, TC, HDL, and TAG by enzymatic kits. LDL concentrations were calculated using the Friedwald formula. A small test tube of

serum was also aliquoted and immediately stored at -80 °C until analyzed for cytokines. Commercially available enzyme linked immunosorbent assay (ELISA) kits were used to analyze the IL-6 and TNF-α concentrations in the serum of the stored samples (Cayman Chemical Company, Ann Arbor, Michigan). Each assay is based on a double-antibody 'sandwich' technique with intra and interassay coefficients of variance ≤ 10% as reported by the manufacturer. The detection limit of each kit is 1.5 $pg \cdot mL^{-1}$. Hematopoietic measurements were analyzed using an automated cell counter (Cell Dyne, Abbott Laboratories, Abbott Park, Illinois).

Anthropometrics and body composition

Height and weight were measured by a standard stadiometer and a scale at baseline and 6 months and recorded to the nearest 0.1 centimeter and 0.1 kilogram respectively. Body mass index (BMI) was obtained by dividing subject's total body mass (kg) to the square of height in meters.

Body composition was assessed at baseline and after 6 months with dual energy X-ray absorptiometry (DEXA) with QDR software for Windows XP (QDR Discovery, Hologic, Inc., Bedford, Maryland) by a certified radiation technologist. Abdominal fat was determined from DEXA by following the protocol utilized by Svendsen et al. (Svendsen et al. 1993). Briefly, a region of interest was generated with the QDR software for Windows XP between the 1^{st} and 4^{th} intervertebral lumbar disks and the inter-costal margins of the whole body scan. The region of interest was then analyzed by the QDR software to assess abdominal fat mass. The coefficient of variation for DEXA derived abdominal fat mass was assessed by having the same research assistant create a region of interest on a total of n = 30 DEXA scans in older men and women at two different time points (one day apart). For each DEXA scan a coefficient of variation was

determined from the two time-points using the following formula: Coefficient of Variation = (Standard Error of the Mean/Absolute Mean Value) × 100. The coefficient of variation of this technique was 4.7%. Blood measures were assessed every two months throughout the study because they were expected to change quickly; whereas body composition was assessed only at baseline and 6 months. This part of the thesis was part of a larger study that also measured bone mineral and the DEXA measure was only done at 6 months as bone would not be expected to change before this.

Dietary assessment

For dietary assessment a food frequency questionnaire (FFQ) was completed by the subjects at baseline and after the 6 month study intervention. This FFQ is a 110 item survey designed to estimate the customary dietary intake for a wide variety of food items and nutrients in adults (Block Dietary Data Systems - Block 98.2, Berkeley, California). The FFQ was modified by the addition of foods unique to Canada. Portion size pictures were provided to subjects to aid in estimation of foods consumed. As a control, all subjects were informed at the beginning of the study to avoid ingesting any supplement or food product containing flaxseed or supplements that contain flaxseed (i.e. combination ω-3 fatty acid supplements) while they were enrolled in the study.

Blood pressure

After 5 minutes of sitting, resting blood pressure was measured at baseline and 6 months with a standard sphygmomanometer by a trained exercise specialist (Certified Exercise Physiologist – Canadian Society for Exercise Physiology) at the same time of day on each occasion.

Total exercise time

Subjects were instructed to record the amount of time, in minutes, they completed in terms of the walking program on forms given to them at the initiation of the intervention. All the time was summed to determine total walking time, in minutes, for the entire 6 months. This data was converted to an estimate of energy expenditure per week by following the guidelines set forth by Ainsworth et al. (Ainsworth et al. 1993). This was done in an attempt to control for any differences in estimated energy expenditure between the treatment groups over the 6 month intervention. Total walking time in minutes was divided by 26 weeks and then by 60 $min \cdot hr^{-1}$ to determine an average total walking time in $hr \cdot wk^{-1}$. It was assumed that most subjects in the study would walk at approximately 5.6 $km \cdot hr^{-1}$ which corresponds to a estimated energy expenditure of 3.3 $kcal \cdot kg^{-1} \cdot hr^{-1}$ (Ainsworth et al. 1993). The estimate of energy expenditure was determined by the following formula: 3.3 $kcal \cdot kg^{-1} \cdot hr^{-1}$ × average hours of $walking \cdot wk^{-1}$ × subject body mass (kg) at baseline.

2.2.6 Metabolic Syndrome Risk Factors

The secondary dependent variable for the study was a composite score of metabolic syndrome. The metabolic syndrome composite score was calculated by determining the sex-specific Z-scores of each of the following variables: glucose, HDL, TAG, blood pressure (i.e. mean of Z-scores for systolic and diastolic blood pressure), abdominal fat mass (as assessed by dual energy x-ray absorptiometry), and inflammatory markers (mean of Z-scores for the cytokines IL-6 and TNF-α). Once the Z-score for each variable was calculated, the mean of the inverse of the Z-score for HDL and the five other Z-scores was determined and termed the metabolic syndrome composite score for each individual. The inverse of the Z-score for HDL

was used because higher levels of HDL cholesterol are considered beneficial for cardiovascular health. Each subject's individual Z-score was calculated by subtracting the mean of the entire group's variable of interest at baseline from the individual subject's value and dividing this value by the standard deviation of the entire group's variable of interest at baseline. The baseline values were used as representative of values from a normal healthy older adult sample. Once a Z-score was calculated for each variable of interest (i.e. inflammatory markers, abdominal fat mass, glucose, HDL, TAG, and blood pressure), a composite score was calculated for each individual subject by summing the six Z-score variables and dividing by six to achieve an average Z-score for each individual subject. This average Z-score was termed the metabolic syndrome composite score and was then utilized in statistical analyses of metabolic syndrome. Previous research has used a similar composite score of metabolic syndrome in accordance with Adult Treatment Panel III and World Health Organization guidelines (Brage et al. 2004; Eisenmann et al. 2004; Franks et al. 2004).

2.2.7 Statistical Analyses

Covariates

Baseline characteristics of the subjects were assessed by a 1 factor (group) ANOVA. It was decided *a priori* that if group statistical differences existed at baseline that the variable or variables showing significant differences would be entered as covariates in the statistical analysis. Also, the dietary data (baseline and 6 months) was analyzed by a 2 (group) × 2 (sex) × 2 (time) ANOVA with repeated measures on the last factor to determine if differences existed in self-reported dietary intake at the beginning and end of the study. If there were significant changes between groups in dietary intake over the 6 month intervention, the nutrient indicating

significant differences was entered as a covariate. Further, a 1 factor (group) ANOVA was used to evaluate differences in estimated energy expenditure (from self-reported data) between groups; if a significant difference between groups was found, this variable was entered as a covariate in the analysis.

Main analyses

If there was a significant difference between baseline characteristics, nutrients, or estimated energy expenditure, these variables were entered as covariates in a 2 (group) × 2 (sex) × 4 (time) ANCOVA with repeated measures on the last factor analyzing the cytokines, leukocytes, glucose, HDL, LDL, TC, and TAG. Also, a 2 (group) × 2 (sex) × 2 (time) ANCOVA with repeated measures on the last factor was used to analyze the metabolic syndrome composite score. If no covariates were found, the data was analyzed using a 2 (group) × 2 (sex) × 4 (time) ANOVA with repeated measures on the last factor for the cytokines, leukocytes, glucose, HDL, LDL, TC, and TAG; while a 2 (group) × 2 (sex) × 2 (time) ANOVA with repeated measures on the last factor was used to analyze the metabolic syndrome composite score. When a significant interaction was indicated Tukey's post-hoc test was used to detect differences between means. If a significant interaction was not found, main effects were assessed for significance. All results are expressed as means ± standard error of the mean (SE). The alpha level was set at $p \leq 0.05$. All statistical analyses were completed using Statistica version 7 (StatSoft Inc., Tulsa, Oklahoma). The data was analyzed with an ANOVA as opposed to a MANOVA as the hypothesis of Experiment I has not been evaluated in the population tested.

2.3 Results

Nineteen of the original one hundred subjects enrolled in the study dropped out. This included nine subjects in the female placebo group, four subjects in the female lignan group, five subjects in the male placebo group, and one subject in the male lignan group. Reasons for dropping out included lack of time (n = 9), exacerbation of osteoarthritis (n = 2), study dissatisfaction (n = 1), commencement of lipid-lowering medication (n = 1), leg amputation (n = 1), and loss to follow-up (n = 5). Eleven of the 19 had measurements of blood parameters at baseline and one or more other time points and these were carried over and included in the final analysis. Thus, total subject number analyzed for the blood parameters was 92 and baseline characteristics of these subjects are presented in Table 2.1. Of the 92 subjects recruited, six females and eight males were defined as having metabolic syndrome while forty-seven females and thirty-one males were not defined as having metabolic syndrome according to Adult Treatment Panel III guidelines (Lorenzo et al. 2007). Separate sub-analyses of this group indicated no substantial alteration to the results reported below (data not shown). The changes in the group defined as having metabolic syndrome were similar to the rest of the group. It is probable that this sub-analysis lacked statistical power due to the low number of subjects defined as having metabolic syndrome; therefore, finding significant changes was unlikely.

There were no differences between groups for age, mass, height, BMI or blood pressure at baseline ($p > 0.05$; see Table 2.1). There were no differences between groups for baseline dietary habits ($p > 0.05$; see Table 2.2). No differences were found between groups for macronutrient intake throughout the study ($p > 0.05$; see Table 2.2) but, there were significant differences between sexes for total kilocalories, protein, and fat with men consuming more when compared to women. There were no differences between groups for the estimated energy

expenditure from the walking program (placebo: 809±96 kcal·wk^{-1} versus SDG: 1033±92 kcal·wk^{-1}; $p > 0.05$).

Table 2.1 Baseline characteristics of subjects randomized to supplement orally with either flaxseed lignan complex (4050 mg·d^{-1}) or placebo (4050 mg·d^{-1}) before 6 months of cardiovascular exercise training (n=92).

	Placebo (n = 44)	Lignan Complex (n = 48)
Age (y)	62.9±1.0	60.7±1.0
Body Mass (kg)	82.8±2.4	78.7±2.3
Height (cm)	168.2±1.3	168.1±1.3
BMI (kg/m^2)	29.3±0.7	27.7±0.7
SBP (mmHg)	127±2	128±2
DBP (mmHg)	80±1	83±1

Mean ± SE. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Table 2.2 Dietary intake of subjects randomized to supplement orally with either flaxseed lignan complex (4050 mg·d^{-1}) or placebo (4050 mg·d^{-1}) during 6 months of cardiovascular exercise training (n=75).

	Placebo		Lignan Complex	
	Female (n=20)	Male (n=15)	Female (n=21)	Male (n=19)
Energy (kcal·d^{-1})				
Baseline	1738±150	*1777±173	1828±146	*2176±154
6 month	1646±134	1677±155	1609±130	2074±138
Protein (g·d^{-1})				
Baseline	73±6	*75±7	70±6	*86±7
6 month	68±6	67±6	63±5	80±6
Carbohydrate (g·d^{-1})				
Baseline	202±18	*186±21	227±17	*241±19
6 month	197±16	185±18	210±16	233±16
Fat (g·d^{-1})				
Baseline	72±8	*80±9	75±7	*97±8
6 month	68±7	72±8	61±7	92±7
Saturated Fat (g·d^{-1})				
Baseline	20±2	21±2	24±3	28±3
6 month	19±2	18±2	20±2	27±3
MUFA (g·d^{-1})				
Baseline	27±2	29±3	33±3	39±3
6 month	26±3	23±2	28±3	37±3

ω-6 Fatty Acid ($g \cdot d^{-1}$)				
Baseline	18±2	19±2	21±2	21±2
6 month	17±2	15±2	18±2	21±2
ω-3 Fatty Acid ($g \cdot d^{-1}$)				
Baseline	1.9±0.2	2.0±0.3	2.0±0.2	1.9±0.2
6 month	1.6±0.2	1.8±0.2	1.7±0.2	1.8±0.2
Vitamin A (I.U.)				
Baseline	16580±1999	14294±1855	9918±1555	12385±1497
6 month	15128±1639	14091±1665	11014±1635	12893±1528
Vitamin B_6 ($mg \cdot d^{-1}$)				
Baseline	1.96±0.12	1.80±0.13	1.89±0.20	2.20±0.17
6 month	1.92±0.19	1.87±0.13	1.74±0.21	2.02±0.14
Folate ($mg \cdot d^{-1}$)				
Baseline	389±28	364±32	340±41	418±37
6 month	361±29	363±29	328±38	431±35
Vitamin C ($mg \cdot d^{-1}$)				
Baseline	127±11	126±10	92±14	126±10
6 month	110±11	128±9	89±16	127±13
Vitamin D (I.U.)				
Baseline	194±27	145±20	218±42	191±30
6 month	194±31	165±25	167±32	168±22
Vitamin E ($mg \cdot d^{-1}$)				
Baseline	11±1	11±1	10±1	12±1

6 month	10±1	9±1	9±1	12±7
Zinc ($mg \cdot d^{-1}$)				
Baseline	13±1	11±1	12±1	14±1
6 month	12±1	11±1	11±1	14±1
Iron ($mg \cdot d^{-1}$)				
Baseline	14±1	13±1	14±2	16±1
6 month	13±1	12±1	12±1	16±1

Mean±SE. * Indicates significant sex main effect from 2 factor analysis of variance ($p < 0.05$) at baseline (males > females).

2.3.1 Cytokines and Leukocytes

No main effects or interactions for either IL-6 or TNF-α were observed (see Figures 2.1-2.4). No interactions were noted for any of the leukocyte sub-sets analyzed ($p > 0.05$). There was a main effect of time for basophils ($p < 0.05$) with basophil numbers higher at the 2 month, 4 month, and 6 month time-point when compared to baseline counts (see Tables 2.3 and 2.4).

2.3.2 Blood Lipids and Glucose

The blood lipid and glucose data are presented in Tables 2.5 and 2.6 for men and women, respectively. No differences between groups were observed at baseline except the lignan group had a higher concentration of HDL than the placebo group ($p = 0.031$). Males had higher glucose concentrations than females ($p = 0.002$) and females had higher concentrations of HDL than males at baseline ($p = 0.001$). There was a significant group × sex × time interaction for TAG ($p = 0.017$). Post-hoc analyses indicated no differences between any pair of individual means. The interaction was due to a trend for the males on placebo to increase TAG over time relative to males on flaxseed lignan complex (see Table 2.5). Time main effects were found for high-density lipoprotein cholesterol ($p < 0.0001$) which decreased over the intervention and the ratio of total cholesterol to high-density lipoprotein cholesterol ($p < 0.0001$) which increased over the intervention. There were main effects for group on HDL ($p = 0.049$) and total cholesterol to HDL ratio ($p = 0.032$) with the lignan group having a higher HDL and lower total cholesterol to HDL ratio than the placebo group. No main effects or interactions for total cholesterol or LDL were found.

2.3.3 Metabolic Syndrome Composite Score

No baseline differences between the flaxseed lignan polymer and placebo groups for metabolic composite score were found. Changes for the metabolic composite Z-score from baseline to 6 months are presented in Figures 2.5 and 2.6 for males and females, respectively. A significant group × sex × time interaction ($p = 0.037$) was found. Post-hoc analysis indicated that only the men on placebo increased their metabolic composite score (Figure 2.5).

2.3.4 Abdominal Fat and Blood Pressure

The analysis of abdominal fat indicated no differences between the flaxseed lignan complex (females: 17.4±1.7 to 17.8±1.8 kilograms; males: 26.0±1.8 to 25.2±1.9 kilograms) and placebo (females: 20.6±1.8 to 21.0±1.9 kilograms; males: 26.0±2.0 to 26.7±2.1 kilograms) groups. No difference in systolic blood pressure was found between the flaxseed lignan complex (females: 128±3 to 128±3 mmHg; males: 130±3 to 130±3 mmHg) or placebo (females: 123±3 to 122±3 mmHg; males: 129±3 to 127±4 mmHg) groups ($p > 0.05$). There was a significant group × sex × time interaction for diastolic blood pressure ($p < 0.05$). Post hoc analysis did not suggest any significant differences between the individual means (females flaxseed lignan complex: 81±2 to 82±2 mmHg; males flaxseed lignan complex: 85±2 to 81±2 mmHg; females placebo: 77±2 to 76±2 mmHg; males placebo: 82±2 to 82±2 mmHg). Again, the interaction was due to a trend for males on the flaxseed lignan complex to decrease diastolic blood pressure over the 6 months when compared to the female flaxseed lignan complex and male placebo groups.

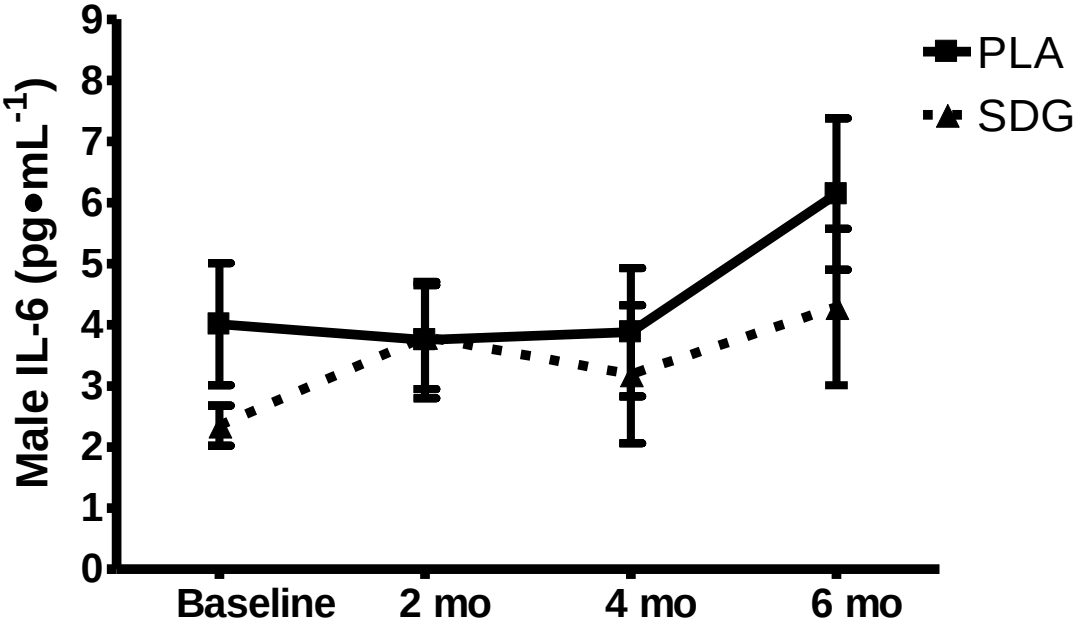

Figure 2.1 Male serum interleukin-6 concentration for each group over 6 month cardiovascular exercise program with either placebo (PLA; n = 17; 4050 mg·d^{-1}) or flaxseed lignan complex (SDG; n = 20; 4050 mg·d^{-1}) oral supplementation. Values are reported as means ± SE.

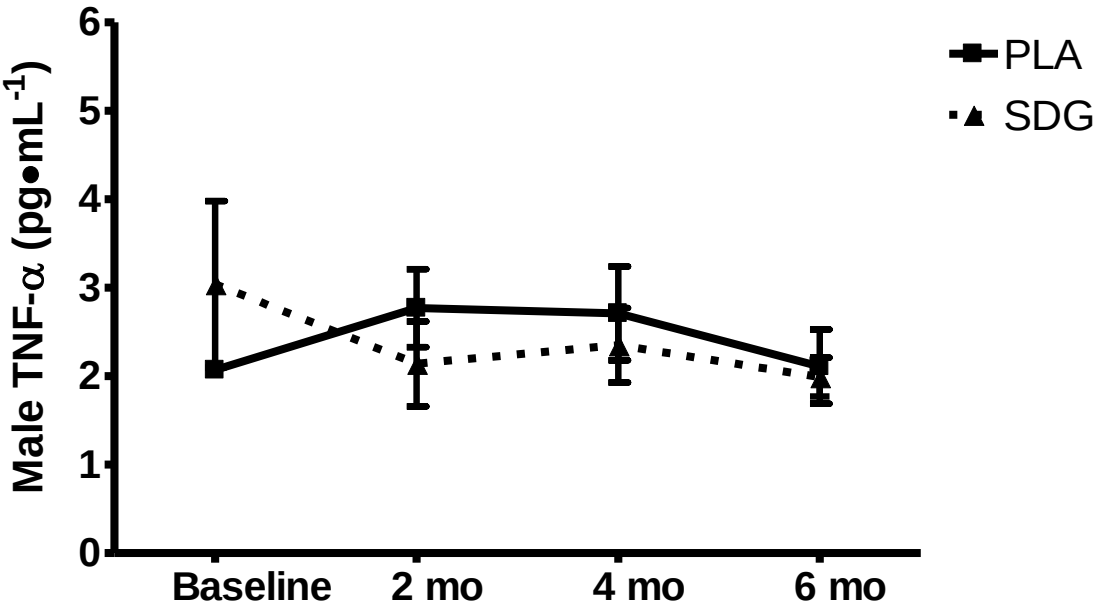

Figure 2.2 Male serum tumor necrosis factor-α concentration for each group over 6 month cardiovascular exercise program with either placebo (PLA; n = 17; 4050 mg·d^{-1}) or flaxseed lignan complex (SDG; n = 20; 4050 mg·d^{-1}) oral supplementation. Values are reported as means ± SE.

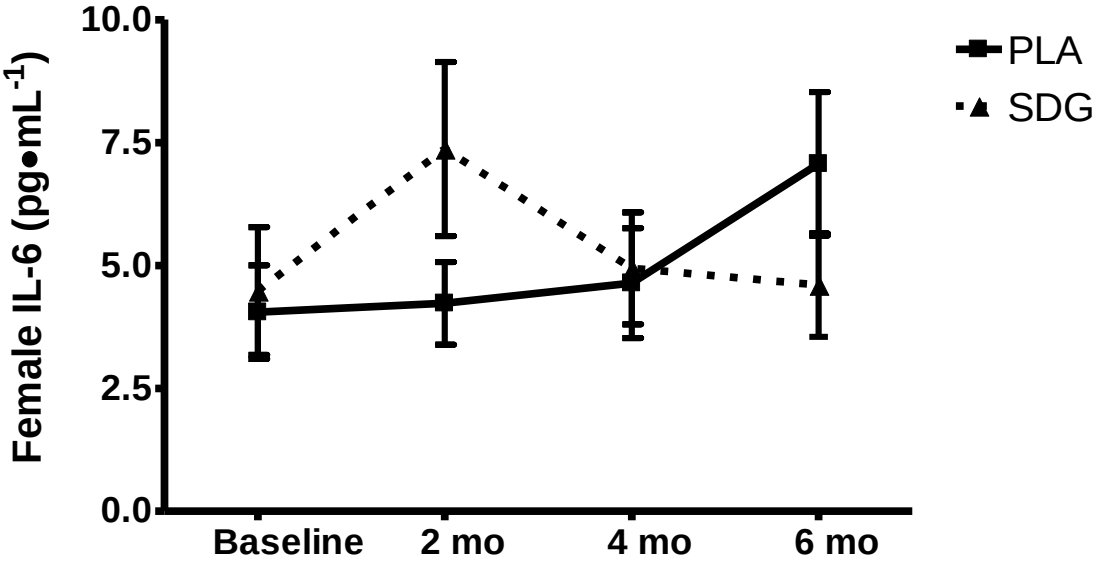

Figure 2.3 Female serum interleukin-6 concentration for each group over 6 month cardiovascular exercise program with either placebo (PLA; n = 23; 4050 mg·d^{-1}) or flaxseed lignan complex (SDG; n = 25; 4050 mg·d^{-1}) oral supplementation. Values are reported as means ± SE.

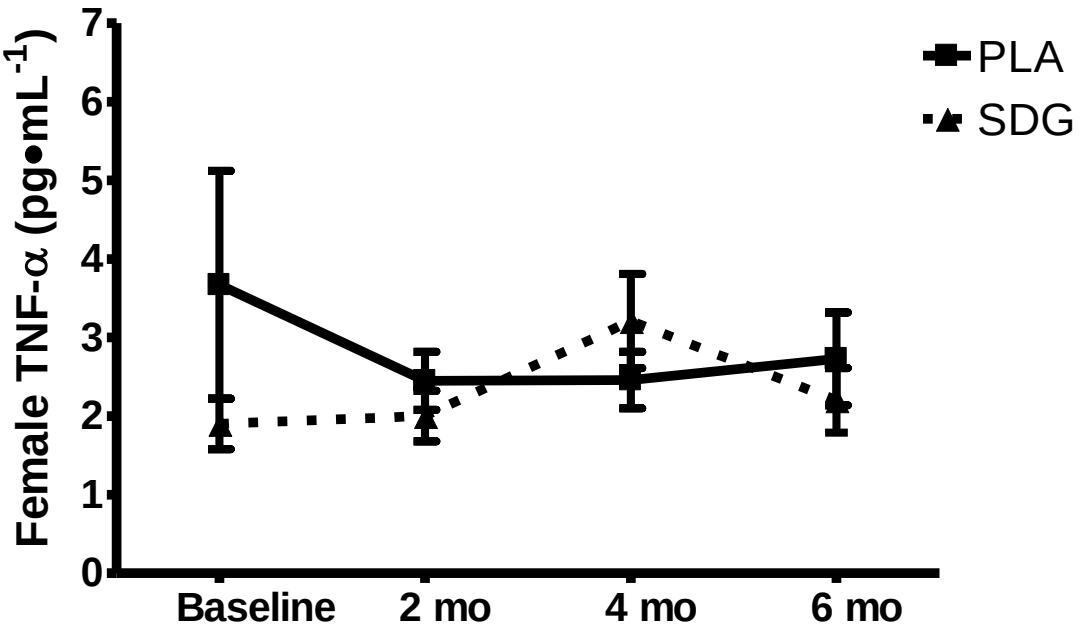

Figure 2.4 Female serum tumor necrosis factor-α concentration for each group over 6 month cardiovascular exercise program with either placebo (PLA; n = 23; 4050 mg·d^{-1}) or flaxseed lignan complex (SDG; n = 25; 4050 mg·d^{-1}) oral supplementation. Values are reported as means ± SE.

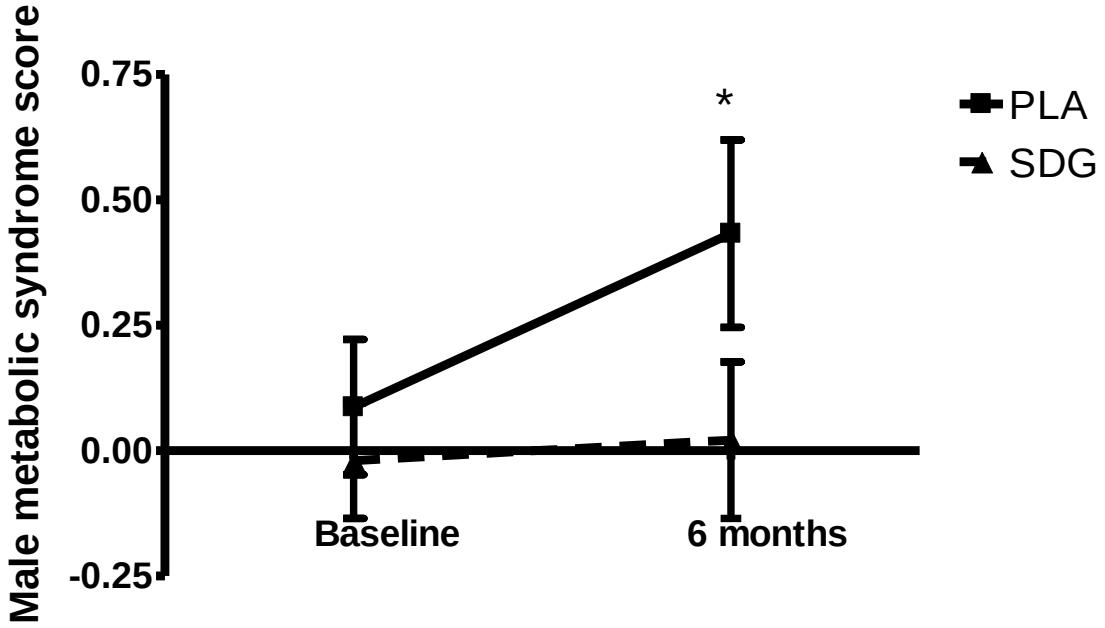

Figure 2.5 Male metabolic syndrome composite score for each group over 6 month cardiovascular exercise program with either placebo (PLA; n = 19; 4050 mg·d^{-1}) or flaxseed lignan complex (SDG; n = 20; 4050 mg·d^{-1}) oral supplementation. Values are reported as means ± SE. *Significant difference between baseline and 6 month values for the male placebo group $p < 0.05$.

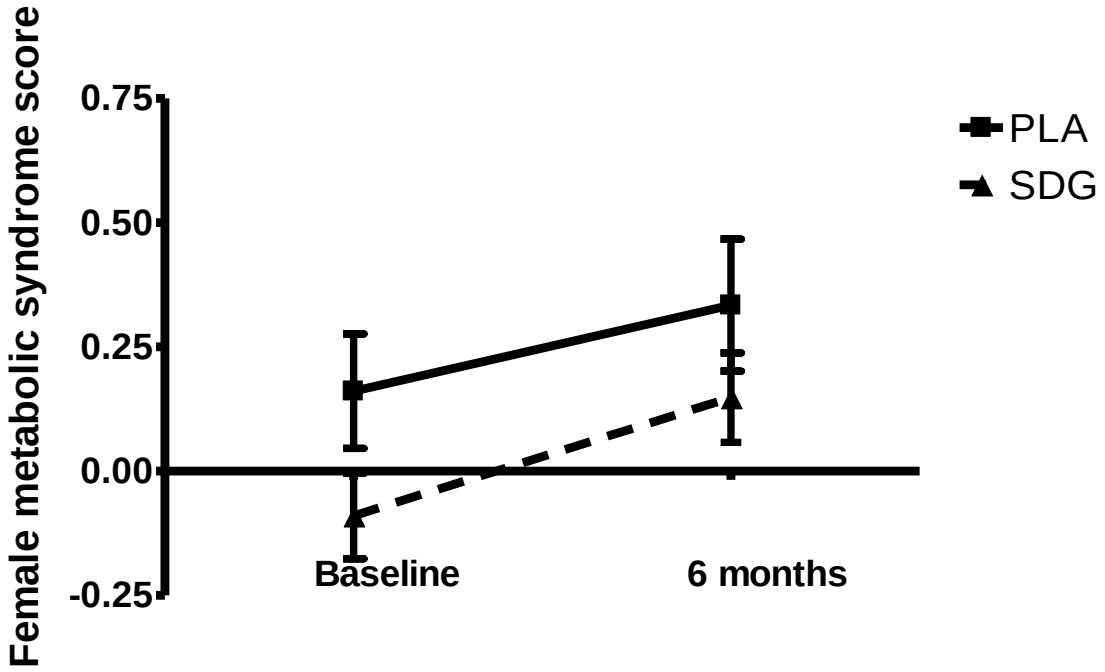

Figure 2.6 Female metabolic syndrome composite score for each group over 6 month cardiovascular exercise program with either placebo (PLA, n = 25; 4050 mg·d^{-1}) or flaxseed lignan complex (SDG; n = 28; 4050 mg·d^{-1}) oral supplementation. Values are reported as means ± SE.

Table 2.3 Leukocyte cell counts ($\times 10^9$) from male participants over 6 month cardiovascular exercise intervention supplementing orally with either placebo (n = 18; 4050 $mg \cdot d^{-1}$) or flaxseed lignan complex (n = 20; 4050 $mg \cdot d^{-1}$).

Variable	Baseline	2 month	4 month	6 month
WBC				
Placebo	5.44±0.33	5.36±0.27	5.40±0.31	5.24±0.30
Lignan	5.78±0.76	5.48±0.23	5.82±0.27	5.68±0.26
Neutrophil				
Placebo	3.17±0.22	3.14±0.15	3.08±0.20	3.02±0.21
Lignan	3.14±0.31	2.89±0.17	3.09±0.21	2.97±0.17
Lymphocyte				
Placebo	1.60±0.09	1.61±0.11	1.60±0.10	1.57±0.10
Lignan	2.00±0.11	2.00±0.09	2.05±0.11	2.07±0.11
Monocyte				
Placebo	0.44±0.03	0.44±0.02	0.44±0.02	0.43±0.03
Lignan	0.45±0.03	0.44±0.03	0.48±0.03	0.46±0.03
Eosinophil				
Placebo	0.22±0.06	0.22±0.05	0.26±0.08	0.19±0.05
Lignan	0.16±0.02	0.16±0.02	0.18±0.03	0.15±0.02
Basophil*				
Placebo	0.01±0.01	0.03±0.01	0.03±0.01	0.03±0.01
Lignan	0.02±0.01	0.02±0.01	0.03±0.01	0.02±0.01

Values are mean ± SE. *Indicates significant time main effect (increasing over time, $p < 0.05$) from 3 factor analysis of variance.

Table 2.4 Leukocyte cell counts ($\times 10^9$) from female participants over 6 month cardiovascular exercise intervention supplementing orally with either placebo (n = 22; 4050 $mg \cdot d^{-1}$) or flaxseed lignan complex (n = 27; 4050 $mg \cdot d^{-1}$).

Variable	Baseline	2 month	4 month	6 month
WBC				
Placebo	5.31±0.32	5.50±0.29	5.39±0.31	5.64±0.31
Lignan	5.87±0.25	5.51±0.20	5.70±0.20	5.58±0.24
Neutrophil				
Placebo	2.95±0.18	3.15±0.20	3.00±0.18	3.17±0.18
Lignan	3.32±0.21	3.08±0.15	3.26±0.16	3.11±0.19
Lymphocyte				
Placebo	1.73±0.13	1.73±0.13	1.77±0.14	1.78±0.13
Lignan	1.88±0.08	1.80±0.09	1.81±0.09	1.82±0.09
Monocyte				
Placebo	0.45±0.03	0.43±0.03	0.45±0.03	0.47±0.03
Lignan	0.49±0.03	0.47±0.02	0.47±0.02	0.47±0.02
Eosinophil				
Placebo	0.18±0.02	0.18±0.02	0.17±0.02	0.19±0.02
Lignan	0.15±0.01	0.15±0.01	0.15±0.01	0.15±0.01
Basophil*				
Placebo	0.01±0.01	0.01±0.01	0.03±0.01	0.03±0.01
Lignan	0.00±0.00	0.01±0.01	0.01±0.01	0.01±0.01

Values are mean ± SE. *Indicates significant time main effect (increasing over time, $p < 0.05$) from 3 factor analysis of variance.

Table 2.5 Blood glucose and lipid concentrations of male participants over 6 month cardiovascular exercise intervention supplementing orally with either placebo (4050 $mg \cdot d^{-1}$) or flaxseed lignan complex (4050 $mg \cdot d^{-1}$).

Variable	Baseline	2 months	4 months	6 months
Glucose ($mmol \cdot L^{-1}$)				
Placebo (n=19)	5.69±0.11	5.59±0.18	5.55±0.12	5.51±0.12
Lignan (n=20)	5.55±0.19	5.55±0.19	5.54±0.11	5.63±0.21
TC ($mmol \cdot L^{-1}$)				
Placebo (n=19)	5.68±0.31	5.72±0.39	5.76±0.39	5.67±0.40
Lignan (n=20)	5.66±0.23	5.43±0.26	5.40±0.21	5.52±0.26
TAG ($mmol \cdot L^{-1}$)				
Placebo (n=19)	1.58±0.25	1.77±0.37	1.83±0.38	1.74±0.38
Lignan (n=20)	1.48±0.17	1.28±0.17	1.34±0.13	1.35±0.17
HDL ($mmol \cdot L^{-1}$)				
Placebo (n=19)	1.26±0.05	1.27±0.04	1.16±0.04	1.15±0.04
Lignan (n=20)*	1.43±0.09	1.37±0.08	1.24±0.07	1.28±0.07
LDL ($mmol \cdot L^{-1}$)				
Placebo (n=18)	3.59±0.17	3.47±0.19	3.61±0.18	3.57±0.18
Lignan (n=20)	3.56±0.20	3.47±0.22	3.55±0.19	3.63±0.20
TC:HDL				
Placebo (n=19)	4.59±0.26	4.45±0.34	5.05±0.33	4.98±0.32
Lignan (n=20)*	4.25±0.27	4.15±0.27	4.55±0.25	4.52±0.25

Values are mean ± SE. TC, total cholesterol; TAG, triacylglycerol; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol. *Indicates significant group main effect ($p < 0.05$) from 3 factor analysis of variance.

Table 2.6 Blood glucose and lipid concentrations for female participants over 6 month cardiovascular exercise intervention supplementing orally with either placebo (4050 mg·d^{-1}) or flaxseed lignan complex (4050 mg·d^{-1}).

Variable	Baseline	2 months	4 months	6 months
Glucose (mmol·L^{-1})				
Placebo (n=25)	5.19±0.08	5.22±0.08	5.18±0.08	5.25±0.08
Lignan (n=27)	5.17±0.11	5.21±0.09	5.20±0.08	5.27±0.10
TC (mmol·L^{-1})				
Placebo (n=25)	6.14±0.21	6.27±0.28	6.09±0.24	6.20±0.24
Lignan (n=27)	5.87±0.17	5.84±0.17	5.86±0.18	5.77±0.17
TAG (mmol·L^{-1})				
Placebo (n=25)	1.77±0.22	1.93±0.30	1.75±0.21	1.71±0.22
Lignan (n=27)	1.19±0.13	1.12±0.10	1.28±0.16	1.28±0.13
HDL (mmol·L^{-1})				
Placebo (n=25)	1.54±0.08	1.52±0.09	1.36±0.06	1.39±0.06
Lignan (n=27)*	1.74±0.08	1.63±0.09	1.60±0.08	1.56±0.08
LDL (mmol·L^{-1})				
Placebo (n=24)	3.77±0.16	3.80±0.23	3.91±0.20	4.01±0.19
Lignan (n=27)	3.60±0.17	3.71±0.16	3.68±0.15	3.63±0.15
TC:HDL				
Placebo (n=25)	4.27±0.27	4.46±0.34	4.69±0.31	4.71±0.32
Lignan (n=27)*	3.53±0.17	3.81±0.19	3.88±0.20	3.88±0.19

Values are mean ± SE. TC, total cholesterol; TAG, triacylglycerol; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol. *Indicates significant group main effect ($p < 0.05$) from 3 factor analysis of variance.

2.3.5 Adverse Events and Compliance

There were no serious adverse events that occurred during the study that were considered related to the intervention. One female subject taking the placebo experienced constipation (classified as mild) which resolved without incident. One male subject from the placebo group had a lower leg amputation (classified as severe) during the study due to pre-existing peripheral arterial disease. Also, one female subject experienced a flare up of osteoarthritis in her ankle joint (mild) and one female subject experienced an exacerbation of osteoarthritis symptoms in a knee joint (moderate) due to the walking program. Compliance monitoring indicated that the placebo group ingested 88 ± 2 % of their tablets while the flaxseed lignan group ingested 91 ± 1 % of their tablets.

2.4 Discussion

The data from this study indicate that flaxseed lignan complex supplementation has no effect on the cytokine concentration of IL-6 and TNF-α or on leukocyte cell counts. As flaxseed lignan is a phytoestrogen, it may act in a manner similar to estrogen by up-regulating estrogen receptors which have been shown to have reciprocal cross-talk with nuclear factor kappa B (NFκB), a transcription factor for many pro-inflammatory cytokines (Evans et al. 2001). If NFκB is down-regulated by increased estrogen receptor number, the possibility of decreased risk of inflammation and associated cardiovascular disease is likely (Evans et al. 2001). Supplementing a diet with high amounts of soy isoflavones increased the serum concentrations of IL-6 in middle-aged women but not men (Jenkins et al. 2002). An increase in IL-6 may pre-dispose older adults to a greater risk of many chronic conditions (Dijsselbloem et al. 2004) but it is debated whether IL-6 is pro- or anti-inflammatory in nature as it is a negative feedback signal,

decreasing the release of TNF-α and reducing the amount of this powerful pro-inflammatory cytokine (Pedersen et al. 2003). Previous work using partially defatted flaxseed as a dietary supplement indicated an increase in oxidative stress as measured by a decrease in protein thiol groups compared to a control (Jenkins et al. 1999). Although an increase in oxidative stress could induce an up-regulation of pro-inflammatory cytokines, previous work suggests that the lignan from flaxseed is either an anti-oxidant (Kitts et al. 1999; Prasad 1997b; Prasad 2000a; Prasad 2000c; Prasad 2005b) or has no effect on antioxidant capacity (Hallund et al. 2006a). The results of this study indicate that flaxseed lignan complex had no significant effects on inflammation as measured by IL-6 and TNF-α (Figure 2.1 - 2.4) or leukocyte cell count (Table 2.3 & 2.4).

The results also indicate that flaxseed lignan complex supplementation may be effective for preventing risk of metabolic syndrome in older men but not older women. This is in agreement with several animal models of supplementation with other phytoestrogens such as soy isoflavones which reduce metabolic syndrome in both lean and obese male rats (Davis et al. 2007; Davis et al. 2005). Also, a recent *in vitro* study culturing adipocytes in puerarin (another phytoestrogen) found that this isoflavone is capable of producing anti-metabolic syndrome effects (Xu et al. 2005). A cross-sectional study utilizing the Framingham Offspring found that postmenopausal women with the highest intake of phytoestrogens (either isoflavones or lignans) in their diet had the least possibility of metabolic syndrome risk (de Kleijn et al. 2002). The results in this thesis indicate that metabolic syndrome risk is attenuated in older males, but not females, taking a flaxseed lignan complex supplement, but the reason for this sex effect is not known. There is a dramatic decrease in estrogen and likely a decrease in the number of estrogen receptors at menopause in women (Castelo-Branco and Rostro 2006). Although speculative,

SDG or its metabolites may not have been able to bind as effectively in the women versus the men due to a lack of receptors. Also, evidence suggests that isoflavones from soy may bind with greater affinity to estrogen receptor β than does SDG (Turner et al. 2007) which may explain the contrasting results between metabolic syndrome risk factors in women from this study versus the Framingham Offspring Study. Finally, enterolactone, a metabolite of SDG, effectively binds with estrogen receptor α and induces transcription both *in vitro* and in an *in vivo* animal model (Penttinen et al. 2007). If estrogen receptors are reduced with aging, especially at menopause, then the ability of SDG or its metabolites to bind with estrogen receptor α would also be compromised and not likely to produce a favorable health effect in post-menopausal women.

The interaction noted for TAG in this study most likely occurred due to a decrease in TAG concentration in men on flaxseed lignan combined with a increase in men on placebo, with no change in the female groups (see Tables 2.5 & 2.6). Previous work in animals suggests that SDG or flaxseed consumption will either raise (Prasad et al. 1998) or have no effect (Prasad 1999) on TAG. The results of this study confirm earlier research indicating no effect of lignan supplementation on TAG in postmenopausal women (Hallund et al. 2006a) but suggest that TAG concentration may be lowered in older men ingesting a flaxseed lignan supplement. Further, in type II diabetics, TAG was not altered with 12 weeks of flaxseed lignan complex supplementation (Pan et al. 2007). There are several mechanisms by which flaxseed lignan may lower TAG. Other plant lignans (i.e. sesamin) lower TAG levels by modulating the activity of acyl CoA cholesterol acetyl transferase, the enzyme involved in packaging cholesterol ester and TAG into very low density lipoproteins for export from the liver (Bloedon and Szapary 2004). Lignans also increase hepatic fatty acid oxidation and down regulate hepatic lipogenic enzymes

thereby decreasing fatty acid availability for TAG synthesis and secretion from the liver (Ide et al. 2001).

This study revealed no changes between groups in lipoprotein concentrations. This is in direct contrast to research in animal models which found a decrease in total cholesterol and LDL with a corresponding increase in HDL in animals supplemented with flaxseed lignan (Prasad 1999; Prasad 2005b). These studies found SDG had its greatest effect on animals with abnormal lipid profiles induced by a high cholesterol diet. This was recently confirmed in a human study where 8 weeks of SDG supplementation in hypercholesterolaemic subjects significantly lowered TC, LDL and glucose versus placebo (Zhang et al. 2007). In the current study subjects had a mix of normal and abnormal lipid profiles; however, sub-analyses on those subjects with lipid levels outside the normal reference range indicated no effect of flaxseed lignan complex on lipid profiles, although the power of this analysis was low due to low subject numbers (data not shown). The current results are in agreement with the outcomes of Hallund et al. (2006a) and Pan et al. (Pan et al. 2007) who found no changes in concentrations of lipids in post-menopausal women or type II diabetics supplementing with flaxseed lignan complex. Estrogen is known to increase LDL clearance by up-regulating hepatic LDL receptors (Inukai et al. 2000; Parini et al. 1997). Enterolactone, a metabolite produced by SDG, may act similarly to estrogen by stimulating hepatic and macrophage cells to increase uptake of LDL (Owen and Abbey 2004; Owen et al. 2004). The results indicate that 6 months of flaxseed lignan complex supplementation had no effect on LDL cholesterol concentrations. It may be that the lignan supplementation did not result in a significant metabolic conversion to enterolactone, due to decreased intestinal bacteria activity, which could account for the lack of an effect on LDL concentration or that the dose of supplementation was not enough to produce a physiological

effect. A peculiar finding of this study is the decrease in HDL and increase in TC:HDL over time in all participants. Each group was instructed to exercise by walking 5-6 $d \cdot wk^{-1}$, a dose of exercise recommended as that needed to increase HDL (Durstine et al. 2002; Durstine et al. 2001). The unexpected changes in HDL and TC:HDL over time may have been due to seasonal effects (Gordon et al. 1987). HDL is higher and TC:HDL lower in warmer compared to colder months of the year. The majority of subjects in the current study started the intervention in a warmer month and finished in a colder month.

This study is limited in its interpretation. The study lacked a non-exercise flaxseed lignan complex and placebo group, was constrained to self-reported methods of dietary analysis as well as self-reported amounts of exercise, and, due to participant staggering, may reflect more seasonal effects on metabolic syndrome risk factors (i.e. blood lipids) than effects due to flaxseed lignan supplementation.

In conclusion, this study indicates that 6-months of supplementation with flaxseed lignan complex (543 $mg \cdot d^{-1}$ of SDG) had no effect on inflammatory cytokines. Flaxseed lignan complex supplementation attenuates the risk of metabolic syndrome in older men while there is no effect on metabolic syndrome criteria in older females. Of the components of the metabolic syndrome, flaxseed lignan complex had its greatest effect on TAG and diastolic blood pressure, while having no statistical effect on inflammatory cytokines, HDL, abdominal fat, systolic blood pressure, or glucose. More research on the biological health effects of flaxseed lignan complex supplementation in humans is warranted.

Chapter 3: Experiment II

3.1 Introduction

Skeletal muscle loss is a major health concern associated with aging. Sarcopenia refers to the age related loss of muscle mass and strength (Borst 2004). Recently, it has been determined that the costs associated with health-care due to sarcopenia are 1.5% of the total health care costs per year in the United States (Janssen et al. 2004). Also, it is estimated that this cost will continue to rise as the population ages.

Chronic low grade inflammation is one factor contributing to decreased muscle mass and strength with age (Roubenoff 2001) but others such as decreased neural function, altered nutrition in old age, reduced physical activity as well as decreases in anabolic hormones may all contribute to sarcopenia (Alway and Siu 2008). The inflammatory cytokine interleukin-6 (IL-6) is associated with a decrease in muscle mass, strength, and fiber number in older adults (Bautmans et al. 2005; Deschenes 2004; Ferrucci et al. 2002; Payette et al. 2003). A second inflammatory cytokine, tumor necrosis factor-α (TNF-α) is considered a catabolic signaling agent and increasing concentrations due to the aging process result in a decreased muscle mass and strength (Ladner et al. 2003). Apoptosis, or programmed cell death, has been indicated as a possible contributor to the etiology of sarcopenia (Alway and Siu 2008; Leeuwenburgh 2003) and as age increases, TNF-α stimulates the progressive deterioration of myocytes via apoptosis (Ladner et al. 2003).

Alpha-linolenic acid (18:3ω-3; ALA) is an 18 carbon fatty acid considered an essential ω-3 polyunsaturated fatty acid and the parent to longer carbon chain ω-3 fatty acids. Flaxseed oil is composed of between 45-60% ALA and is considered the most abundant source of ALA (Cunnane 2003). ALA functions as a precursor to other ω-3 fatty acids (eicosapentaeonic acid and docosahexaenoic acid) via carbon chain elongation and further desaturation (Burdge 2004).

Supplementation with ω-3 fatty acids has been used to decrease the amount of inflammation associated with various disease processes (Simopoulos 1999; Simopoulos 2002b). It has been proposed that a high ratio of ω-6 to ω-3 fatty acids in the normal Western diet is associated with an increased risk for many chronic diseases (Simopoulos 2002a). Increasing consumption of ALA will increase eicosapentaenoic acid (EPA) concentration in men and women but the conversion is lower in men than women (Burdge 2004; Burdge et al. 2003; Burdge and Wootton 2003). Theoretically, increasing exogenous consumption of ALA, with the associated increase in EPA, will reduce inflammation by competing for the same enzymatic pathways (cyclooxygenase and 5-lipoxygenase) as arachidonic acid (Alexander 1998). Arachidonic acid produces an eicosanoid series considered to be more pro-inflammatory than EPA. The eicosanoids, which are lipid mediators of inflammation, secreted by arachidonic acid metabolism are considered pro-inflammatory and will up-regulate pro-inflammatory cytokine release while the eicosanoids from EPA metabolism are less inflammatory in nature (Alexander 1998).

The purpose of this study was to evaluate the effectiveness of ALA supplementation on markers of inflammation and muscle mass and strength in older adults completing a progressive resistance training program. The main hypothesis of this study was that ALA supplementation would decrease inflammation, as measured by IL-6 and TNF-α concentration in blood plasma, which in turn would result in a greater increase in muscle mass and strength in older adults completing a strength training program.

3.2 Subjects and Methods

3.2.1 Research Design

A 2 group randomised controlled double blind trial was used to compare the effects of 12 weeks of resistance training with and without ALA supplementation in older males and females. Dependent variables included plasma concentrations of IL-6 and TNF-α, 1-repetition maximum (1 RM) chest and leg press strength, muscle thickness of the elbow and knee extensors and flexors assessed by ultrasound, and body composition (lean tissue and fat mass) as assessed by dual energy X-ray absorptiometry (DEXA). Subjects were matched for sex and randomly assigned by computer to either supplement with ALA or placebo. Each participant completed a progressive resistance training program designed to improve muscle mass and strength.

3.2.2 Subjects, Randomization, and Supplementation

Untrained male and female participants were recruited from the healthy population (n=51; 65.4 ± 0.8 yrs). Participants were included only if they were 60 years of age or older because this is the approximate age at which there is a significant increase in sarcopenia (Castillo et al. 2003). Subjects were excluded if they: 1) were taking any medication that may affect inflammation; 2) had an inflammatory disease; 3) were taking any flaxseed supplements; or 4) were currently participating in a resistance training program ≥2 times/week. Once participants were screened, they were stratified by sex and then randomized to either the ALA or placebo group by a research assistant not involved in any other aspect of the research project. A random number generator (Microsoft Excel) was used to randomize individuals to either the ALA or the placebo treatment group. This was done in blocks of 2, so if one subject was randomized to ALA, the next person was randomized to placebo, and vice versa. A Physical Activity Readiness

Questionnaire (PAR-Q) was completed to ensure there were no other health risks precluding their participation in the resistance training program (Thomas et al. 1992). A sample size of n = 24 per treatment group was calculated by using previous research (Caughey et al. 1996) demonstrating a decrease in LPS stimulated peripheral blood mononuclear cells TNF-α concentration from 30.1±6.2 $pg \cdot mL^{-1}$ to 22.1±5.4 $pg \cdot mL^{-1}$ after 4 weeks of flaxseed oil supplementation in healthy young males at 80% power and an alpha level of 0.05 (Statistica version 7, StatSoft Inc., Tulsa, Oklahoma). A baseline TNF-α concentration of 30.1±9.5 $pg \cdot mL^{-1}$ was entered as the population mean and standard deviation respectively. An increased standard deviation was used as it was expected that the variation in older adults would be greater than young adults. The post-intervention TNF-α concentration of 22.1 $pg \cdot mL^{-1}$ was entered as the expected mean in the intervention group after supplementation. Recruitment was increased to n = 30 per group due to an expected drop-out rate of 25%. Ethical approval was granted for the study through the University of Saskatchewan's Biomedical Research Ethics Board and a certificate of approval was issued (Bio# 06-211; see appendix C). All participants signed a consent form before being enrolled into the research project (see appendix D).

ALA and placebo oil was similar in color and calories. Subjects received a plastic opaque bottle of oil after baseline testing and were instructed to store the bottle in a refrigerator to reduce the chance of the oil oxidizing. Participants supplemented their regular diets with either 30 mL of flaxseed oil (containing ~14 grams of ALA) or 30 mL of placebo oil (corn oil) per day throughout the 12 week trial. This dose was chosen as previous research using ALA found a significant decrease in inflammatory cytokines with the same approximate dose (Caughey et al. 1996). Also, this is the maximum dose allowed by Health Canada. Participants were asked to return the bottles at the end of the study for compliance monitoring.

3.2.3 Resistance Training

Resistance training was 3 days per week with at least one day of rest between training days, for 12 weeks. The resistance training sessions included exercises for all major muscle groups with the following 13 exercises: chest press, lateral pull down, shoulder press, biceps curl, triceps push down, back extension, hip extension, hip flexion, hip adduction and hip abduction using Lever equipment (Pulse Fitness Systems; Winnipeg, MB, Canada) and leg press, knee extension, and knee flexion using Hammer Strength equipment (Life Fitness; Franklin Park, IL, U.S.A.). A periodized resistance training program involving 4 blocks was utilized for this study. The first block included 6 training sessions and was devoted to anatomical adaptation with subjects performing 3 sets of 10-12 repetitions at 60-65% of their 1RM. Block two was devoted to higher volume training designed to stimulate muscle hypertrophy and had subjects complete 6 training sessions utilizing 4 sets of 10-12 repetitions at 65-70% of their 1RM. Block three included12 training sessions and was devoted to strength development with the subjects performing 3-4 sets of 6-10 repetitions at 70-85% of their 1RM. The fourth block included 12 training sessions and was devoted to maintenance of the strength and hypertrophy gains with subjects performing 3 sets of 10-12 repetitions at 65-80% of their 1RM. This program was similar to a program previously utilized which demonstrated significant increases in strength and lean tissue mass in older men (Newton et al. 2002). Participants were allowed to progress in load when they were able to successfully complete all prescribed sets and repetitions with proper form.

3.2.4 Data Collection

Subjects were required to attend the laboratory for testing over 3 days at baseline and after the 12 week intervention. Before and after the 12 week intervention subjects were required to give blood samples for cytokine analysis (Day 1), complete tests of muscle thickness and then maximum chest press and leg press strength (Day 2), and provide measurements of body composition, as well as complete a food frequency questionnaire (Day 3).

Cytokines

Overnight fasting (12 hour) blood samples, of approximately 10 mL were drawn by venipuncture from the antecubital vein into cooled (4˚C) vacutainer tubes containing EDTA. After inversion, the tubes were kept on ice for ~ 15 minutes and then centrifuged at 2300 rpm for 12 minutes at 4˚C. Approximately 4 mL of plasma was aliquoted into Eppendorf microtubes (~1 mL per tube × 4 tubes) and immediately frozen at -80˚C until analyzed. An enzyme linked immunosorbent assay (ELISA) was used to analyze IL-6 and TNF-α concentration in the plasma (Cayman Chemical Company, Ann Arbor, Michigan, U.S.A.) according to the manufacturer's instructions. Each assay is based on a double-antibody 'sandwich' technique. The intraassay coefficients of variation for IL-6 were less than 4% and for TNF-α were less than 6%. The detection limit of each kit is 1.5 $pg \cdot mL^{-1}$. Completed assays were read for absorbance on a Synergy HT microplate reader (BioTek Instruments, Winooski, Vermont, U.S.A.) at a wavelength of 405 nm. All samples of each subject (i.e. both at baseline and after 12 weeks) were assayed in triplicate within the same microplate to reduce variation.

Anthropometrics and Body Composition

Height was measured utilizing a standard stadiometer and weight was measured using a calibrated scale. Body composition was assessed by DEXA (Discovery W version 12.3, Hologic

Inc., Bedford, Maryland). Coefficients of variation for repeated measures in our lab were 0.5% for lean tissue mass, and 3% for fat mass.

Muscle Thickness

Muscle thickness was evaluated on the elbow and knee flexors and extensors on the right limbs by B-mode ultrasound using a 5 MHz scanning transducer (Aloka SSD-500, Tokoyo, Japan) as has been previously described in detail (Candow and Chilibeck 2005; Farthing and Chilibeck 2003). The coefficients of variation for repeated muscle thickness measurements were 2.5% (elbow flexors), 2.2% (elbow extensors), 3.6% (knee flexors), and 2.1% (knee extensors).

Muscle Strength

One-repetition maximum (1-RM) strength was assessed by using the same chest press and leg press equipment used with training, by a protocol previously described in detail (Chrusch et al. 2001). Briefly, all 1-RM strength testing was performed by a qualified exercise professional (Certified Exercise Physiologist) who gave proper instruction on exercise technique before beginning the testing session and made it clear to the subjects that a maximal effort was required for the two strength tests. One-repetition maximum strength was assessed by using standard chest press (Pulse Fitness Systems Inc., Winnipeg, Manitoba) and leg press (Life Fitness; Franklin Park, IL, U.S.A.) equipment. Subjects performed a very light warm up set of 10 repetitions on the equipment being used for the test and then 1-2 minutes of static stretching exercises for the muscle groups being tested. Then another warm-up set of 5 repetitions was performed with a higher load. The equipment was then loaded with more weight and the subject was instructed to make a 1 repetition attempt. If the attempt was successful, the research assistant loaded more weight on to the equipment. This was repeated until the subject was unable to perform one repetition with the load on the equipment. Subjects rested quietly between

1RM attempts for between 30-60 seconds. If the subject was unsuccessful on the first attempt the load was lowered and the same protocol was followed (i.e. 1RM attempts were made until the subject was unable to lift the load). The coefficients of variation for chest and leg press 1-RM were 3.6% and 3.0%, respectively.

Dietary Assessment

Typical dietary intake was evaluated by a food frequency questionnaire (FFQ). The FFQ used was a 110 item survey, with modifications made for the typical Canadian diet, designed to estimate the customary dietary intake for macronutrients and micronutrients in adults (Block 98.2, Block Dietary Data Systems, Berkeley, California). The FFQ utilizes portion size pictures to increase the accuracy of dietary intake estimation.

3.2.5 Statistical Analyses

Covariates

Baseline characteristics of the subjects were assessed by a 1 factor (group) ANOVA. It was decided *a priori* that if group statistical differences existed at baseline that the variable(s) showing significant differences would be entered as covariates in the statistical analysis. Also, the dietary data (baseline and 12 weeks) was analyzed by a 2 (group) × 2 (sex) × 2 (time) ANOVA with repeated measures on the last factor to determine if differences existed in self-reported dietary intake at the beginning and end of the study. If there were significant changes in dietary intake between groups over the 12 week intervention, the nutrient indicating a significant difference was entered as a covariate.

Main analyses

A 2 (groups) × 2 (sex) × 2 (time, before and after 12 weeks of supplementation) ANOVA with repeated measures on the last factor was used to analyze the data in relation to

changes in cytokine concentration, 1 repetition maximum strength, muscle thickness, body composition and dietary status. Relative change scores for dependent variables were analyzed with a 2 (groups) × 2 (sex) factorial ANOVA. Relative change scores were determined by subtracting baseline from final results, dividing by baseline and multiplying by 100 to derive a percentage. If a significant interaction was found an LSD post hoc test was performed to determine differences between means. A more liberal post hoc analysis was used in this study as the number of subjects was not as large as in Experiment I which would reduce the power of our analysis. If a significant interaction was not found, main effects were assessed for significance. The alpha level for the study was set at $p \leq 0.05$. All data is displayed as mean ± standard error (SE). All statistical analyses were performed using Statistica version 7 (Statsoft, Tulsa, OK). The data was analyzed with an ANOVA as opposed to a MANOVA as the hypothesis of Experiment II had not been evaluated in the population tested.

3.3 Results

The number of individuals that responded to the media advertisement was one hundred and forty. Seventy-seven were excluded from the study after the initial interview. Three individuals decided not to participate after the initial interview. Sixty individuals were enrolled in the study at baseline, which included baseline testing. Nine of the sixty original subjects dropped out during the study, leaving fifty-one to complete the intervention. Reasons given for drop out included: lack of time (n = 5), medical problems unrelated to the study intervention (n = 2), family medical problems (n = 1), and unable to tolerate supplement consumption (n = 1). Of the fifty-one remaining, twenty-five were in the ALA group and twenty-six were in the placebo

group. According to returned portions of oil, the placebo group was 78.2 ± 4.1 % compliant while the ALA group was 83.6 ± 2.9 % compliant with the supplementation protocol.

There were no differences between ALA and placebo groups at baseline (Table 3.1) except that females on placebo had a greater elbow extensors muscle thickness than the females on ALA ($p = 0.027$).

3.3.1 Dietary Intake

There were no differences between groups from baseline to 12 wk for intake of kilocalories, protein, total fat, monounsaturated fat, polyunsaturated fat, or carbohydrate (see Table 3.2). There were time main effects for protein (decreasing, $p < 0.05$), total fat (decreasing, $p < 0.05$), saturated fat (decreasing, $p < 0.05$), and monounsaturated fat (decreasing, $p < 0.05$).

3.3.2 Cytokines

A significant group × sex × time interaction for IL-6 was found ($p = 0.05$; Figure 3.1). Post-hoc analysis indicated that the male ALA group had a significant reduction in IL-6 concentration from baseline to 12 weeks ($p = 0.003$) while the male placebo group and female groups did not change significantly over time. The ALA and placebo groups did not differ over time for TNF-α concentration (Figure 3.2). Analysis did reveal a significant sex × time interaction for the absolute changes in TNF-α concentration ($p = 0.03$) with males decreasing (7.80 ± 0.67 to 6.98 ± 0.65 pg·mL^{-1}) and females increasing (4.42 ± 0.74 to 5.72 ± 0.71pg·mL^{-1}) over the 12 weeks.

3.3.3 Muscle Strength

One-repetition maximum strength did not differ between ALA and placebo groups over time. There was a significant sex × time interaction for chest press strength ($p = 0.006$) with females increasing (27.6 ± 3.5 to 42.2 ± 3.9 kg) less than males (73.5 ± 3.1 to 96.8 ± 3.6 kg), see Figure 3.3. Also, a main effect of time ($p < 0.01$) for leg press strength was found (see Figure 3.4).

3.3.4 Body Composition

Body composition measures did not differ between ALA and placebo groups over time. There were main effects of time with lean tissue mass increasing (see Figure 3.5; $p < 0.05$). Also, percent body fat (29.7 ± 0.8 to 29.0 ± 0.7 %) and total body mass (75.8 ± 1.9 to 74.7 ± 1.8 kg) decreased significantly over time (both $p < 0.01$). It was determined that neither the interaction for differences in fat mass were significant ($p > 0.05$) nor were main effects for fat mass significant ($p > 0.05$).

3.3.5 Muscle Thickness

There was a significant group × sex × time interaction ($p \leq 0.05$) for knee flexor muscle thickness with the female placebo group and male ALA group increasing from baseline to 12 weeks with no other significant changes in the other groups over time. Main effects of time were observed for elbow flexor, elbow extensor, and knee extensor thicknesses (all $p < 0.01$; see Table 3.3).

Table 3.1 Baseline characteristics of older adults randomized to supplement orally with either flaxseed oil (ALA; 30 ml·d^{-1}) or corn oil (PLA; 30 ml·d^{-1}) for 12 weeks while completing a strength training exercise program (n = 51).

	ALA (n = 25)	PLA (n = 26)
Age (yr)	65±1	66±1
Height (cm)	168±2	169±2
Mass (kg)	75±3	78±3
Fat (%)	29±2	30±2
Fat Mass (kg)	22±2	23±2
LTM (kg)	51±2	52±2

Data are means ± SE. ALA, alpha-linolenic acid group; PLA, placebo group; LTM, lean tissue mass.

Table 3.2 Dietary intake of older adults randomized to supplement orally with either flaxseed oil (ALA; 30 ml·d^{-1}) or corn oil (PLA; 30 ml·d^{-1}) for 12 weeks while completing a strength training exercise program not including the supplement ingested (n = 51).

	Male		Female	
	(n = 14 PLA; n = 14 ALA)		(n = 12 PLA; n = 11 ALA)	
	Baseline	12 weeks	Baseline	12 weeks
Energy(kcal·d^{-1})				
PLA	2121±157	1949±148	1633±170	1611±159
ALA	1671±157	1517±148	1628±177	1514±167
Protein (g·d^{-1}) †				
PLA	79±7	73±7	66±8	61±7
ALA	64±7	62±7	66±8	57±8
Carbohydrate (g·d^{-1})				
PLA	274±20	251±18	199±22	199±19
ALA	186±20	168±18	195±23	190±20
Total Fat (g·d^{-1})†				
PLA	81±8	74±8	67±9	66±8
ALA	71±8	62±8	70±9	63±9
Saturated Fat (g·d^{-1}) †				
PLA	23±2	21±2	19±3	19±3
ALA	22±2	19±2	19±3	17±3
MUFA (g·d^{-1}) †				
PLA	33±3	29±3	25±4	25±3
ALA	28±3	24±3	28±4	26±4
ω-6 Fatty Acid (g·d^{-1})				
PLA	19±2	17±2	18±2	18±2

ALA	15±2	14±2	17±2	15±2
ω-3 Fatty Acid ($g \cdot d^{-1}$)				
PLA	1.8±0.2	1.8±0.2	1.7±0.2	1.8±0.2
ALA	1.4±0.2	1.3±0.2	1.6±0.2	1.4±0.2
Vitamin A (I.U.)				
PLA	14646±2507	13710±1897	15213±2708	12807±2049
ALA	7724±2507	8009±1897	14254±2829	12207±2140
Vitamin B_6 ($mg \cdot d^{-1}$)				
PLA	2.2±0.2	2.1±0.2	1.8±0.2	1.8±0.2
ALA	1.8±0.2	1.7±0.2	1.7±0.2	1.7±0.2
Folate ($mg \cdot d^{-1}$)				
PLA	448±37	407±37	391±40	375±40
ALA	330±37	303±37	370±42	337±41
Vitamin C ($mg \cdot d^{-1}$)				
PLA	143±16	138±14	139±18	129±15
ALA	99±16	91±13	116±19	117±16
Vitamin D (I.U.)				
PLA	181±35	170±30	158±38	128±32
ALA	158±35	173±30	229±40	156±34
Vitamin E ($mg \cdot d^{-1}$)				
PLA	11.6±1.3	10.4±1.0	10.8±1.3	10.5±1.1
ALA	8.3±1.2	7.6±1.0	10.8±1.3	10.0±1.1
Zinc ($mg \cdot d^{-1}$)				

PLA	13.4±1.3	13.0±1.5	11.3±1.4	10.8±1.6
ALA	11.0±1.3	10.4±1.5	11.5±1.5	10.2±1.7
Iron ($mg \cdot d^{-1}$)				
PLA	15.9±1.3	14.6±1.3	12.7±1.5	11.8±1.4
ALA	13.3±1.3	12.5±1.3	12.5±1.5	11.9±1.5

Data are means ± SE. ALA, alpha-linolenic acid. † Significant main effect of time ($p < 0.05$).

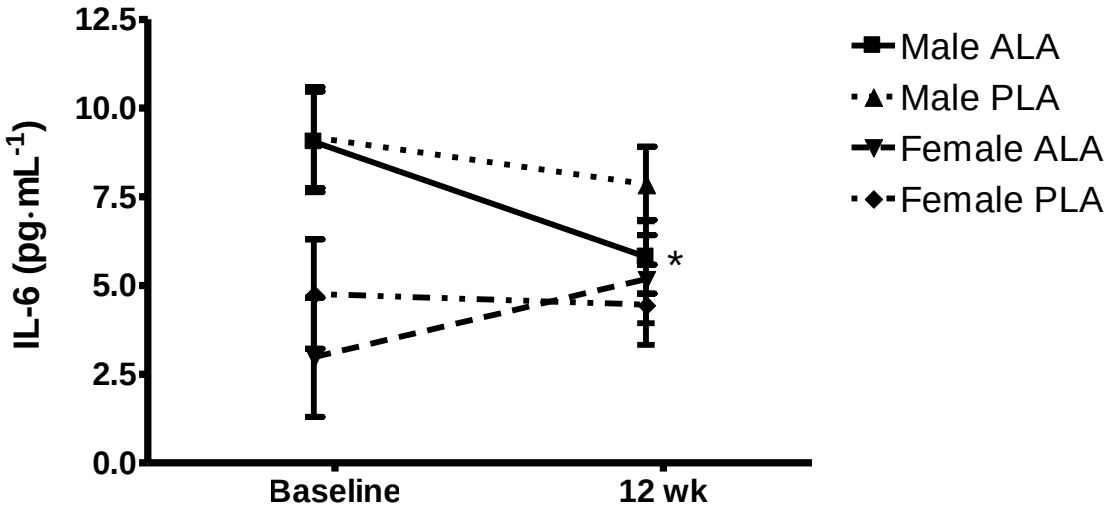

Figure 3.1 IL-6 concentration at baseline and 12 weeks of oral supplementation with flaxseed oil (ALA; 30 ml·d^{-1}) or corn oil (PLA; 30 ml·d^{-1}) in older adults completing a strength training program. * Male ALA value at 12 wk significantly lower than baseline ($p < 0.01$). Values are group means ± standard error.

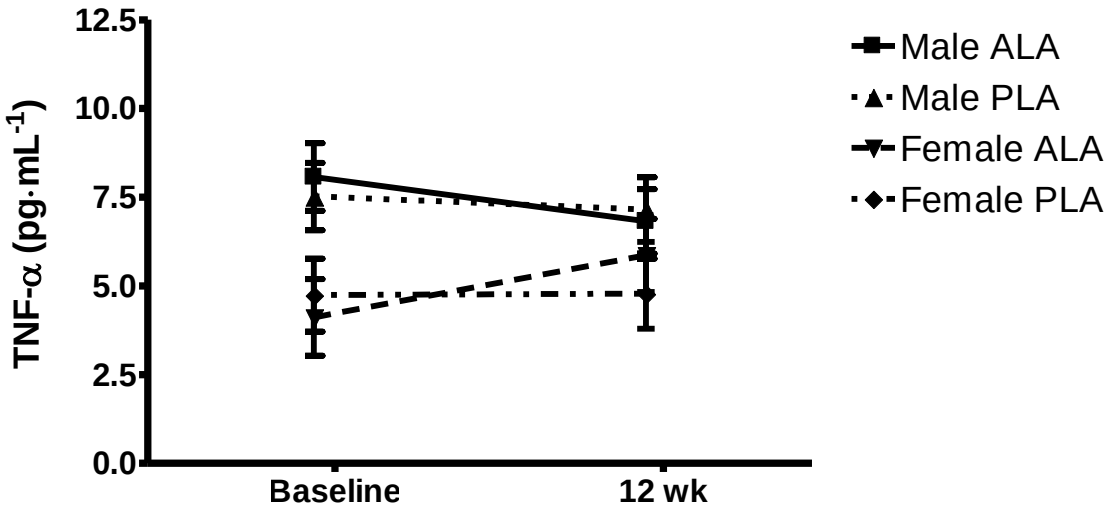

Figure 3.2 TNF-α concentration at baseline and 12 weeks of oral supplementation with flaxseed oil (ALA; 30 ml·d^{-1}) or corn oil (PLA; 30 ml·d^{-1}) in older adults completing a strength training program. Values are group means ± standard error.

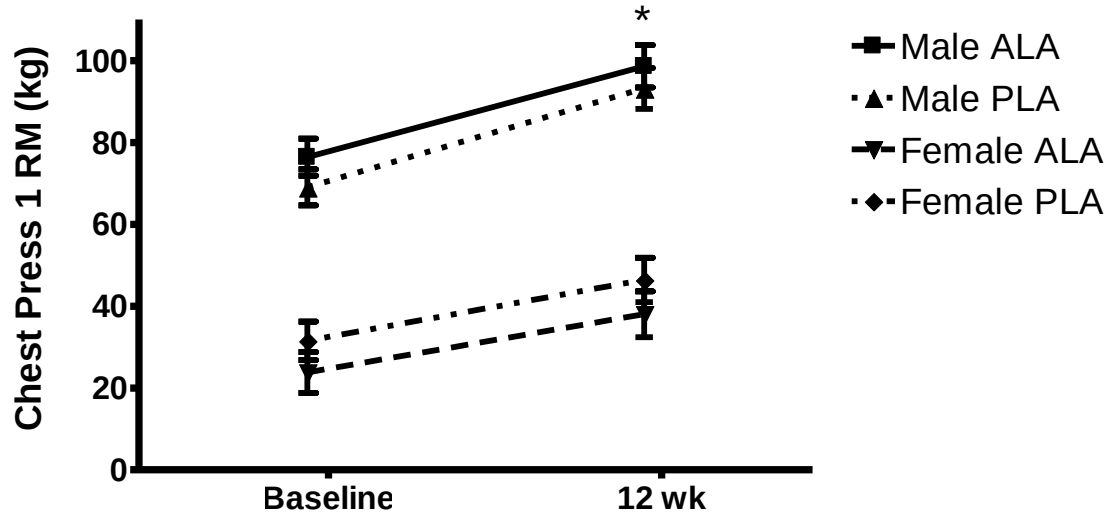

Figure 3.3 Chest press 1 repetition maximum at baseline and 12 weeks of oral supplementation with flaxseed oil (ALA; 30 ml·d^{-1}) or corn oil (PLA; 30 ml·d^{-1}) in older adults completing a strength training program. * Significant time × sex interaction, females increased less than males ($p < 0.01$). Values are group means ± standard error.

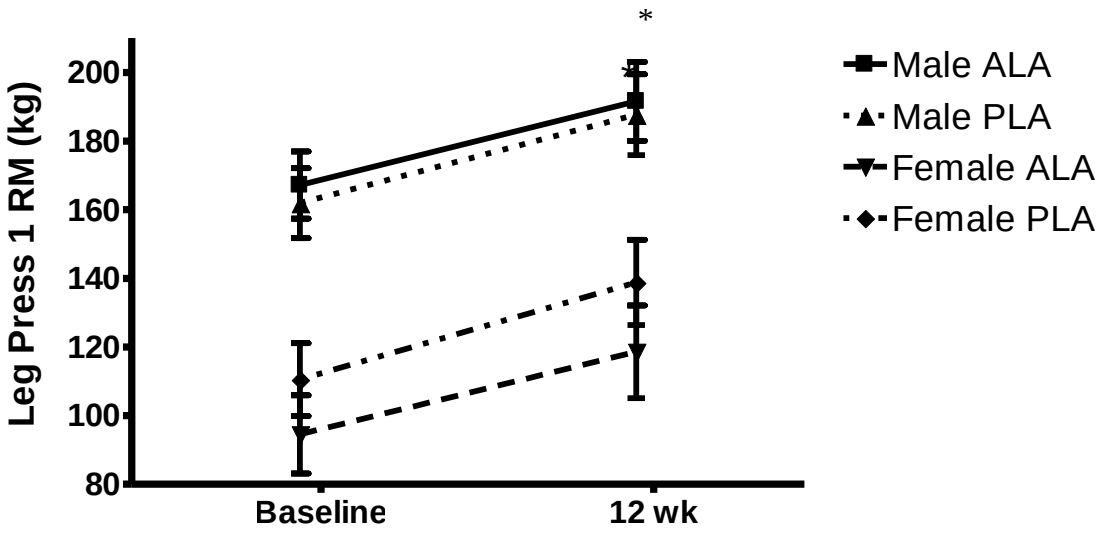

Figure 3.4 Leg press 1 repetition maximum at baseline and 12 weeks of oral supplementation with flaxseed oil (ALA; 30 ml·d^{-1}) or corn oil (PLA; 30 ml·d^{-1}) in older adults completing a strength training program. *Significant time main effect, increasing strength in whole group ($p < 0.01$). Values are group means ± standard error.

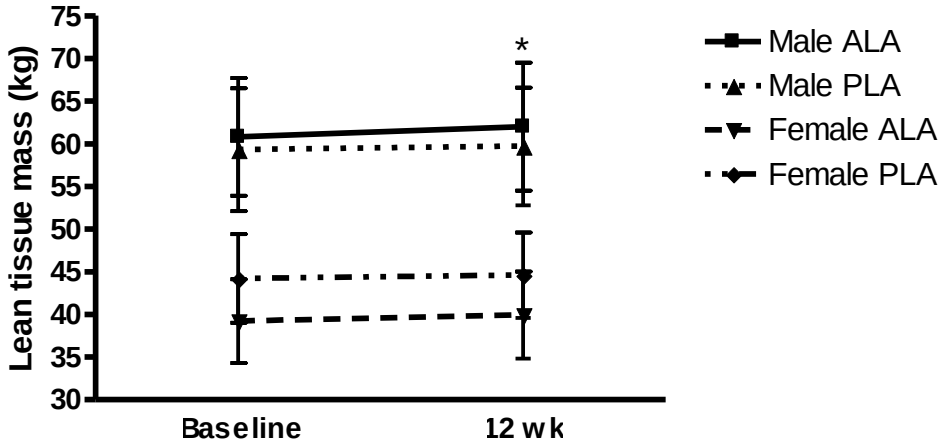

Figure 3.5 Lean tissue mass at baseline and 12 weeks of oral supplementation with flaxseed oil (ALA; 30 ml·d^{-1}) or corn oil (PLA; 30 ml·d^{-1}) in older adults completing a strength training program. * Significant time main effect, increasing lean tissue mass in whole group ($p < 0.05$). Values are group means ± standard error.

Table 3.3. Muscle thickness measurements for the flexor and extensor muscles surrounding the elbow and knee joints of older adults randomized to supplement orally with either flaxseed oil (ALA; 30 ml·d^{-1}) or corn oil (PLA; 30 ml·d^{-1}) for 12 weeks while completing a strength training exercise program (n = 51).

	Male		Female	
	(n = 14 PLA; n = 14 ALA)		(n = 12 PLA; n = 11 ALA)	
	Baseline	12 week	Baseline	12 week
Elbow flexors (cm) †				
PLA	3.5 ± 0.2	3.7 ± 0.1	3.6 ± 0.3	3.8 ± 0.3
ALA	3.6 ± 0.1	4.0 ± 0.2	2.9 ± 0.2	3.2 ± 0.2
Elbow extensors (cm) †				
PLA	4.4 ± 0.3	4.5 ± 0.3	3.5 ± 0.4	4.6 ± 0.4
ALA	4.4 ± 0.3	5.2 ± 0.3	3.7 ± 0.4	4.6 ± 0.2
Knee flexors (cm)				
PLA	5.1 ± 0.4	5.3 ± 0.5	4.5 ± 0.5	5.8 ± 0.5*
ALA	5.2 ± 0.4	6.1 ± 0.3*	5.1 ± 0.5	5.1 ± 0.5
Knee extensors (cm) †				
PLA	3.6 ± 0.2	3.9 ± 0.2	3.6 ± 0.3	3.9 ± 0.1
ALA	3.4 ± 0.1	3.4 ± 0.1	3.5 ± 0.2	4.0 ± 0.2

Data are means ± SE. PLA (placebo); ALA (alpha-linolenic acid). *Group × sex × time, females on placebo and males on ALA increased over time with no changes in the other groups ($p \leq 0.05$). †Significant main effect of time ($p < 0.01$).

3.3.6 Adverse Events

Three subjects in the placebo group reported adverse events that were deemed related to the supplement which included upset stomach/nausea (n = 1) and diarrhea (n = 2). Two subjects experienced adverse events that resulted in a reduced resistance training volume. One subject in the placebo group was diagnosed as having a first degree sprain in the left ACL ligament due to pre-existing joint laxity and 'over-exertion' on the knee flexion machine and one subject in the ALA group fractured her patella in an accident unrelated to the study intervention. These two subjects did not complete the leg press 1-RM post-testing due to these injuries and were unable to complete all the lower body exercises in the exercise program.

3.4 Discussion

It was hypothesized that ALA supplementation would reduce inflammation and that this would be beneficial for increasing muscle mass and strength. The results of this study indicate that older men supplementing with ALA for 12 weeks decreased the concentration of interleukin-6 with no effect in women, but this did not appear to have beneficial effects on muscle mass or strength. ALA acts as a substrate for the longer chain fatty acids EPA and DHA and it has been shown that women convert higher amounts of ALA to the longer chain fatty acids because the conversion is mediated by estrogen (Burdge 2006). As the women recruited in this study were all post-menopausal, the conversion of ALA to the longer chain fatty acids may have been inhibited due to the decrease in the estrogen production after menopause (Ottosson et al. 1984; Silfverstolpe et al. 1981; Stark et al. 2003). Earlier data has indicated that estrogen intake during menopause stimulates delta-6 desaturase and inhibits delta-5 desaturase activity which would result in an increased conversion of ALA to EPA but a reduction in DHA (Stark et al.

2003). Further, research in lactating women, who have increased estrogen concentrations, indicated that EPA was significantly increased while DHA was not in the plasma (Francois et al. 2003). These observations indicate that estrogen has a mediating role in the conversion of ALA to the longer chain fatty acids and that post-menopausal women may therefore have a different response to ALA supplementation. Previous research evaluating the effects of either dietary supplementation or dietary modification to include more ALA in humans has shown a decrease (Caughey et al. 1996; Mantzioris et al. 2000; Nelson and Hickey 2004) or no change (Kew et al. 2003; Wallace et al. 2003) in various pro-inflammatory cytokines including IL-6, TNF-α, IL-1β, and the acute phase protein C-reactive protein. The changes in TNF-α concentration mirrored the same pattern as IL-6 but did not reach statistical significance for differences between groups. It is well established that TNF-α is responsible for the release of IL-6 and that the two cytokines are highly correlated (Pedersen et al. 2000). Likely, this study lacked statistical power to detect significant differences between ALA and placebo for TNF-α. Nevertheless, these results seem to further substantiate the anti-inflammatory effect of ALA in older men but not in older women.

The results indicate that progressive resistance training is effective for increasing muscle thickness, muscle strength, and lean tissue mass in older adults. The addition of ALA to the strength training program resulted in a significantly greater increase in knee flexor muscle thickness in men but not women. There was no greater improvement in the other muscle thickness measures, lean tissue mass, or muscle strength with ALA. Interventions that decrease the amount of pro-inflammatory cytokines such as TNF-α and IL-6 may be effective at decreasing the amount of myocyte apoptosis in older adults thus preserving muscle mass and strength into older age (Dirks and Leeuwenburgh 2006; Pistilli et al. 2006). It has been suggested that older adults are at increased risk of sarcopenia due to the hyper-inflammatory

state associated with aging and increased concentrations of TNF-α will act as a catabolic agent for death-cell receptors on myocytes signaling an increase in apoptosis (Dirks and Leeuwenburgh 2006) and a decrease in total skeletal muscle mass and consequently strength (Pistilli et al. 2006). The ALA only resulted in increased muscle thickness of the knee flexors in the older men, with no greater increase in other measures of muscle thickness, muscle strength or lean tissue mass compared to placebo. This indicates that, even though ALA supplementation seemed to be beneficial for lowering IL-6 concentration, ALA resulted in only minimal changes in muscle mass with no changes in strength. Although there were no statistical differences between groups in protein intake, the men consuming ALA consumed, on average, a lower amount of protein in their diets than the current RDA (~0.76 $g \cdot d^{-1}$). This may have influenced the results by putting these men at a disadvantage for inducing skeletal muscle hypertrophy as protein consumption beyond the RDA is usually recommended (Andrews et al. 2006; Campbell and Geik 2004).

Unexpectedly, the women in the placebo group increased knee flexor muscle thickness while the women in the ALA group did not. This conflicts with the study hypothesis. Muscle regeneration has been inhibited in animal models utilizing non-steroidal anti-inflammatory medication which inhibits cyclooxygenase activity and decreases the regenerative ability of muscle (Bondesen et al. 2004; Bondesen et al. 2006). Also, ibuprofen (1200 $mg \cdot d^{-1}$) administration to young men resulted in a blunted postexercise fractional synthesis rate of skeletal muscle and decreased prostaglandin $F_{2\alpha}$, indicating a decrease in muscle protein synthesis, while having no effect on prostaglandin E_2, which if up-regulated would result in increased skeletal muscle degradation (Trappe et al. 2001; Trappe et al. 2002). This research also suggested that there was no change in inflammatory cell response to skeletal muscle tissue

injury with or without ibuprofen ingestion (Peterson et al. 2003). This proposes that cyclooxygenase (COX) inhibition may be deleterious to muscle hypertrophy. Specifically, it seems that inhibition of the COX-2 isoform results in the greatest decrease in muscle regenerative ability (Bondesen et al. 2004; Bondesen et al. 2006; Mendias et al. 2004). Recently, ALA has been found to act on COX-2 in a similar manner which could explain the results of an increase in knee flexor thickness in the female placebo group and no increase in the female ALA group but does not provide evidence for the effect observed on knee flexor thickness in men on ALA in this study (Ren and Chung 2007; Ren et al. 2007).

Cross-sectional research in older adults has indicated that physical activity is anti-inflammatory in nature (Colbert et al. 2004; McFarlin et al. 2006) which may decrease the risk of chronic health conditions (Bruunsgaard and Pedersen 2003). Relatively little prospective research has been performed in older adults to evaluate the effects of progressive resistance training exercise on markers of inflammation. Twelve weeks of progressive resistance training was found to have no effect on markers of inflammation in older healthy and frail elderly subjects (Bruunsgaard et al. 2004; Rall et al. 1996) but a 6 week intensive resistance training program was able to decrease heat shock protein 70 and eliminate the decrease in IL-10 (an anti-inflammatory cytokine) after an acute bout of exercise in older adults (Bautmans et al. 2005). Our results would suggest that resistance training is unable to alter inflammatory markers; however, our study is limited because it did not include a non-training control group.

In conclusion, the results of this study suggest that ALA supplementation for 12 weeks reduces the plasma concentration of IL-6 in older men but not older women while progressive resistance training remains an effective method for reducing the risk of sarcopenia in older adults. High doses of ALA may reduce skeletal muscle regenerative ability by inhibiting the

COX-2 enzyme but more research on the mechanistic differences between men and women ingesting ALA is warranted.

Chapter 4: General Discussion

4.1 Summary of Major Findings

The constituents of flaxseed used in these experiments seem to exert some favorable health effects in older men while having no effect in older women when all subjects were completing standardized exercise programs. The change in hormonal status experienced by older women (i.e. post-menopause) may moderate the effects of supplementing diets with components of flaxseed. Decreasing estrogen (estrone, estradiol, estrione) concentrations may influence the effectiveness of flaxseed supplementation on inflammation in older women.

The results of Experiment I indicate that a composite score of metabolic syndrome may be attenuated in older men consuming a flaxseed lignan complex (containing SDG) supplement during 6 months of cardiovascular exercise training with no effect on the concentration of the cytokines IL-6 and TNF-α. Further, the attenuation of the metabolic syndrome score was accompanied by a decrease in TAG and diastolic blood pressure in the men consuming the flaxseed lignan complex supplement. The flaxseed lignan complex supplement appears to have no effect on metabolic syndrome or cytokines in older women. This experiment is unique as no other study to date has evaluated the effects of flaxseed lignan complex supplementation on blood lipids, metabolic syndrome, leukocytes, and inflammatory cytokines in older men and women completing standardized cardiovascular exercise. Further, this experiment in humans is the longest intervention, to date, evaluating the cardiovascular health effects of combined cardiovascular exercise and flaxseed lignan complex supplementation.

Experiment II indicates that older men supplementing with flaxseed oil (ALA) during 12 weeks of strength training have a significant decrease in the concentration of IL-6 but not in TNF-α while there was no effect in older women. Further, the hypothesized greater increase in

lean tissue mass, muscle strength, and muscle size did not occur in the subjects supplementing their diet with ALA. Experiment II is unique to the literature as no study has evaluated the effects of ALA supplementation in older adults completing a standardized resistance training program.

4.2 Inflammation and Flaxseed

The immune system is responsible for coordinating and controlling the overall health of an organism. Components of flaxseed have exhibited some beneficial health modifying effects via their positive influence on the inflammatory response controlled by the immune system, but more knowledge in regards to safety, efficacy, and dosing with components of flaxseed is required to establish the actual influence of flaxseed on human health. Evidence from the two experiments conducted suggests that components of flaxseed may exert some positive health effects, beyond the beneficial effects of exercise training, in older men but not older women.

4.2.1 Inflammation and Secoisolariciresinol Diglucoside

The data from the first experiment indicate that flaxseed lignan complex supplementation during cardiovascular exercise training has no effect on the cytokines IL-6 and TNF-α. The theory of the first experiment was that SDG would act as an anti-oxidant, by increasing the scavenging of the hydroxyl radical, which in turn would result in a decreased amount of systemic inflammation. The research is scarce on the effects of the lignan component of flaxseed on the immune system and inflammation. The effects of a flaxseed lignan complex on the hematopoietic system has been evaluated in rabbits (Prasad 2005a). This research concluded that the lignan complex had no adverse effects on the counts of leukocytes, granulocytes,

lymphocytes, or monocytes in healthy or hyper-cholesterolaemic rabbits. The results of the first experiment confirmed this in humans (see Tables 2.3 & 2.4). Likely, the health benefits associated with flaxseed lignan consumption are through another mechanism. SDG seems to possess good potential for modifying immune function as it possesses anti-carcinogenic effects in some hormone and non-hormonal cancers (Bergman Jungestrom et al. 2007; Li et al. 1999; Serraino and Thompson 1991; Sung et al. 1998; Thompson et al. 2005) but the mechanism(s) remain poorly understood. It has been speculated that the anti-oxidant ability of SDG may be responsible for decreasing development of cancer but the anti-estrogenic effects of SDG may also be involved (Bergman Jungestrom et al. 2007). Other types of phytoestrogens affect immune system function and markers of inflammation. In humans, studies with soy isoflavones are contradictory. One study indicated that IL-6 was increased in postmenopausal women, with no effect on men (Jenkins et al. 2002), while a second study indicated IL-6 and TNF-α in the blood of postmenopausal women were decreased with soy isoflavone (phytoestrogen) supplementation (Huang et al. 2005). Monocytes cultured with genistein and daidzein (the two main phytoestrogens from soy) from the women in this study were subjected to endotoxin and exhibited an increased response of TNF-α. Recently, *in vitro* analysis indicated that NFκB is blocked by isoflavones independent of estrogen activity (Vanden Berghe et al. 2006) suggesting that isoflavones have effects on this inflammatory transcription factor via a mechanism other than cross-talk with estrogen receptors. A study evaluating the effects of flaxseed lignan complex on health was done in post-menopausal women (Hallund et al. 2006a; Hallund et al. 2006b). Supplementation for 6 weeks had no effect on anti-oxidant capacity, blood lipids or endothelial function. The study is limited due to a small sample size, a short duration of supplementation, and it only included women in the sample. Another study evaluated the effects

of flaxseed lignan complex supplementation in type II diabetic patients over 12 weeks and found no effect on blood lipids but improved glucose control as measured by HbA_{1c} (Pan et al. 2007). These results are limited in interpretation as the majority of the subjects were taking oral hypoglycemic agents, antihypertensives or hypolipidemics. Efficacy in lowering LDL, TC, and glucose has been demonstrated for flaxseed lignan supplementation in hypercholesterolaemic subjects (Zhang et al. 2007). It is premature to speculate on all the effects that phytoestrogens have on immune system function and inflammation. More research in this area, especially with flaxseed lignan, is required. The results of Experiment I indicate that oral supplementation with a flaxseed lignan complex during 6 months of cardiovascular exercise training had no significant effect on IL-6, TNF-α or leukocyte cell number.

As Experiment I was done over different seasons, the effects that the environmental conditions during varying times of the year have on cytokine concentrations is of interest. A substantial amount of evidence from Antarctic exploration indicates that cold exposure changes cytokine concentrations and immune function (Francis et al. 2002; Muller et al. 1995; Shearer et al. 2002; Shirai et al. 2003; Tingate et al. 1997; Tringali et al. 2000). Exposure to Antarctic isolation suppresses anti-inflammatory cytokines and stimulates pro-inflammatory cytokines (Shearer et al. 2002). Further, cold exposure combined with strenuous exercise may combine to produce an increased suppression of immune function (Shephard 1998; Shephard and Shek 1998). It is theorized that cold exposure promotes a hypothalamic-pituitary-adrenal stress response which influences the immune response (Shephard 1998; Shephard and Shek 1998). It is unlikely that subjects involved in this study were exposed to the same extremes in environment as an Antarctic winter or that they participated in exercise strenuous enough to

cause immunosuppression. Thus, it is unlikely that there was an effect of season on cytokine concentrations in this group of older adults.

A seasonal effect on blood lipid concentrations is not a new phenomenon (Gordon et al. 1987). HDL is higher and TC:HDL lower in warmer compared to colder months of the year. Also, LDL and TAG have been shown to be elevated in colder months in the elderly (Woodhouse et al. 1993). The majority of subjects in the current study started their intervention in a warmer month and finished in a colder month. The change in blood lipids with season may be explained by changes in plasma volume (Frohlich et al. 1997). Plasma volume is expanded in summer months to aid with thermoregulation and possibly due to increased physical activity levels, and this could dilute lipid concentrations (Ockene et al. 2004). Experiment I is limited in its interpretation as the time of year was not controlled during the intervention. The confounding factor of season was controlled for in Experiment I by randomly assigning subjects to the treatment groups.

4.2.2 Inflammation and Alpha-Linolenic Acid

The results of the Experiment II indicate that older men supplementing with ALA during 12 weeks of strength training decreased the concentration of interleukin-6 with no effect in older women. Also, this experiment indicated that TNF-α was modified in a similar pattern as IL-6 although the results were not significant. As supplementation with ALA did not result in a significant decrease in the cytokines measured in older women it is possible that the conversion of ALA to the longer ω-3 fatty acids was inhibited due to the decrease in the estrogen production after menopause (Burdge 2006). Data indicates that delta-6 desaturase activity (i.e. the enzyme responsible for the conversion of ALA to EPA) is increased in postmenopausal women on

hormone replacement therapy (Stark et al. 2003) and lactating women (Francois et al. 2003). This evidence suggests that postmenopausal women not on hormone replacement therapy would have decreased amounts of ALA conversion to EPA due to decreased estrogen concentrations. Earlier research with ALA in humans has shown a decrease (Caughey et al. 1996; Mantzioris et al. 2000; Nelson and Hickey 2004) or no change (Kew et al. 2003; Thies et al. 2001a; Wallace et al. 2003) in pro-inflammatory cytokines including IL-6, TNF-α, IL-1β, and the acute phase protein C-reactive protein. There are a few methodological differences between the studies to explain varying results. The dose of ALA given in each study varied substantially. In the studies indicating a decrease in inflammatory markers a minimum dose of 9 $g \cdot d^{-1}$ (range 9.0 – 13.7 $g \cdot d^{-1}$) was used, while the studies indicating no effect used doses ranging from 2.0 to 9.5 $g \cdot d^{-1}$. A recent review on ALA metabolism to the longer chain fatty acids concluded that ALA conversion to EPA is approximately 5% while its conversion to DHA is never more than 1% (Plourde and Cunnane 2007). Subsequently, studies evaluating the anti-inflammatory effect of ALA supplementation using smaller doses are unlikely to see an effect as conversion to the longer chain metabolites, which likely mediate the inflammatory reaction, will not be as great. Assuming that there was 5% conversion of ALA to EPA, our study would result in an increase of approximately 700 mg of EPA per day. Recent reviews suggest that EPA and DHA fatty acids have positive beneficial effects with doses less than 1 $g \cdot d^{-1}$ on risk factors for cardiovascular and inflammatory disease (Breslow 2006; Ergas et al. 2002). Further there may be a sex effect with ALA supplementation. All of the studies that showed a decrease in inflammatory markers were completed in men (Caughey et al. 1996; Mantzioris et al. 2000; Nelson and Hickey 2004) while two of the studies indicating no effect of ALA supplementation on inflammatory markers were done in mixed populations of women and men (Kew et al. 2003; Thies et al. 2001a) and the

statistical analyses used did not account for a sex effect. Thus, there may have been a sex difference present in these studies, but it could not be determined with the analysis utilized. One of these studies (Kew et al. 2003) evaluated a sample population with a large age range (25-72 years) and did not determine whether there was a beneficial effect on inflammation in relation to different age groups. Older adults are at an increased risk of inflammation while healthy younger adults are not likely "hyper-inflamed", unless there is underlying pathology. The results from Experiment II suggest that IL-6 and possibly TNF-α are decreased in older men but not older women supplementing with ALA during 12 weeks of strength training exercise.

4.3 Other Mechanisms

The first experiment indicated that flaxseed lignan complex supplementation during cardiovascular exercise has no effect on IL-6, TNF-α, or leukocyte cell count but may have an effect on metabolic syndrome risk factors, mainly by lowering TAG concentrations in older men. Previous *in vitro* research in macrophages has indicated that savinin, another type of lignan, is able to reduce TNF-α formation and T-cell propagation (Cho et al. 2001). Thus, although Experiment I did not demonstrate efficacy in lowering markers of inflammation, there seems to remain potential for phytoestrogens to impact immune system function. The most possible mechanisms for control of metabolic syndrome by phytoestrogens seem to be the ligand binding activity of various phytoestrogens to nuclear receptors involved in fat and glucose metabolism (PPARs) (Kwon et al. 2006; Ricketts et al. 2005; Shen et al. 2006), while the effects of phytoestrogens on inflammation or immune system function are most likely regulated through NFκB (Dijsselbloem et al. 2004; Evans et al. 2001; Garcia Palacios et al. 2005; Harnish et al. 2000; Kang et al. 2005; Vanden Berghe et al. 2006). Interestingly, some *in vitro* research has

demonstrated reciprocal cross-talk between PPARγ and NFκB suggesting a novel pathway for the reduction of metabolic syndrome due to exacerbation of inflammation (Takada et al. 2005).

The results of Experiment I did demonstrate an attenuation of metabolic syndrome risk along with a decrease in TAG concentration in older men. Research on other phytoestrogens has indicated that the favorable modification of blood lipids and cholesterol is due to the ligand binding ability of phytoestrogens to perioxisome proliferator-activated receptors (PPAR). Up-regulation of PPARα and PPARγ, which would result in increased fatty acid β-oxidation via increased enzyme activity and enhanced glucose control, has been demonstrated with isoflavones *in vitro* (Kwon et al. 2006; Shen et al. 2006). A recent human study in hypercholesterolaemic subjects indicated that 8 weeks of supplementation with flaxseed lignan complex lowered TC, LDL, and fasting glucose to a greater extent than placebo (Zhang et al. 2007). Thus, flaxseed lignan complex may be effective in altering blood lipids in those with an increased risk of cardiovascular disease while having minimal effect in those without health risk due to abnormal blood lipids. Likely, the effects observed in Experiment I on older men were mediated by one of the aforementioned mechanisms. No direct measure of mRNA or protein content of the PPARs was done in this study so this mechanism remains speculative.

Experiment II demonstrated that ALA supplementation was effective at lowering IL-6 concentration in older men. The conversion of ALA to the longer chain fatty acids may be but one mechanism whereby ALA exerts its anti-inflammatory effects. Recent *in vitro* and animal data indicate ALA is effective at inhibiting gene expression of iNOS, COX-2, and TNF-α by blocking NFκB and mitogen activated protein kinases (Ren and Chung 2007; Ren et al. 2007). Thus, although the metabolic conversion of ALA to EPA and DHA may be one mechanism whereby ALA is anti-inflammatory, ALA may have direct effects via other mechanisms

resulting in a decreased amount of inflammation. More research should be completed to establish the mechanism(s) by which ALA reduces inflammation. Also, more randomized controlled trials evaluating ALA effects on inflammation in various ages, genders, and diseased conditions is warranted. In agreement with the above research, the results of the second experiment suggest that ALA supplementation during strength training reduces IL-6 in older men but has no effect in women.

4.4 Limitations

Although the research conducted in Experiment I and II was done in a randomized, controlled fashion there are limitations associated with the studies. Each experiment was lacking a non-exercise control group. The sample reflects the attributes of largely urban, socio-economically middle class, healthy, Caucasian older adults and thus, would only be generalizable to this demographic. Also, the participants recruited for the studies were highly motivated to exert health change or improvement by the dietary and exercise protocols used in the research. Thus, the results may only be generalizable to older individuals who are health conscious. The food frequency questionnaires utilized reflect self-reported data of typical dietary habits which may under or over-predict characteristic nutrient intake. In the second experiment subject motivation could have influenced the 1-RM strength testing. Also, a stress response could be associated with drawing blood in some individuals which might affect the cytokine concentration.

Another limitation of this thesis is that the intensity of exercise prescribed between the two studies was different. In the first experiment, intensity would be considered light to moderate while in the second experiment, intensity would be considered moderate to vigorous.

Nieman (Nieman 2003) has suggested that there is a J-curve relationship between exercise intensity and immune function. This theory states that sedentary individuals may be at an increased risk of immune function abnormalities as opposed to those who are moderately active. Also, the theory states that heavy physical exertion, such as that type of exercise observed in highly trained athletes (over-reaching, over-training), will reduce immune system function and could leave an individual immuno-compromised. Although the intensity of exercise was different between the two studies in this thesis, it is unlikely that the intensity of exercise was high enough in either study to promote immunosuppression. The difference between low and moderate intensity exercise in terms of immune function is not known, especially in older exercising adults. The current recommendations for health benefits associated with physical activity in older adults include accumulating at least 30 minutes of moderate intensity activity on most days of the week or a volume of exercise corresponding to 1000 $kcal \cdot wk^{-1}$ of energy expenditure (1998; Nelson et al. 2007; Paterson et al. 2007). Nieman (Nieman 2003) argues that this dose of exercise has health benefits and that it is likely effective for improving immune function. The strategy employed in Experiment I was to prescribe enough exercise to elicit a minimum of 1000 kilocalories of energy expenditure for each week of the study intervention. Although there is evidence to support the J-curve theory, especially in highly trained athletes, there is a shortage of clinical evidence suggesting that moderate intensity activity is able to enhance immune function. An analysis of the scientific evidence supporting the positive role of moderate intensity exercise in reducing inflammation is only beginning to emerge. More long-term clinical and basic research is needed to establish how the type, intensity, duration, and frequency of exercise will influence inflammation, especially in populations that are already immunocompromised such as the elderly.

4.5 Future Research

Some, (de Kleijn et al. 2002; Kreijkamp-Kaspers et al. 2004), but not all (van der Schouw et al. 2005) epidemiological data suggests a positive association between dietary phytoestrogen consumption and risk factors for metabolic syndrome and cardiovascular disease in post-menopausal women. Also, phytoestrogen supplementation has been found to alter markers of inflammation in prospective research (Huang et al. 2005; Jenkins et al. 2002). The current research shows potential for the effectiveness of phytoestrogens to exert positive health effects in older men. Longer, prospective randomized trials utilizing various types of phytoestrogens are needed to elucidate which type(s) is/are most valuable for improving the health of older adults. However, more research on the possible negative health effects of high amounts of phytoestrogen consumption is warranted as some data indicates negative effects on the immune system in animal models (Selvaraj et al. 2005; Yellayi et al. 2002; Yellayi et al. 2003). Finally, it is suggested that further basic research on the mechanisms involved in the beneficial or detrimental effects of phytoestrogens on health be intensified to determine the usefulness of phytoestrogens in health maintenance.

Higher intakes of ALA in the diet are consistently associated with lower amounts of inflammatory mediators in epidemiological data (Ferrucci et al. 2006; Lopez-Garcia et al. 2004; Yoneyama et al. 2007). Prospective interventional research on ALA is limited and demonstrates conflicting results in relation to ALA's effectiveness in lowering inflammatory markers. Data from Experiment II suggests ALA may have a negative effect on muscle, especially in older women. Further research evaluating the effects of ALA supplementation on skeletal muscle is needed. Different inflammatory modification mechanisms may be at work with ALA supplementation in older men versus women; this problem needs to be addressed more fully.

Age differences with respect to ALA supplementation should also be addressed. Although Dietary Reference Intake values are developed for essential polyunsaturated fatty acids, the establishment of an appropriate dietary balance between ω-3 and ω-6 fatty acids requires further investigation especially in specific groups (inflammatory conditions, aging, and frailty). Ultimately, the issue of dietary ratio between essential fatty acids is not a new research debate, but it is a problem that requires intensive investigation to establish the necessary equilibrium between these essential nutrients for optimal health.

4.6 General Conclusion

The results of these two experiments suggest that the components of flaxseed used as supplements were effective at alleviating some of the risk factors associated with chronic disease in older men. It is unknown why flaxseed did not have a similar effect in older women. It is suggested that older men ingest flaxseed or its components to produce favorable effects in reducing disease risk. Older women should not follow the same recommendation until more research is done to determine the underlying mechanism whereby flaxseed may be ineffective in reducing disease risk in older women. From this research, one can conclude that the lignan component of flaxseed will attenuate an increase in metabolic syndrome risk observed with aging and that the oil component of flaxseed will reduce inflammatory markers in older men. Further, it can be concluded that the lignan component has no effect on inflammation whereas the oil component is effective at reducing inflammation. Those older male individuals interested in reducing inflammation should choose flaxseed oil as an effective means of doing so. Those older female individuals interested in supplementing with flaxseed components would be prudent to refrain from doing so as the lignan or oil from flaxseed did not demonstrate efficacy in this

research and thus, represents an unnecessary cost for older females interested in nutritional supplementation for improving health.

Although there was no true control group to compare results to in this research, the evidence from the placebo groups in both experiments suggest that cardiovascular exercise training or resistance training in older adults was not effective for reducing inflammation as assessed by inflammatory cytokines. This is in contrast to previous research which suggests that exercise may be effective in lowering inflammation in older adults. Although speculative, it may be that the intensity or volume of exercise was either not enough or too much to elicit an anti-inflammatory effect. Further research on the mechanisms and clinical evidence for or against the anti-inflammatory nature of exercise in older adults is warranted.

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Glossary

Term	Explanation
Acute phase protein	Early inflammatory proteins made by hepatocytes in response to IL-6, TNF and IL-1β. Acute phase proteins (particularly C-reactive protein) bind to cell wall components of microbes and activate complement.
Aglycone	Is the noncarbohydrate group (as a phenol or alcohol) combined with the sugar portion of a glycoside.
Antibody	Secreted immunoglobulin that is produced by B lineage plasma cells and binds to specific antigens. Able to recognize antigens that are either soluble or fixed in a tissue or on a cell surface.
Antigen	Entity (such as an element of an infectious pathogen, cancer, or inert injurious material, or of a self-tissue) that can bind to the antigen receptor of a T or B cell. This binding does not necessarily lead to lymphocyte activation.
Apoptosis	The controlled death of a cell mediated by certain intracellular proteases (including caspases) that cause the orderly breakdown of the cell nucleus and its DNA. Death occurs without the release of

	internal contents and without triggering inflammation. The intrinsic apoptotic pathway involves the release of mitochondrial cytochrome c, while the extrinsic apoptotic pathway is triggered by the engagement of death receptors such as Fas and TNFR. Apoptosis is also called "programmed cell death".
Arachidonic Acid	Arachidonic acid is a ω-6 polyunsaturated fatty acid that is present in the phospholipids (especially phosphatidylethanolamine, phosphatidylcholine and phosphatidylinositides) of membranes of the body's cells, and is highly enriched in the brain. It is a precursor in the production of eicosanoids: the prostaglandins, thromboxanes, prostacyclin and the leukotrienes (through enzymes including cyclooxygenase, lipoxygenase and peroxidase). The production of these derivatives, and their action in the body, are collectively known as the arachidonic acid cascade which affects inflammation and other cellular functions.
Basophil	Circulating granulocyte with an irregularly shaped nucleus. Contains granules that stain a dark blue color with basic dyes. Basophil granules contain heparin and vasoactive amines, as well as many enzymes capable of promoting inflammation.
B-cell	Lymphocytes that have antibody molecules on the surface and

	comprise the antibody-secreting plasma cells when mature; also known as B-lymphocyte. B-cells are distinguished phenotypically by the cell surface expression of B-cell receptors.
Bradykinin	One of a family of small peptide inflammatory mediators that circulate in inactive form in the blood until cleaved by blood clotting enzymes or enzymes released from damaged cells. These peptides cause increased vascular permeability, smooth muscle contraction, and pain.
Cachexia	Wasting of the body due to uncontrolled cellular catabolism. Cachexia is usually induced by high levels of TNF.
Chemokine	Chemotactic cytokines. Structural families include the C, CC, CXC, and CX3C subgroups. *See also chemotaxis.*
Chemotactic factors	Factors derived from either host or bacterial metabolism that can induce chemotaxis. Includes chemokines, kallikrein, platelet activating factor, thrombin, and the anaphylatoxin C5a.
Chemotaxis	The directed movement of cells along the concentration gradient of a chemotactic factor. Serves to draw neutrophils, lymphocytes, and

	other leukocytes from the circulation into an injured or infected tissue.
Cluster of differentiation (CD)	A CD number in the unique designation given to a cell surface protein identified serologically by the binding to that protein of a cluster of different antibodies. That is, the CD number points to the specific collection of serological epitopes furnished by the protein.
CD3	The CD3 antigen protein complex is composed of three distinct chains (CD3γ, CD3δ and CD3ε) in mammals, that associate with molecules known as the T cell receptor (TCR) and the ζ-chain to generate an activation signal in T lymphocytes. The TCR, ζ-chain and CD3 molecules together comprise the TCR complex.
CD4	Glycoprotein found on the surface of helper T-cells which usually functions to facilitate recognition by T cell receptors of antigens complexed with molecules of a class that are found on the surface of antigen presenting cells (as B cells and macrophages) and are the product of genes of the major histocompatibility complex class II proteins.
CD8	Glycoprotein found on the surface of cytotoxic T-cells which usually functions to facilitate recognition of cytotoxic T cell receptors of

	antigens complexed with molecules of a class that are found on the surface of most nucleated cells and are the product of genes of the major histocompatibility complex class I proteins
CD16	Alson known as Fc receptor. A transmembrane glycoprotein that is a member of the immunoglobulin superfamily which exists in two isoforms that 1) functions to mediate phagocytosis and antigen dependent cell-mediated cytotoxity and 2) does not mediate phagocytosis or antigen dependent cell-mediated cytotoxity but serves as a trap for immune complexes.
CD56	Also known as neural cell adhesion molecule. A membrane glycoprotein and member of the immunoglobulin superfamily that has various isoforms on a variety of cell lines which functions as an adhesion molecule in neuronal tissue and NK cells but its function in T-cells is not known.

Complement	A series of thirty serum and membrane proteins that are involved in humoral immunity and inflammation.
C-reactive protein	An acute phase protein released by hepatocytes into the blood in response to stimulation by TNF and IL-6 usually in response to various abnormal states including inflammation. Its release will result in activation of the complement cascade of events.
Cyclooxygenase	An enzyme that catalyzes the conversion of arachidonic acid to prostaglandins which has three isoforms (COX-1, COX-2, and COX-3) of which COX-2 is involved in the cascade of events producing pain and inflammation while COX-1 is not involved in this cascade of events. COX-1 is considered a constitutive enzyme, being found in most mammalian cells. More recently it has been shown to be upregulated in various carcinomas and to have a central role in tumorigenesis. COX-2, on the other hand, is undetectable in most normal tissues. It is an inducible enzyme, becoming abundant in activated macrophages and other cells at sites of inflammation. Both COX-1 and -2 also oxygenate two other essential fatty acids – DGLA (ω-6) and EPA (ω-3) – to give the series-1 and series-3 prostanoids, which are less inflammatory than those of series-2. DGLA and EPA are competitive inhibitors with AA for the COX pathways. This inhibition is a major mode of action in the way that dietary sources of

	DGLA and EPA (e.g. borage, fish oil) reduce inflammation.
Cytotoxicity	Having the ability to kill cells.
Dendritic cells (DCs)	Irregularly shaped phagocytic leukocytes with finger-like processes resembling the neuronal dendrites. DC subsets arise from both the myeloid and lymphoid lineages and include conventional and plasmacytoid DCs. Immature DCs in the tissues capture antigen. DCs activated by pro-inflammatory cytokines migrate to the draining lymph node and mature, upregulating expression of costimulatory molecules. Mature DCs are the only antigen presenting cell capable of activating naïve T-cells.
Eicosapentaenoic acid (EPA)	A ω-3 polyunsaturated fatty acid found especially in fish oils.
Endotoxic shock	A sometimes fatal collapse of circulatory and metabolic systems induced by an overwhelming amount of cytokines (particularly IL-1 and TNF) released into the circulation by macrophages in response to infection with gram-negative bacteria or their components (especially lipopolysaccharide). Also known as septic shock.

Eosinophils	Connective tissue granulocytes with bilobed nuclei and granules that stain reddish with acidic dyes. The granules contain highly basic proteins and enzymes effective in the killing of larger parasites. Eosinophils also play a role in allergy.
Erythrocytes	Red blood cells.
Extravasation	To exude from a vessel (such as blood or lymph) into the surrounding tissue.
Fibrin	A white insoluble fibrous protein formed from fibrinogen by the action of thrombin especially in the clotting of blood.
Fibroblasts	A connective tissue cell of mesenchymal origin that secretes proteins and especially molecular collagen from which the extracellular fibrillar matrix of connective tissue forms.
Fibrosis	The formation of excessive fibrous tissue, as in a reparative or restorative process.
Glycoprotein	A conjugated protein in which the nonprotein group is a carbohydrate. Also known as a glucoprotein.
Granulocytes	Myeloid leukocytes that harbor large intracellular granules containing

	microbe-destroying hydrolytic enzymes. Include neutrophils, basophils, and eosinophils.
Granulocyte-colony stimulating factor (G-CSF)	A colony stimulating factor produced by macrophages, endothelial cells, and fibroblasts that acts to promote the maturation of precursor cells into granulocytes.
Hematopoietic cells	Red and white blood cells.
Heparin	A glycosaminoglycan sulfuric acid ester that occurs especially in the liver and lungs, that prolongs the clotting time of blood by preventing the formation of fibrin.
Hepatocyte	Cells of the liver.
Histamine	A nitrogen containing compound in mammalian tissues that causes dilatation of capillaries, contraction of smooth muscle, and stimulation of gastric acid secretion, that is released during allergic reactions, and that is formed by the decarboxylation of histidine.
5-Hydroxy Tryptamine	Serotonin. A monoamine neurotransmitter synthesized in serotonergic neurons in the central nervous system (CNS) and enterochromaffin cells in the gastrointestinal tract.

Interferons (IFN)	Family of cytokines produced by cells upon infection by virus. Type I (IFN α, β, and ω) are important in inhibiting proliferation and establishing an antiviral state in bystander cells. Type II IFN (IFNγ) has multiple immunoregulatory and pro-inflammatory effects in addition to its anti-proliferative and anti-viral activities, and is essential for a hyperactivated macrophage.
IL-1ra	Interleukin-1 receptor antagonist; will bind to IL-1 receptors but will not produce an effect thus reducing the ability of IL-1 to exert an effect.
Ig isotype switching	During the initial stages of a B-cell's primary response to antigen, it produces and secretes IgM. Later in the primary response of during subsequent responses, different heavy chain isotypes may be expressed by the progeny of the original IgM-producing clone. Such "switching" occurs at the DNA level, resulting in the production of an Ig protein with the capability of producing antibodies of the same specificity but different isotypes.
Immuoglobulin (Ig)	Y-shaped antigen binding protein expressed by B lineage cells. An Ig monomer is composed of two identical light and two identical heavy chains. In its plasma membrane-bound form, an Ig is the

	antigen-binding component of the B-cell receptor complex. In its secreted form, an Ig is an antibody. Igs were originally named for both their involvement in immunity and their presence in the gamma globulin fraction of plasma proteins.
Immunosenescence	The process whereby the immune system diminishes its functional ability as a result of the aging process or some other immunological/inflammatory disease.
Inducible nitric oxide synthase (iNOS)	Enzyme induced mainly in phagocytes by the presence of microbial products or pro-inflammatory cytokines. Converts arginine to citrulline and nitric oxide, which is toxic to endocytosed cells.

Intercellular adhesion molecule (ICAM)	Molecules that promote adhesion between cells. There are several different forms of ICAM. ICAM-1 is continuously present in low concentrations in the membranes of leukocytes and endothelial cells. Upon cytokine stimulation, the concentrations greatly increase. ICAM-1 can be induced by IL-1 and TNFα and is expressed by the vascular endothelium, macrophages and lymphocytes.
Kupffer cells	Macrophage-like cells in liver sinusoids.
Leukocytes	White blood cells, including lymphocytes, granulocytes, monocytes, macrophages, NK, and NKT cells.
Leukotrienes	Lipid inflammatory mediators whose formation is initiated when phospholipids in the membranes of macrophages, monocytes, neutrophils, and mast cells are degraded and converted to arachidonic acid. Metabolism of arachidonic acid via the lipoxygenase pathway then produces leukotrienes.
Lignan	A chemical compound found in plants. Lignans are one of the two major classes of phytoestrogens, which are antioxidants found in a variety of plants which includes flaxseeds, pumpkin seeds, sesame seeds, rye, soybeans, broccoli, beans, and some berries. The other class of phytoestrogens is the isoflavones. Plant lignans are

	polyphenolic substances derived from phenylalanine via dimerization of substituted cinnamic alcohols to a dibenzylbutane skeleton.
Lipopolysaccharide (LPS)	Component of gram-negative bacteria bacterial cell walls that generates endotoxin and induces endotoxic shock. Will bind to toll like receptor-4.
Lipoxygenase	Are iron-containing enzymes that catalyse the dioxygenation of polyunsaturated fatty acids. 5-lipoxygenase (5-LO), which transforms EFAs into leukotrienes. Substrates and leukotriene products of 5-LO include: 1) Arachidonic acid yields the 4-series leukotrienes (LTB4, LTC4, LTD4, LTE4 — generally proinflammatory); 2) Eicosapentaenoic acid yields the 5-series (LTB5, LTC5, LTD5, LTE5 — antiinflammatory); 3) Gamma-linolenic acid yields the 3-series via the DGLA intermediary. 5-LO catalyzes oxidation of AA at the 5-position to yield 5-hydroperoxyeicosatetraenoic acid (5-HPETE). Two other lipoxygenases, 12-LO and 15-LO, act at the 12- and 15-positions, yielding 12- and 15-HPETE. These pathways lead to the leukotriene 12-hydroxyeicosatetraenoic acid (12-HETE) and to the lipoxins, respectively.
Lymphoid cells	Cells that develop from the common lyphoid progenitor, including T

	and B lymphocytes, NK cells, and NKT cells.
Lysis	Refers to the death of a cell by bursting, often by viral or osmotic mechanisms that compromise the integrity of the cellular membrane. A process of of disintegration or dissolution particularly of cellular processes.
Macrophage	Powerful phagoctye that also secretes a large array of proteases, cytokines, and growth factors and can act as an antigen-presenting cell.
Macrophage activating factor	Factors secreted by stimulated lymphocytes that prime macrophages to become nonspecifically cytotoxic to tumors and modulate the expression of macrophage cell surface Ig antigens.
Macrophage inflammatory protein	A chemokine of the CC subgroup produced by monocytes, macrophages, neutrophils, and endothelium

Major histocompatibility complex (MHC)	Region of the genome containing genes encoding the chains composing the MHC class I, class II, and class III proteins. Originally defined as a cluster of genes encoding proteins controlling tissue compatibility between individuals. The function of the proteins encoded in the MHC class I and class II regions is to combine with antigenic peptides, both self and nonself, and display them on the surface of host cells for perusal by T-cells. MHC class I molecules are expressed on most nucleated cells and present peptides to CD8-expressing Tc cells. MHC class II molecules are expressed only on antigen presenting cells that present peptides to CD4-expressing Th cells. The class I and class II MHC genes are highly polymorphic in many species, including mice and humans. The class III MHC genes encode various protiens important in complement activation, inflammation, and stress responses.

Mast cells	Granule-containing leukocytes with non-lobed nucleus derived from a lineage seprate from that giving rise to other granulocytes. The cytoplasmic granules of mast cells stain like those of basophils and also contain heparin and histamines, but are more numerous and smaller than those of basophils. Mast cells are found in both mucosal and non-mucosal tissues and their degranulation is important for inflammation and allergy.
Monocytes	Myeloid cells in the blood. Monocytes circulate in the blood for approximately 1 day before entering the tissues and serous cavities and maturing into macrophages.
Myeloid cells	Cells that develop from the common myeloid progenitor, including erythrocytes, neutrophils, monocytes/macrophages, eosinophils, basophils, and megakaryocytes.

Natural killer cells (NK-cells)	Lymphoid lineage cells that recognize nonself entities with broad specificity. NK cells are activated when a target cell expresses ligands that bind to NK activatory receptors but lacks MHC class I to engage NK inhibitory receptors. NK cells bear cytoplasmic granules that allow them to kill targets such as virus-infected and tumor cells by natural cytotoxicity. NK cells also secrete inflammatory cytokines and carry Fc receptrs mediating antibody dependent cell-mediated cytotoxicity.
Neutrophils	Most common leukocytes in the body. Respond immediately in great numbers to tissue injury or pathogen attack. Neutrophils are both granulocytes and phagocytes, and are distinguished by their irregularly shaped multi-lobed nuclei and cytoplasmic granules that stain neutrally. Also called “polymorphonuclear cells” (PMNs).
Neutrophilia	Greatly increased number of neutrophils in the circulation.

Nuclear factor kappa B (NF-κB)	A family of heterodimeric transcription factors. NF-κB activation is induced by engagement of the B-cell receptor, T-cell receptor, and many cytokine/growth factor receptors. In resting cells, NF-κB is held inactive in the cytoplasm by binding to the IκB inhibitor. Receptor engagement stimulates intracellular signaling that activates the IKK kinase, which phosphorylates IκB, triggering its degradation. Free NF-κB enters the nucleus and binds to the κB DNA binding motif, initiating new gene transcription.
Nutraceutical	A combination of "nutritional" and "pharmaceutical" and refers to foods thought to have a beneficial effect on human health. It can also refer to individual chemicals which are present in common foods (and therefore may be delivered in a non-drug form). Many such nutraceuticals are phytonutrients. Sometimes called functional foods.
Opsonization	Enhanced phagocytosis of a pathogen or macromolecule due to the binding of molecules that interact with cell surface receptors on phagocytes.
Phagocytosis	The engulfing and ingesting of bacteria and other foreign particles by phagocytes.

Pleiotropic	Usually used in genetics to refer to the ability of one gene to express multiple phenotypic characteristics. In relation to immunology and cytokines, the word refers to a cytokine that is able to produce an effect in multiple cell types.
PMNL	Polymorphonuclear leukocytes. A type of white blood cell (e.g., neutrophil) containing more than one nucleus. PMNL are phagocytes (scavenger cells) important in immune defense, particularly against cell-free organisms such as fungi.
Prostaglandin	Any of various oxygenated unsaturated cyclic fatty acids of animals that are formed as cyclooxygenase metabolites especially from unsaturated fatty acids (as arachidonic acid) composed of a chain of 20 carbon atoms and that perform a variety of hormonelike actions (as in controlling blood pressure or smooth muscle contraction).

Reactive oxygen species (ROS)	Species such as superoxide, hydrogen peroxide, and hydroxyl radical that contain unpaired electrons and are associated with cell damage. ROS will 'steal' electrons from nearby tissue (such as lipid membranes or proteins) to increase their stability. If the ability to neutralize increased amounts of ROS is compromised it may result in increased cellular damage via apoptosis.
Septic shock	See endotoxic shock.
T-cell	Also known as T-lymphocyte generally concentrated in the secondary lymphoid tissue in a resting state. T-cells can be distinguished phenotypically by the cell surface expression of T-cell receptors.
T-cytotoxic cells (Tc cells)	Cytotoxic T-cells that recognize nonself-peptide presented on MHC class I molecules expressed by a target cell. Upon activation, Tc cells differentiate into cytotoxic T lymphocyte effector cells that kill target cells by perforin/granzyme-mediated cytotoxicity or by secretion of cytotoxic cytokines. Tc cells generally express the CD8 coreceptor.

TGF-β	Transforming growth factor β. A cytokine with three isoforms that has multiple roles including adhesion, proliferation, differentiation, transformation, chemotaxis, and immunoregulation. Particularly important for stimulating angiogenesis.
Th1	An isoform of Th cells. See T-helper cells.
Th2	An isoform of Th cells. See T-helper cells.
T-helper cell	T-cells that express the CD4 glycoprotein and are involved in stimulating and directing the immune system by aiding antibody cell switching, activating cytotoxic T-cells, and by supporting the bactericidal action of macrophages and phagocytes.

Thromboxane	A member of the family of lipids known as eicosanoids. It is produced in platelets by thromboxane-A synthase, which is produced from the endoperoxides by the cyclooxygenase (COX) enzyme from arachidonic acid. Thromboxane is a vasoconstrictor and a potent hypertensive agent, and it facilitates the clumping of platelets. It is in homeostatic balance in the circulatory system with prostacyclin, a related compound.
Thymus gland	A small bilobed organ located above the heart, consisting of the medulla, the inner and outer cortex, and the subcapsule. The thymus is the primary lymphoid tissue in which thymocytes develop into mature T-cells.
ω-3 fatty acid	A polyunsaturated fatty acid that has the final double bond in the hydrocarbon chain between the third and fourth carbon atoms from the end of the molecule opposite that of the carboxylic acid group. Predominantly found in fish oils, vegetable oils, and green leafy vegetables.

Compiled from Mak & Saunders, 2006

Appendix A Certificate of Approval Flaxseed Lignan Study

University of Saskatchewan
Biomedical Research Ethics Board (Bio-REB) 02-Feb-
2005

Certificate of Approval

PRINCIPAL INVESTIGATOR	DEPARTMENT	Bio #
Philip D. Chilibeck	Kinesiology	04-169

INSTITUTION (S) WHERE RESEARCH WILL BE CARRIED OUT

College of Kinesiology
105 Gymnasium Place
Saskatoon SK S7N 5C2

Royal University Hospital
103 Hospital Drive
Saskatoon SK S7N OW8

SUB-INVESTIGATOR(S)

JayBiem
Punam Pahwa
Susan J. Whiting

SPONSORING AGENCIES

HEART AND STROKE FOUNDATION OF SASKATCHEWAN

TITLE:

Protocol Flaxseed Lignan and Exercise Training for Improving Blood Lipid Levels

ORIGINAL APPROVAL DATE	CURRENT EXPIRY DATE
08-Sep-2004	Ol-Sep-2005
CERTIFICATION UPDATE	APPROVED ON
Consent Form (28 Jan 05)	02-Feb-2005

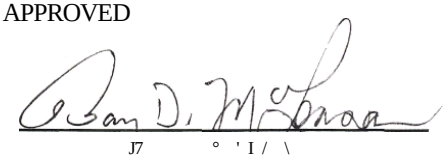
CERTIFICATION

The University of Saskatchewan Biomedical Research Ethics Board (Bio- REB) has reviewed the above-named research project including the protocol and consent form, where applicable. The proposal was found to be acceptable on ethical grounds. The principal investigator has the responsibility of ensuring that the authorized research is carried out according to governing law. This Approval is valid for the above time period provided there is no change in experimental protocol or in the consent process.

ONGOING REVIEW REQUIREMENT(S) / REB ATTESTATION

In order to receive annual renewal, a status report must be submitted to the Chair for Committee consideration within one month of the current expiry date each year the study remains open, and upon study completion. Please refer to the following website for further instructions: http://www.usask.ca/research/ethics.shtml. In respect to clinical trials, the University of Saskatchewan Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations and carries out its functions in a manner consistent with Good Clinical Practices. This approval and the views of this REB have been documented in writing.

APPROVED



Barry D. McLennan, Ph.DC, Chair
University of Saskatchewan
Biomedical Research Ethics Board (Bio-REB)

Please send all correspondence to:

Office of Research Services, University of Saskatchewan
Room 3403, 110 Gymnasium Place Box 5000
RPO University Saskatoon, SK S7N 4J8 Phone:

Appendix B Consent Form Flaxseed Lignan Study

Consent Form

Title: Effectiveness of flax lignan and exercise training for improving lipid profiles

Names of Researchers: Philip D. Chilibeck, Ph.D., Associate Professor, College of Kinesiology, University of Saskatchewan (966-1072 or 343-6577), role: principal researcher; H. Jay Biem, M.D., Department of Medicine, Royal University Hospital, University of Saskatchewan (966-7951), role: clinical trials consultant, Susan Whiting, Ph.D., Professor, College of Pharmacy and Nutrition, University of Saskatchewan (966-5837), role: nutritional analyses, Punam Pahwa, Assistant Professor, Department of Community Health and Epidemiology, University of Saskatchewan (966-7941), role: statistical consultant, Stephen Cornish, M.Sc. (student researcher supervised by Dr. Chilibeck), College of Kinesiology, University of Saskatchewan, phone: 966-6505.

Sponsor: Heart and Stroke Foundation of Saskatchewan; Archer Daniels Midland (supplier of the flax lignan nutritional supplement)

You are being asked to participate in a research study because you are of the age where you may be at risk for high cholesterol levels. This study involves the use of a dietary supplement derived from flaxseed (flax lignan) and exercise training (brisk walking or slow jogging) for improving cholesterol levels. Exercise training is thought to raise the levels of "good" cholesterol (high density lipoproteins) while the flax supplement is thought to lower the level of "bad" cholesterol (low density lipoproteins).

Before you decide, it is important for you to understand what the research involves. This consent form will tell you about the study, why the research is being done, what will happen to you during the study and the possible benefits, risks and discomforts. If you wish to participate, you will be asked to sign this form. Your participation is entirely voluntary, so it is up to you to decide whether or not to take part in this study. If you do decide to take part in this study, you are free to withdraw at any time without giving any reasons for your decision nor will you lose the benefit of any medical care to which you are entitled or are presently receiving. Please take time to read the following information carefully and to discuss it with your family, friends, and doctor before you decide.

Purpose of the study: The purpose of the study is to determine the effectiveness of combining exercise training and flax lignan supplementation for improving blood cholesterol levels in individuals who are at risk for high cholesterol levels.

Possible benefits of the study: You may improve you blood cholesterol levels and lower your body fat level. These results are not guaranteed.

Procedures: You will initially be given a questionnaire that asks whether you are at risk for performing exercise. This will take several minutes to complete. If it is determined you are at risk, we will ask you to obtain permission from your family physician before entering the study.

You will be randomized by chance to one of two groups (i.e. There will be an equal chance of being assigned to either group). You will have an equal (one in two) chance of being assigned to either group. The first group will receive flax lignan dietary supplement (500 mg per day) and will participate in an exercise program of brisk walking or slow jogging six days per week, 60 minute per day, for 6 months. The second group will receive a placebo (i.e. an inactive substance that looks identical to the flax lignan dietary supplement but contains no active ingredients) and will participate in the same exercise training program. The study is "double blind", that is, neither you nor the investigators will know whether you are on the flax lignan supplement or placebo until the study is over. In case of emergency, the code on the double blind can be broken so we can tell whether you are on the flax lignan or placebo. If you agree to be in the study, we will do the following measurements on you:

1) Blood cholesterol, and glucose levels will be measured at the start of the study, after 2 months, after 4 months, and after 6 months. Approximately 11 mL of (about 2.5 teaspoons) blood will be collected at each time point, after a 12 hour fast. This procedure will take about 5 minutes.
2) Body composition (i.e. lean tissue, fat, and bone mass) will be assessed at the start of the study and 6 months by dual energy X-ray absorptiometry. This involves lying still on a table for approximately 10 minutes while your body composition is assessed. Dual energy X-ray absorptiometry uses X-ray beams that are absorbed differently by fat, muscle, and bone. From this we can determine your fat, muscle, and bone mass.
3) Body mass, height, waist girth, and two skinfolds from your trunk area (measured with calipers, that is, an instrument which pinches your fat skinfold) will be assessed at the start of the study and after 6 months. This procedure takes about 5 minutes.

4) Your diet will be assessed at the start of the study and after 6 months by a food frequency questionnaire. This will take about 30 minutes to complete.
5) Your aerobic fitness will be assessed at the start of the study and after 6 months with a "step test". This involves stepping up and down a set of 2 steps at a rate which increases every 3 minutes. The test stops when you reach a heart rate level corresponding to 85% of your age-predicted maximal heart rate. This test can last between 3 and 20 minutes, depending on your fitness level.

With your permission, your family physician will be informed of your enrolment and of your test results.

Foreseeable risks, side effects or discomfort: There is radiation exposure from the dual energy X-ray absorptiometry but this is considered minimal. This is about the same amount of radiation as you would be exposed to during a return airplane flight from Saskatoon to Toronto, and is less than the amount of radiation you would be exposed to during a regular X-ray.

There is a risk of injury during exercise testing or training. This will be minimized by proper warm-up procedures and supervision by qualified exercise trainers.

There will be some discomfort when blood is drawn for testing cholesterol levels. Bruising or infection at the sight of the blood draw is a possibility, but care will be taken to minimize these risks.

The side effects of flax lignan are unknown.

There may be unforeseen and unknown risks during the study, or after the study has been completed.

Alternatives to this study:
You do not have to participate in this study to improve your cholesterol levels or body composition. Your cholesterol levels can be improved by altering your diet (i.e. lowering saturated fat intake) or by a variety of drugs that may be prescribed by your family physician. Your body composition can also be improved by altering your diet. You can discuss these options with your doctor before deciding whether or not to participate in this research project.

Research-Related Injury: There will be no cost to you for participation in this study. You will not be charged for the study nutritional supplements or any

research procedures. In the event you become ill or injured as a result of participating in this study, necessary medical treatment will be made available at no additional cost to you. By signing this document you do not waive any of your legal rights.

Confidentiality: Precautions will be taken to protect your anonymity during the study. All data collected will be stored in a locked office in the College of Kinesiology. While absolute confidentiality cannot be guaranteed, every effort will be made to ensure that the information you provide for this study is kept entirely confidential. Your name will not be attached to any information, nor mentioned in any study report, nor be made available to anyone except the research team. It is the intention of the research team to publish results of this research in scientific journals and to present the findings at related conferences and workshops, but your identity will not be revealed.

If you have questions concerning the study you can contact Dr. Philip Chilibeck at 966-1072 or 343-6577 or Stephen Cornish (student researcher) at 966-6505.

If you have any questions about your rights as a research subject or concerns about the study, you should contact the Chair of the Biomedical Research Ethics Board, c/o the Office of Research Services, University of Saskatchewan at (306) 966-4053.

If, during the course of this study, new information becomes available that may be related to your willingness to continue to participate, this information will be provided to you by the investigator.

Your participation in the research is entirely voluntary. You are free to withdraw from this study at any time and this withdrawal will not affect your access to health care or other services. If you choose to enter the study and then decide to withdraw at a later time, all data collected about you during your enrolment in the study will be retained for analysis. If you do withdraw from the study you will be informed of the treatment you were receiving (i.e. flax lignan or placebo).

Please read the following before signing this consent form:

- I have read or have had this read to me and understood the research subject information and consent form.
- I have had sufficient time to consider the information provided and to ask for advice if necessary.
- I have had the opportunity to ask questions and have had satisfactory responses to my questions.
- I understand that all of the information collected will be kept confidential and that the result will only be used for scientific objectives.
- I understand that my participation in this study is voluntary and that I am completely free to refuse to participate or to withdraw from this study at any time without changing in any way the quality of care that I receive.
- I understand that I am not waiving any of my legal rights as a result of signing this consent form.
- I understand that there is no guarantee that this study will provide any benefits to me (if applicable).
- I have read this form and I freely consent to participate in this study.
- I have been told that I will receive a dated and signed copy of this form.

Please notify my primary care physician of my participation in this study:
Yes_____
No_____

Research Subject's Signature:_____

Signature of the Individual responsible for conducting the consent process:

_____

Date: _____

Appendix C Cardiovascular (Walking) Exercise Prescription Flaxseed Lignan Study

Determining Your Heart Rate

There are two simple ways to take your heart rate. The first way is on your radial artery (on the side of your wrist closet to the thumb). You will want to press down with the index and middle finger to find the "beating" of the artery. You will count the number of "beats" you feel with this method in 15 seconds. Always start counting at zero (0). Once you determine the number of beats in 15 seconds multiply the number by 4. This will give you the number of beats per minute (bpm) that your heart is beating. The second way to take your heart rate is on your carotid artery (on the neck). The procedure is the same as above. Use whatever method is easiest for you. You are also able to determine your heart rate with a heart rate monitor. This will display your heart rate on a watch that is worn on the wrist.

Determining Your Training Zone

When performing aerobic activity, a good way to monitor your progression in fitness is by utilizing target heart rate "zone" training. This is the method of exercise prescription that we will use in prescribing exercise intensity in this study. When using the target heart rate (THR) zone method of training, each individual will need to determine their maximum heart rate (MHR). MHR is calculated from the following formula:

MHR = 220-age

Once you determine your maximum heart rate you will then be able to insert this value into the following table to determine what intensity (i.e. number of beats per minute) you should strive for in each of your exercise training sessions.

Zone	***Type of Training***	***Percent of MHR***
I	Health Zone*	50-55% MHR
II	Base Fitness Zone*	55-65% MHR
III	Aerobic Zone (Extensive)*	65-70% MHR
IV	Aerobic Zone (Intensive) ☻	70-85% MHR
V	Anaerobic Zone (Sport Training) ☻	85-90% MHR
VI	Maximum Zone (Sport Training) ☻	90-100% MHR

* These are the only three zones that will be used in this study.

☻ These zones are used for sport and elite fitness training and will not be used in this study.

TRAINING ZONES

*Zone I: Health Zone (Warm up) --- 50 - 55% of maximum heart rate: The easiest zone and probably the best zone for people just starting a fitness program. It can also be used as a warm up for more serious walkers. This zone has been shown to help decrease body fat, blood pressure and cholesterol. It also decreases the risk of degenerative diseases and has a low risk of injury. 85% of kilocalories burned in this zone are fats.

*Zone II: Base Fitness Zone (Fat Burning) --- 55 - 65% of maximum heart rate: This zone provides the same benefits as the healthy heart zone, but is more intense and burns more total kilocalories. The percent of fat kilocalories is still 85%.

*Zone III: Aerobic Zone (Extensive Training) --- 65-70% of maximum heart rate: The extensive aerobic zone will improve your cardiovascular and respiratory system AND increase the size and strength of your heart. This is the preferred zone if you are training for an endurance event. More kilocalories are burned with 50% from fat.

☻ *Zone IV: Aerobic Zone (Intensive Training) --- 70-85% of maximum heart rate: The intensive aerobic zone will improve VO_2 maximum (the highest amount of oxygen one can consume during exercise), increase the efficiency of the cardiovascular system, and elevate your ability to sustain more intense activity. The difference between the extensive and intensive zones is the amount of time an individual would be able to sustain the activity. More kilocalories are used in this zone with less of the energy derived from fat than the extensive zone.*

☻ *Zone V: Anaerobic Zone (Sport Training) --- 85 - 90% of maximum heart rate: Benefits of this zone include an improved VO_2 maximum (the highest amount of oxygen one can consume during exercise) and thus an improved cardiorespiratory system, and a higher lactate tolerance ability which means your endurance will improve and you'll be able to fight fatigue better. This is a high intensity zone burning more kilocalories, 15 % from fat.*

☻ *Zone VI: Maximum (Sport Training) --- 90 - 100% of maximum heart rate: Although this zone burns the highest number of calories, it is very intense. Most people can only stay in this zone for short periods. You should only train in this zone if you are in very good shape and have been cleared by a physician to do so.*

Week	Prescription	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1	Duration	5'/20'/5'	5'/20'/5'	5'/22'/5'	5'/22'/5'	5'/24'/5'	5'/24'/5'	
	Intensity	I/II/I	I/II/I	I/II/I	I/II/I	I/II/I	I/II/I	
	Actual Time Distance/Steps							
2	Duration	5'/24'/5'	5'/24'/5'	5'/26'/5'	5'/26'/5'	5'/28'/5'	5'/28'/5'	
	Intensity		I/II/ I/II/III	I/II/ I/II/III	I/II/I I/II/I			
	Actual Time Distance/Steps							
3	Duration	5'/28'/5'	5'/28'/5'	5'/30'/5'	5'/30'/5'	5'/32'/5'	5'/32'/5'	
	Intensity	I/II/I	I/II/I	I/II/I	I/II/I	I/II/I	I/II/I	
	Actual Time Distance/Steps							
4	Duration	5'/32'/5'	5'/32'/5'	5'/34'/5'	5'/34'/5'	5'/36'/5'	5'/36'/5'	
	Intensity		I/II/ I/II/III	I/II/ I/II/III	I/II/I I/II/I			
	Actual Time Distance/Steps							
5	Duration	5'/36'/5'	5'/36'/5'	5'/38'/5'	5'/38'/5'	5'/40'/5'	5'/40'/5'	
	Intensity	I/II/I	I/II/I	I/II/I	I/II/I	I/II/I	I/II/I	
	Actual Time Distance/Steps							
6	Duration	5'/40'/5'	5'/40'/5'	5'/42'/5'	5'/42'/5'	5'/44'/5'	5'/44'/5'	
	Intensity		I/II/ I/II/III	I/II/ I/II/III	I/II/I I/II/I			
	Actual Time Distance/Steps							
7	Duration	5'/44'/5'	5'/44'/5'	5'/46'/5'	5'/46'/5'	5'/48'/5'	5'/48'/5'	
	Intensity	I/II/I	I/II/I	I/II/I	I/II/I	I/II/I	I/II/I	
	Actual Time Distance/Steps							
8	Duration	5'/48'/5'	5'/48'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	
	Intensity		I/II/ I/II/III	I/II/ I/II/III	I/II/I I/II/I			
	Actual							

	Time						
	Distance/Steps						
9	Duration	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'
	Intensity	I/II/I	I/II/I	I/II/I	I/II/I	I/II/I	I/II/I
	Actual Time						
	Distance/Steps						
10	Duration	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'
	Intensity		I/II/ I/II/I I	I/II/ I/II/I I	I/II/I I/II/I		
	Actual Time						
	Distance/Steps						
11	Duration	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'
	Intensity	I/II/I	I/II/I	I/II/I	I/II/I	I/II/I	I/II/I
	Actual Time						
	Distance/Steps						
12	Duration	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'
	Intensity		I/II/ I/II/I I	I/II/ I/II/I I	I/II/I I/II/I		
	Actual Time						
	Distance/Steps						
13	Duration	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'
	Intensity	I/II/I	I/II/I	I/II/I	I/II/I	I/II/I	I/II/I
	Actual Time						
	Distance/Steps						

Duration: 5' = 5 mintues

Intensity = Your target heart rate (see other handout)

Actual Time = for you to record the actual amount of time walked each day.

Distance = distance in kilometers and/or number of steps taken from pedometer

Week	Prescription	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
14	Duration	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	
	Intensity	I/II/I	I/II/I	I/II/I	I/II/I	I/II/I	I/II/I	
	Actual Time							
	Distance/Steps							
15	Duration	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	
	Intensity	I/II/I	I/II/I	I/II/I	I/II/I	I/II/I	I/II/I	
	Actual Time							
	Distance/Steps							
16	Duration	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	
	Intensity	I/II/I	I/II/I	I/II/I	I/II/I	I/II/I	I/II/I	
	Actual Time							
	Distance/Steps							
17	Duration	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	
	Intensity	I/II/I	I/II/I	I/II/I	I/II/I	I/II/I	I/II/I	
	Actual Time							
	Distance/Steps							
18	Duration	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	
	Intensity	I/II/I	I/II/I	I/II/I	I/II/I	I/II/I	I/II/I	
	Actual Time							
	Distance/Steps							
19	Duration	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	
	Intensity	I/II/I	I/II/I	I/II/I	I/II/I	I/II/I	I/II/I	
	Actual Time							
	Distance/Steps							
20	Duration	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	
	Intensity	I/II/I	I/II/I	I/II/I	I/II/I	I/II/I	I/II/I	
	Actual Time							
	Distance/Steps							
21	Duration	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	
	Intensity	I/II/I	I/II/I	I/II/I	I/II/I	I/II/I	I/II/I	
	Actual Time							
	Distance/Steps							
22	Duration	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	
	Intensity	I/II/I	I/II/I	I/II/I	I/II/I	I/II/I	I/II/I	
	Actual Time							
	Distance/Steps							
23	Duration	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	
	Intensity	I/II/I	I/II/I	I/II/I	I/II/I	I/II/I	I/II/I	
	Actual Time							
	Distance/Steps							

24	Duration	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'
	Intensity	I/II/I	I/II/I	I/II/I	I/II/I	I/II/I	I/II/I
	Actual Time						
	Distance/Steps						
25	Duration	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'
	Intensity	I/II/I	I/II/I	I/II/I	I/II/I	I/II/I	I/II/I
	Actual Time						
	Distance/Steps						
26	Duration	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'	5'/50'/5'
	Intensity	I/II/I	I/II/I	I/II/I	I/II/I	I/II/I	I/II/I
	Actual Time						
	Distance/Steps						

Duration: 5' = 5 mintues

Intensity = Your target heart rate (see other handout)

Actual Time = for you to record the actual amount of time walked each day.

Distance = distance in kilometers and/or number of steps taken from pedometer

Appendix D Certificate of Approval Flaxseed Oil Study

UNIVERSITY OF SASKATCHEWAN

Certificate of Approval

PRINCIPAL INVESTIGATOR

DEPARTME

Bio# 06-211

NT
Philip D. Chilibeck
Kinesiology

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT College of Kinesiology 105
Gymnasium Place Saskatoon SK S7N 5C2

STUDENT RESEARCHER(S)
Stephen Cornish

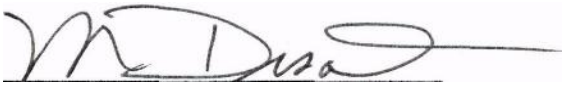
SPONSORING AGENCIES
GATORADE SPORTS SCIENCE INSTITUTE

TITLE
The Combined Effects of Resistance Training and Flax Oil Supplementation Upon Inflammation in Older Adults

ORIGINAL APPROVAL DATE	CURRENT EXPIRY DATE	APPROVAL OF
18-Oct-2006	17-Oct-2007	Researcher's Summary as submitted Revised Consent Form as submitted

CERTIFICATION
The University of Saskatchewan Biomedical Research Ethics Board has reviewed the above-named research project at a full-board meeting (any research classified as minimal risk is reviewed through the expedited review process). The proposal was found to be acceptable on ethical grounds. The principal investigator has the responsibility for any other administrative or regulatory approvals that may pertain to this research project, and for ensuring that the authorized research is carried out according to governing law. This Approval is valid for the above time period provided there is no change in experimental protocol or in the consent process.

ONGOING REVIEW REQUIREMENTS/REB ATTESTATION
In order to receive annual renewal, a status report must be submitted to the Chair for Committee consideration within one month of the current expiry date each year the study remains open, and upon study completion. Please refer to the following website for further instructions: http://www.usask.ca/research/ethics.shtml. In respect to clinical trials, the University of Saskatchewan Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations and carries out its functions in a manner consistent with Good Clinical Practices. This approval and the views of this REB have been documented in writing.



Michel Desautels, Ph.D., Chair
University of Saskatchewan
Biomedical Research Ethics Board

Appendix E Consent Form Flaxseed Oil Study

Consent Form

Title: The combined effects of resistance training and flax oil supplementation upon inflammation in older adults

Names of Researchers: Philip D. Chilibeck, Ph.D. (principal investigator), Associate Professor, College of Kinesiology, University of Saskatchewan (966-1072 or 343-6577); Lisa Paus-Jensson, M.D., Department of Medicine, University of Saskatchewan (966-7951) Stephen Cornish, M.Sc. (student investigator supervised by Dr. Chilibeck), College of Kinesiology, University of Saskatchewan, phone: 966-1123 or 978-5735

You are being asked to participate in a research study because you are of the age where you may be at risk for increased inflammation and reduced muscle strength and mass. This study involves the use of a dietary supplement derived from flaxseed (flax oil) and exercise training (weight training) for improving blood markers of inflammation, muscle strength, and muscle mass. Exercise training is thought to increase muscle mass and strength while the flax supplement is thought to lower the level of blood markers of inflammation.

A total of 60 subjects are expected to participate in this trial.

Before you decide, it is important for you to understand what the research involves. This consent form will tell you about the study, why the research is being done, what will happen to you during the study and the possible benefits, risks and discomforts. If you wish to participate, you will be asked to sign this form. Your participation is entirely voluntary, so it is up to you to decide whether or not to take part in this study. If you do decide to take part in this study, you are free to withdraw at any time without giving any reasons for your decision nor will you lose the benefit of any medical care to which you are entitled or are presently receiving. Please take time to read the following information carefully and to discuss it with your family, friends, and doctor before you decide.

Purpose of the study: The purpose of the study is to determine the effectiveness of combining exercise training and flax oil supplementation for reducing inflammation, and increasing muscle mass and strength, in those at risk of having a higher amount of inflammation.

Possible benefits of the study: You may improve your blood inflammatory status, increase your muscle mass, increase your muscle strength, and lower your body fat level. These results are not guaranteed.

Procedures: You will initially be given a questionnaire that asks whether you are at risk for performing exercise. This will take several minutes to complete. If it is determined you are at risk, we will ask you to obtain permission from your family physician before entering the study.

You will be randomized by chance (i.e. by a computer program) to one of two groups (i.e. There will be an equal chance of being assigned to either group). The first group will receive flax oil dietary supplement (30 mL or 2 Tablespoons per day) and will participate in an exercise program of resistance training (weight training) 3 days per week, 60 minutes per day for 12 weeks. The second group will receive a placebo (i.e. a substance that looks identical to the flax oil dietary supplement but does not contain the active ingredients of flax oil. The placebo we are using in our study is corn oil) and will participate in the same exercise training program. The study is "double blind", that is, neither you nor the investigators will know whether you are on the flax oil supplement or placebo until the study is over. In case of emergency, the code on the double blind can be broken so we can tell whether you are on the flax oil or placebo. If you agree to be in the study, we will do the following measurements on you:

6) Blood (to assess markers of inflammation) will be taken at the start of the study and after 12 weeks. Approximately 11 mL of (about 2.5 teaspoons) blood will be collected at each time point, after a 12 hour fast. This procedure will take about 5 minutes.
7) Body composition (i.e. lean tissue, fat, and bone mass) will be assessed at the start of the study and after 12 weeks by dual energy X-ray absorptiometry. This involves lying still on a table for approximately 10 minutes while your body composition is assessed. Dual energy X-ray absorptiometry uses X-ray beams that are absorbed differently by fat, muscle, and bone. From this we can determine your fat, muscle, and bone mass.
8) Body mass, height, waist girth, and two skinfolds from your trunk area (measured with calipers, that is, an instrument which pinches your fat skinfold) will be assessed at the start of the study and after 12 weeks. This procedure takes about 5 minutes.

9) Muscle thickness will be determined using ultrasound on your arm and leg at the start of the study and after 12 weeks. This procedure takes about 10 minutes.
10) Your diet will be assessed at the start of the study and after 12 weeks by a food frequency questionnaire. This will take about 30 minutes to complete.
11) Your muscle strength will be assessed at the start of the study and after 12 weeks by completing a one repetition maximum strength test (i.e. determining the maximal amount of weight you can lift). One test (chest press) will be done to assess your upper body strength and another test (leg press) will be done to assess your lower body strength. These tests involve progressively increasing the amount of weight you lift for one repetition until you are no longer able to complete a full repetition of the specific exercise.

All of the above measurements will be done at one visit, both before and after the 12 weeks of flax oil/placebo supplementation and training, except for the muscular strength assessment. This will be done on a separate day so that you are not fasted.

With your permission, your family physician will be informed of your enrolment and of your test results.

You will be asked to maintain a consistent diet throughout the study.

You will be prohibited from taking the following during the trial: Flax oil (besides that given as part of the trial), any other flaxseed product (i.e. whole or ground flaxseed), fish oil, evening primrose oil supplements, other marine oil supplements (i.e. seal oil), foods that are supplemented with omega-3 fatty acids (i.e. omega-3 supplemented dairy and eggs) and prescription or over-the counter medication (non-steroidal anti-inflammatory drugs, prednisone etc.) or natural health products (i.e. bromelain) that are anti-inflammatory in nature.

Foreseeable risks, side effects or discomfort: There is radiation exposure from the dual energy X-ray absorptiometry but this is considered minimal. This is about the same amount of radiation as you would be exposed to during a return airplane flight from Saskatoon to Toronto, and is less than the amount of radiation you would be exposed to during a regular X-ray.

There is a risk of injury during exercise testing or training. This will be minimized by proper warm-up procedures and supervision by qualified exercise trainers.

There will be some discomfort when blood is drawn for testing inflammation levels. Bruising or infection at the sight of the blood draw is a possibility, but care will be taken to minimize these risks.

One study reported that flax oil supplementation induced diarrhea or loose stools in 20% of individuals; however, this study used four times the dose to be used in the current study.

Increased bleeding time is a possible risk associated with use of flax seed oil. If you have a bleeding disorder or are taking blood thinners you should consult a health care practitioner before using the investigational product.

There may be other side effects of flax oil supplementation which are unknown.

There may be unforeseen and unknown risks during the study, or after the study has been completed.

Alternatives to this study:
You do not have to participate in this study to improve your inflammatory status or body composition. Your level of inflammation can be improved by altering your diet (i.e. lowering saturated fat intake and increasing the amount of essential fatty acids) or by a variety of drugs that may be prescribed by your family physician. Your body composition can also be improved by altering your diet or participating in an alternative fitness program. You can discuss these options with your doctor before deciding whether or not to participate in this research project.

Research-Related Injury: There will be no cost to you for participation in this study. You will not be charged for the study nutritional supplements or any research procedures. In the event you become ill or injured as a result of participating in this study, necessary medical treatment will be made available at no additional cost to you. By signing this document you do not waive any of your legal rights.

Confidentiality: Precautions will be taken to protect your anonymity during the study. All data collected will be stored in a locked office in the College of Kinesiology. While absolute confidentiality cannot be guaranteed, every effort will be made to ensure that the information you provide for this study is kept entirely confidential. Your name will not be attached to any information, nor mentioned in any study report, nor be made available to anyone except the research team. Health Canada may be granted direct access to your original medical records for verification of clinical trial procedures and/or data. It is the intention of the research team to publish results of this research in scientific journals and to present the findings at related conferences and workshops, but your identity will not be revealed.

If you have questions concerning the study you can contact Stephen Cornish (student researcher) at 966-1123 or 978-5735 or Dr. Philip Chilibeck at 966-1072, 343-6577, or 230-3849 (24 hour number). Any adverse events you experience during the study should be reported immediately by calling one of the above numbers.

If you have any questions about your rights as a research subject or concerns about the study, you should contact the Chair of the Biomedical Research Ethics Board, c/o the Office of Research Services, University of Saskatchewan at (306) 966-4053.

If, during the course of this study, new information becomes available that may be related to your willingness to continue to participate, this information will be provided to you by the investigator.

Your participation in the research is entirely voluntary. You are free to withdraw from this study at any time and this withdrawal will not affect your access to health care or other services. If you choose to enter the study and then decide to withdraw at a later time, all data collected about you during your enrolment in the study will be retained for analysis.

Unused study product must be returned to the Investigator for the purpose of compliance monitoring and product accountability.

Please read the following before signing this consent form:

- I have read or have had this read to me and understood the research subject information and consent form.

- I have had sufficient time to consider the information provided and to ask for advice if necessary.
- I have had the opportunity to ask questions and have had satisfactory responses to my questions.
- I understand that all of the information collected will be kept confidential and that the result will only be used for scientific objectives.
- I understand that my participation in this study is voluntary and that I am completely free to refuse to participate or to withdraw from this study at any time without changing in any way the quality of care that I receive.
- I understand that I am not waiving any of my legal rights as a result of signing this consent form.
- I understand that there is no guarantee that this study will provide any benefits to me (if applicable).
- I have read this form and I freely consent to participate in this study.
- I have been told that I will receive a dated and signed copy of this form.

Please notify my primary care physician of my participation in this study:
Yes_____
No_____

Research Subject's Signature:_____

Signature of the Individual responsible for conducting the consent process:

_____

Date: _____

Appendix F Exercise Prescription for Flaxseed Oil Study

Week 1: 3 Sets of 12 Repetitions @ 65% 1RM

Date:			
Day 1	**Reps/Weight Set 1**	**Reps/Weight Set 2**	**Reps/Weight Set 3**
Chest Press			
Lat Pulldown			
Trunk Flexion/Extension			
Leg Press			
Shoulder Press			
Bicep Curl			
Hip Flexion/Extension			
Tricep Press			
Hip Abduction/Adduction			
Leg Extension			
Leg Curl			
Other:			

Week 1: 3 Sets of 12 Repetitions @ 65% 1RM

Date:			
Day 2	**Reps/Weight Set 1**	**Reps/Weight Set 2**	**Reps/Weight Set 3**
Chest Press			
Lat Pulldown			
Trunk Flexion/Extension			
Leg Press			
Shoulder Press			
Bicep Curl			
Hip Flexion/Extension			

Tricep Press			
Hip Abduction/Adduction			
Leg Extension			
Leg Curl			
Other:			

Week 1: 3 Sets of 12 Repetitions @ 65% 1RM

Date:			
Day 3	**Reps/Weight Set 1**	**Reps/Weight Set 2**	**Reps/Weight Set 3**
Chest Press			
Lat Pulldown			
Trunk Flexion/Extension			
Leg Press			
Shoulder Press			
Bicep Curl			
Hip Flexion/Extension			
Tricep Press			
Hip Abduction/Adduction			
Leg Extension			
Leg Curl			
Other:			

Week 2: 3 Sets of 10 Repetitions @ 70% 1RM

Date:			
Day 1	**Reps/Weight Set 1**	**Reps/Weight Set 2**	**Reps/Weight Set 3**

Chest Press			
Lat Pulldown			
Trunk Flexion/Extension			
Leg Press			
Shoulder Press			
Bicep Curl			
Hip Flexion/Extension			
Tricep Press			
Hip Abduction/Adduction			
Leg Extension			
Leg Curl			
Other:			

Week 2: 3 Sets of 10 Repetitions @ 75% 1RM

Date:			
Day 2	**Reps/Weight Set 1**	**Reps/Weight Set 2**	**Reps/Weight Set 3**
Chest Press			
Lat Pulldown			
Trunk Flexion/Extension			
Leg Press			
Shoulder Press			
Bicep Curl			
Hip Flexion/Extension			
Tricep Press			

Hip Abduction/Adduction			
Leg Extension			
Leg Curl			
Other:			

Week 2: 3 Sets of 10 Repetitions @ 70% 1RM

Date:			
Day 3	**Reps/Weight Set 1**	**Reps/Weight Set 2**	**Reps/Weight Set 3**
Chest Press			
Lat Pulldown			
Trunk Flexion/Extension			
Leg Press			
Shoulder Press			
Bicep Curl			
Hip Flexion/Extension			
Tricep Press			
Hip Abduction/Adduction			
Leg Extension			
Leg Curl			
Other:			

Week 3: 4 Sets of 12 Repetitions @ 65% 1RM

Date:				
Day 1	**Reps/Weight Set 1**	**Reps/Weight Set 2**	**Reps/Weight Set 3**	**Reps/Weight Set 4**
Chest Press				

Lat Pulldown				
Trunk Flexion/Extension				
Leg Press				
Shoulder Press				
Bicep Curl				
Hip Flexion/Extension				
Tricep Press				
Hip Abduction/Adduction				
Leg Extension				
Leg Curl				
Other:				

Week 3: 4 Sets of 12 Repetitions @ 75% 1RM

Date:				
Day 1	**Reps/Weight Set 1**	**Reps/Weight Set 2**	**Reps/Weight Set 3**	**Reps/Weight Set 4**
Chest Press				
Lat Pulldown				
Trunk Flexion/Extension				
Leg Press				
Shoulder Press				
Bicep Curl				
Hip Flexion/Extension				
Tricep Press				
Hip Abduction/Adduction				
Leg Extension				

Leg Curl				
Other:				

Week 3: 4 Sets of 12 Repetitions @ 70% 1RM

Date:				
Day 1	**Reps/Weight Set 1**	**Reps/Weight Set 2**	**Reps/Weight Set 3**	**Reps/Weight Set 4**
Chest Press				
Lat Pulldown				
Trunk Flexion/Extension				
Leg Press				
Shoulder Press				
Bicep Curl				
Hip Flexion/Extension				
Tricep Press				
Hip Abduction/Adduction				
Leg Extension				
Leg Curl				
Other:				

Week 4: 4 Sets of 10 Repetitions @ 75% 1RM

Date:				
Day 1	**Reps/Weight Set 1**	**Reps/Weight Set 2**	**Reps/Weight Set 3**	**Reps/Weight Set 4**
Chest Press				
Lat Pulldown				
Trunk Flexion/Extension				

Leg Press				
Shoulder Press				
Bicep Curl				
Hip Flexion/Extension				
Tricep Press				
Hip Abduction/Adduction				
Leg Extension				
Leg Curl				
Other:				

Week 4: 4 Sets of 10 Repetitions @ 75% 1RM

Date:				
Day 1	**Reps/Weight Set 1**	**Reps/Weight Set 2**	**Reps/Weight Set 3**	**Reps/Weight Set 4**
Chest Press				
Lat Pulldown				
Trunk Flexion/Extension				
Leg Press				
Shoulder Press				
Bicep Curl				
Hip Flexion/Extension				
Tricep Press				
Hip Abduction/Adduction				
Leg Extension				
Leg Curl				

Other:				

Week 4: 4 Sets of 10 Repetitions @ 75% 1RM

Date:				
Day 1	**Reps/Weight Set 1**	**Reps/Weight Set 2**	**Reps/Weight Set 3**	**Reps/Weight Set 4**
Chest Press				
Lat Pulldown				
Trunk Flexion/Extension				
Leg Press				
Shoulder Press				
Bicep Curl				
Hip Flexion/Extension				
Tricep Press				
Hip Abduction/Adduction				
Leg Extension				
Leg Curl				
Other:				

Week 5: 3 Sets of 8 Repetitions @ 80% 1RM

Date:			
Day 1	**Reps/Weight Set 1**	**Reps/Weight Set 2**	**Reps/Weight Set 3**
Chest Press			
Lat Pulldown			
Trunk Flexion/Extension			
Leg Press			
Shoulder Press			

Bicep Curl			
Hip Flexion/Extension			
Tricep Press			
Hip Abduction/Adduction			
Leg Extension			
Leg Curl			
Other:			

Week 5: 3 Sets of 8 Repetitions @ 75% 1RM

Date:			
Day 2	**Reps/Weight Set 1**	**Reps/Weight Set 2**	**Reps/Weight Set 3**
Chest Press			
Lat Pulldown			
Trunk Flexion/Extension			
Leg Press			
Shoulder Press			
Bicep Curl			
Hip Flexion/Extension			
Tricep Press			
Hip Abduction/Adduction			
Leg Extension			
Leg Curl			
Other:			

Week 5: 3 Sets of 8 Repetitions @ 80% 1RM

Date:			
Day 3	**Reps/Weight Set 1**	**Reps/Weight Set 2**	**Reps/Weight Set 3**
Chest Press			
Lat Pulldown			
Trunk Flexion/Extension			
Leg Press			
Shoulder Press			
Bicep Curl			
Hip Flexion/Extension			
Tricep Press			
Hip Abduction/Adduction			
Leg Extension			
Leg Curl			
Other:			

Week 6: 3 Sets of 10 Repetitions @ 75% 1RM

Date:			
Day 1	**Reps/Weight Set 1**	**Reps/Weight Set 2**	**Reps/Weight Set 3**
Chest Press			
Lat Pulldown			
Trunk Flexion/Extension			
Leg Press			
Shoulder Press			
Bicep Curl			
Hip Flexion/Extension			

Tricep Press			
Hip Abduction/Adduction			
Leg Extension			
Leg Curl			
Other:			

Week 6: 3 Sets of 10 Repetitions @ 80% 1RM

Date:			
Day 2	**Reps/Weight Set 1**	**Reps/Weight Set 2**	**Reps/Weight Set 3**
Chest Press			
Lat Pulldown			
Trunk Flexion/Extension			
Leg Press			
Shoulder Press			
Bicep Curl			
Hip Flexion/Extension			
Tricep Press			
Hip Abduction/Adduction			
Leg Extension			
Leg Curl			
Other:			

Week 6: 3 Sets of 10 Repetitions @ 75% 1RM

Date:			
Day 3	**Reps/Weight Set 1**	**Reps/Weight Set 2**	**Reps/Weight Set 3**

Chest Press			
Lat Pulldown			
Trunk Flexion/Extension			
Leg Press			
Shoulder Press			
Bicep Curl			
Hip Flexion/Extension			
Tricep Press			
Hip Abduction/Adduction			
Leg Extension			
Leg Curl			
Other:			

RE-TEST STRENGTH THIS WEEK

Week 7: 4 Sets of 8 Repetitions @ 80% 1RM

Date:				
Day 1	**Reps/Weight Set 1**	**Reps/Weight Set 2**	**Reps/Weight Set 3**	**Reps/Weight Set 4**
Chest Press				
Lat Pulldown				
Trunk Flexion/Extension				
Leg Press				
Shoulder Press				
Bicep Curl				
Hip Flexion/Extension				
Tricep Press				

Hip Abduction/Adduction				
Leg Extension				
Leg Curl				
Other:				

Week 7: 4 Sets of 8 Repetitions @ 75% 1RM

Date:				
Day 1	**Reps/Weight Set 1**	**Reps/Weight Set 2**	**Reps/Weight Set 3**	**Reps/Weight Set 4**
Chest Press				
Lat Pulldown				
Trunk Flexion/Extension				
Leg Press				
Shoulder Press				
Bicep Curl				
Hip Flexion/Extension				
Tricep Press				
Hip Abduction/Adduction				
Leg Extension				
Leg Curl				
Other:				

Week 7: 4 Sets of 8 Repetitions @ 80% 1RM

Date:				
Day 1	**Reps/Weight Set 1**	**Reps/Weight Set 2**	**Reps/Weight Set 3**	**Reps/Weight Set 4**
Chest Press				

Lat Pulldown				
Trunk Flexion/Extension				
Leg Press				
Shoulder Press				
Bicep Curl				
Hip Flexion/Extension				
Tricep Press				
Hip Abduction/Adduction				
Leg Extension				
Leg Curl				
Other:				

Week 8: 4 Sets of 6 Repetitions @ 85% 1RM

Date:				
Day 1	**Reps/Weight Set 1**	**Reps/Weight Set 2**	**Reps/Weight Set 3**	**Reps/Weight Set 4**
Chest Press				
Lat Pulldown				
Trunk Flexion/Extension				
Leg Press				
Shoulder Press				
Bicep Curl				
Hip Flexion/Extension				
Tricep Press				
Hip Abduction/Adduction				
Leg Extension				

Leg Curl				
Other:				

Week 8: 4 Sets of 6 Repetitions @ 80% 1RM

Date:				
Day 1	**Reps/Weight Set 1**	**Reps/Weight Set 2**	**Reps/Weight Set 3**	**Reps/Weight Set 4**
Chest Press				
Lat Pulldown				
Trunk Flexion/Extension				
Leg Press				
Shoulder Press				
Bicep Curl				
Hip Flexion/Extension				
Tricep Press				
Hip Abduction/Adduction				
Leg Extension				
Leg Curl				
Other:				

Week 8: 4 Sets of 6 Repetitions @ 85% 1RM

Date:				
Day 1	**Reps/Weight Set 1**	**Reps/Weight Set 2**	**Reps/Weight Set 3**	**Reps/Weight Set 4**
Chest Press				
Lat Pulldown				
Trunk Flexion/Extension				

Leg Press				
Shoulder Press				
Bicep Curl				
Hip Flexion/Extension				
Tricep Press				
Hip Abduction/Adduction				
Leg Extension				
Leg Curl				
Other:				

Week 9: 3 Sets of 12 Repetitions @ 75% 1RM

Date:			
Day 1	**Reps/Weight Set 1**	**Reps/Weight Set 2**	**Reps/Weight Set 3**
Chest Press			
Lat Pulldown			
Trunk Flexion/Extension			
Leg Press			
Shoulder Press			
Bicep Curl			
Hip Flexion/Extension			
Tricep Press			
Hip Abduction/Adduction			
Leg Extension			

Leg Curl			
Other:			

Week 9: 3 Sets of 12 Repetitions @ 75% 1RM

Date:			
Day 2	**Reps/Weight Set 1**	**Reps/Weight Set 2**	**Reps/Weight Set 3**
Chest Press			
Lat Pulldown			
Trunk Flexion/Extension			
Leg Press			
Shoulder Press			
Bicep Curl			
Hip Flexion/Extension			
Tricep Press			
Hip Abduction/Adduction			
Leg Extension			
Leg Curl			
Other:			

Week 9: 3 Sets of 12 Repetitions @ 75% 1RM

Date:			
Day 3	**Reps/Weight Set 1**	**Reps/Weight Set 2**	**Reps/Weight Set 3**
Chest Press			
Lat Pulldown			
Trunk Flexion/Extension			

Leg Press			
Shoulder Press			
Bicep Curl			
Hip Flexion/Extension			
Tricep Press			
Hip Abduction/Adduction			
Leg Extension			
Leg Curl			
Other:			

Week 10: 3 Sets of 10 Repetitions @ 80% 1RM

Date:			
Day 1	**Reps/Weight Set 1**	**Reps/Weight Set 2**	**Reps/Weight Set 3**
Chest Press			
Lat Pulldown			
Trunk Flexion/Extension			
Leg Press			
Shoulder Press			
Bicep Curl			
Hip Flexion/Extension			
Tricep Press			
Hip Abduction/Adduction			
Leg Extension			
Leg Curl			

Other:			

Week 10: 3 Sets of 10 Repetitions @ 70% 1RM

Date:			
Day 2	**Reps/Weight Set 1**	**Reps/Weight Set 2**	**Reps/Weight Set 3**
Chest Press			
Lat Pulldown			
Trunk Flexion/Extension			
Leg Press			
Shoulder Press			
Bicep Curl			
Hip Flexion/Extension			
Tricep Press			
Hip Abduction/Adduction			
Leg Extension			
Leg Curl			
Other:			

Week 10: 3 Sets of 10 Repetitions @ 80% 1RM

Date:			
Day 3	**Reps/Weight Set 1**	**Reps/Weight Set 2**	**Reps/Weight Set 3**
Chest Press			
Lat Pulldown			
Trunk Flexion/Extension			

Leg Press			
Shoulder Press			
Bicep Curl			
Hip Flexion/Extension			
Tricep Press			
Hip Abduction/Adduction			
Leg Extension			
Leg Curl			
Other:			

Week 11: 3 Sets of 10 Repetitions @ 70% 1RM

Date:			
Day 1	**Reps/Weight Set 1**	**Reps/Weight Set 2**	**Reps/Weight Set 3**
Chest Press			
Lat Pulldown			
Trunk Flexion/Extension			
Leg Press			
Shoulder Press			
Bicep Curl			
Hip Flexion/Extension			
Tricep Press			
Hip Abduction/Adduction			
Leg Extension			

Leg Curl			
Other:			

Week 11: 3 Sets of 10 Repetitions @ 80% 1RM

Date:			
Day 2	**Reps/Weight Set 1**	**Reps/Weight Set 2**	**Reps/Weight Set 3**
Chest Press			
Lat Pulldown			
Trunk Flexion/Extension			
Leg Press			
Shoulder Press			
Bicep Curl			
Hip Flexion/Extension			
Tricep Press			
Hip Abduction/Adduction			
Leg Extension			
Leg Curl			
Other:			

Week 11: 3 Sets of 10 Repetitions @ 70% 1RM

Date:			
Day 3	**Reps/Weight Set 1**	**Reps/Weight Set 2**	**Reps/Weight Set 3**
Chest Press			
Lat Pulldown			

Trunk Flexion/Extension			
Leg Press			
Shoulder Press			
Bicep Curl			
Hip Flexion/Extension			
Tricep Press			
Hip Abduction/Adduction			
Leg Extension			
Leg Curl			
Other:			

Week 12: 3 Sets of 12 Repetitions @ 70% 1RM

Date:			
Day 1	**Reps/Weight Set 1**	**Reps/Weight Set 2**	**Reps/Weight Set 3**
Chest Press			
Lat Pulldown			
Trunk Flexion/Extension			
Leg Press			
Shoulder Press			
Bicep Curl			
Hip Flexion/Extension			
Tricep Press			
Hip Abduction/Adduction			
Leg Extension			

Leg Curl			
Other:			

Week 12: 3 Sets of 12 Repetitions @ 70% 1RM

Date:			
Day 2	**Reps/Weight Set 1**	**Reps/Weight Set 2**	**Reps/Weight Set 3**
Chest Press			
Lat Pulldown			
Trunk Flexion/Extension			
Leg Press			
Shoulder Press			
Bicep Curl			
Hip Flexion/Extension			
Tricep Press			
Hip Abduction/Adduction			
Leg Extension			
Leg Curl			
Other:			

Week 12: 3 Sets of 12 Repetitions @ 70% 1RM

Date:			
Day 3	**Reps/Weight Set 1**	**Reps/Weight Set 2**	**Reps/Weight Set 3**
Chest Press			
Lat Pulldown			

Trunk Flexion/Extension			
Leg Press			
Shoulder Press			
Bicep Curl			
Hip Flexion/Extension			
Tricep Press			
Hip Abduction/Adduction			
Leg Extension			
Leg Curl			
Other:			

Appendix G ANOVA Tables from Flaxseed Lignan Study

ANOVA Covariate Analysis at Baseline (Age)

Effect	Univariate Tests of Significance for Age (LignanData Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	*347844.2*	*1*	*347844.2*	*7965.192*	*0.000000*
group	64.6	1	64.6	1.480	0.226926
Error	3930.3	90	43.7		

ANOVA Covariate Analysis at Baseline (Body Mass)

Effect	Univariate Tests of Significance for Weight1 (Lignan Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	*598067.0*	*1*	*598067.0*	*2306.779*	*0.000000*
group	387.2	1	387.2	1.493	0.224882
Error	23333.8	90	259.3		

ANOVA Covariate Analysis at Baseline (Height)

Effect	Univariate Tests of Significance for Height1 (Lignar Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	*2596435*	*1*	*2596435*	*32944.31*	*0.000000*
group	0	1	0	0.00	0.975605
Error	7093	90	79		

ANOVA Covariate Analysis at Baseline (BMI)

Effect	Univariate Tests of Significance for BMI1 (LignanDa Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	*74406.43*	*1*	*74406.43*	*3040.512*	*0.000000*
group	59.80	1	59.80	2.444	0.121499
Error	2202.45	90	24.47		

ANOVA Covariate Analysis at Baseline (Systolic blood pressure)

Effect	Univariate Tests of Significance for SBP1 (LignanD Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	*1463285*	*1*	*1463285*	*9724.849*	*0.000000*
group	3	1	3	0.022	0.881217
Error	13241	88	150		

ANOVA Covariate Analysis at Baseline (Diastolic blood pressure)

Effect	Univariate Tests of Significance for DBP1 (LignanDa Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	*594938.5*	*1*	*594938.5*	*10116.22*	*0.000000*
group	160.9	1	160.9	2.74	0.101667
Error	5175.3	88	58.8		

Repeated Measures ANOVA Dietary Covariate Analysis (Kilocalorie)

Effect	Repeated Measures Analysis of Variance (LignanData[Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	*486273069*	*1*	*486273069*	*776.0981*	*0.000000*
Sex	1789498	1	1789498	2.8561	0.095417
group	1661466	1	1661466	2.6517	0.107868
Sex*group	1277190	1	1277190	2.0384	0.157754
Error	44485854	71	626561		
TIME	608132	1	608132	3.3075	0.073180
TIME*Sex	28036	1	28036	0.1525	0.697342
TIME*group	38626	1	38626	0.2101	0.648106
TIME*Sex*group	36525	1	36525	0.1987	0.657168
Error	13054226	71	183862		

Repeated Measures ANOVA Dietary Covariate Analysis (Total Fat)

Effect	Repeated Measures Analysis of Variance (LignanDa Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	*872121.6*	*1*	*872121.6*	*518.1602*	*0.000000*
Sex	*9650.1*	*1*	*9650.1*	*5.7335*	*0.019287*
group	2336.4	1	2336.4	1.3881	0.242658
Sex*group	3907.6	1	3907.6	2.3217	0.132025
Error	119501.0	71	1683.1		
TIME	*2308.2*	*1*	*2308.2*	*4.6131*	*0.035142*
TIME*Sex	59.9	1	59.9	0.1196	0.730462
TIME*group	94.1	1	94.1	0.1880	0.665914
TIME*Sex*group	357.4	1	357.4	0.7144	0.400843
Error	35525.2	71	500.4		

Repeated Measures ANOVA Dietary Covariate Analysis (Saturated Fat)

Effect	Repeated Measures Analysis of Variance (LignanDa Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	*71585.36*	*1*	*71585.36*	*392.0507*	*0.000000*
Sex	*965.77*	*1*	*965.77*	*5.2892*	*0.024401*
group	275.76	1	275.76	1.5102	0.223160
Sex*group	345.28	1	345.28	1.8910	0.173411
Error	12964.04	71	182.59		
TIME	172.95	1	172.95	3.6919	0.058692
TIME*Sex	2.42	1	2.42	0.0516	0.820902
TIME*group	0.02	1	0.02	0.0004	0.984475
TIME*Sex*group	10.61	1	10.61	0.2265	0.635567
Error	3325.97	71	46.84		

Repeated Measures ANOVA Dietary Covariate Analysis (MUFA)

Effect	Repeated Measures Analysis of Variance (LignanDa Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	*135237.9*	*1*	*135237.9*	*501.2435*	*0.000000*
Sex	*1873.5*	*1*	*1873.5*	*6.9441*	*0.010319*
group	587.9	1	587.9	2.1791	0.144318
Sex*group	933.1	1	933.1	3.4584	0.067075
Error	19156.1	71	269.8		
TIME	301.5	1	301.5	3.7526	0.056704
TIME*Sex	28.5	1	28.5	0.3542	0.553647
TIME*group	46.3	1	46.3	0.5759	0.450430
TIME*Sex*group	47.3	1	47.3	0.5888	0.445430
Error	5704.8	71	80.3		

Repeated Measures ANOVA Dietary Covariate Analysis (Omega-6)

Effect	Repeated Measures Analysis of Variance (LignanDa Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	*50135.97*	*1*	*50135.97*	*472.5188*	*0.000000*
Sex	221.07	1	221.07	2.0835	0.153298
group	29.51	1	29.51	0.2781	0.599592
Sex*group	106.27	1	106.27	1.0015	0.320341
Error	7533.36	71	106.10		
TIME	*139.93*	*1*	*139.93*	*4.3249*	*0.041170*
TIME*Sex	25.84	1	25.84	0.7986	0.374544
TIME*group	10.70	1	10.70	0.3308	0.567006
TIME*Sex*group	35.50	1	35.50	1.0973	0.298404
Error	2297.21	71	32.36		

Repeated Measures ANOVA Dietary Covariate Analysis (Omega-3)

Effect	Repeated Measures Analysis of Variance (omega3d Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	*477.9176*	*1*	*477.9176*	*330.6460*	*0.000000*
Group	0.0218	1	0.0218	0.0151	0.902684
Sex	0.1817	1	0.1817	0.1257	0.723982
Group*Sex	0.2264	1	0.2264	0.1567	0.693431
Error	102.6238	71	1.4454		
TIME	*2.1417*	*1*	*2.1417*	*5.3035*	*0.024217*
TIME*Group	0.0145	1	0.0145	0.0360	0.850122
TIME*Sex	0.0063	1	0.0063	0.0155	0.901235
TIME*Group*Sex	0.0463	1	0.0463	0.1146	0.735993
Error	28.6719	71	0.4038		

Repeated Measures ANOVA Dietary Covariate Analysis (Carbohydrate)

Effect	Repeated Measures Analysis of Variance (LignanD Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	*6526094*	*1*	*6526094*	*702.6492*	*0.000000*
Sex	157	1	157	0.0169	0.897018
group	*45424*	*1*	*45424*	*4.8907*	*0.030222*
Sex*group	9731	1	9731	1.0477	0.309501
Error	659437	71	9288		
TIME	2186	1	2186	0.9310	0.337885
TIME*Sex	374	1	374	0.1593	0.690996
TIME*group	738	1	738	0.3145	0.576702
TIME*Sex*group	72	1	72	0.0305	0.861809
Error	166713	71	2348		

Repeated Measures ANOVA Dietary Covariate Analysis (Protein)

Effect	Repeated Measures Analysis of Variance (LignanDa Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	*782363.6*	*1*	*782363.6*	*699.9091*	*0.000000*
Sex	2703.0	1	2703.0	2.4181	0.124385
group	627.6	1	627.6	0.5615	0.456137
Sex*group	2551.5	1	2551.5	2.2826	0.135269
Error	79364.3	71	1117.8		
TIME	*1445.9*	*1*	*1445.9*	*4.4086*	*0.039313*
TIME*Sex	18.6	1	18.6	0.0566	0.812577
TIME*group	0.6	1	0.6	0.0018	0.966320
TIME*Sex*group	27.0	1	27.0	0.0823	0.775088
Error	23285.7	71	328.0		

Repeated Measures ANOVA Dietary Covariate Analysis (Vitamin A)

Effect	Repeated Measures Analysis of Variance (LignanData[July200 Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	*2.612716E+10*	*1*	*2.612716E+10*	*273.3990*	*0.000000*
Sex	3.628979E+08	1	3.628979E+08	3.7974	0.055284
group	1.546057E+07	1	1.546057E+07	0.1618	0.688730
Sex*group	4.898677E+07	1	4.898677E+07	0.5126	0.476361
Error	6.785060E+09	71	9.556423E+07		
TIME	9.982927E+04	1	9.982927E+04	0.0038	0.951258
TIME*Sex	8.530975E+06	1	8.530975E+06	0.3216	0.572452
TIME*group	1.894941E+06	1	1.894941E+06	0.0714	0.790041
TIME*Sex*group	3.448615E+06	1	3.448615E+06	0.1300	0.719508
Error	1.883545E+09	71	2.652881E+07		

Repeated Measures ANOVA Dietary Covariate Analysis (Vitamin B6)

Effect	Repeated Measures Analysis of Variance (LignanDa Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	*555.4905*	*1*	*555.4905*	*652.8937*	*0.000000*
Sex	0.1820	1	0.1820	0.2140	0.645093
group	0.4679	1	0.4679	0.5500	0.460780
Sex*group	0.8512	1	0.8512	1.0005	0.320589
Error	60.4077	71	0.8508		
TIME	0.4451	1	0.4451	2.1261	0.149214
TIME*Sex	0.2701	1	0.2701	1.2900	0.259859
TIME*group	0.0004	1	0.0004	0.0021	0.963383
TIME*Sex*group	0.0022	1	0.0022	0.0106	0.918291
Error	14.8647	71	0.2094		

Repeated Measures ANOVA Dietary Covariate Analysis (Vitamin C)

Effect	Repeated Measures Analysis of Variance (LignanD Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	*2002329*	*1*	*2002329*	*446.4571*	*0.000000*
Sex	7783	1	7783	1.7353	0.191970
group	*26899*	*1*	*26899*	*5.9976*	*0.016796*
Sex*group	2306	1	2306	0.5142	0.475689
Error	318430	71	4485		
TIME	1300	1	1300	1.3830	0.243518
TIME*Sex	546	1	546	0.5810	0.448438
TIME*group	37	1	37	0.0391	0.843856
TIME*Sex*group	172	1	172	0.1829	0.670170
Error	66753	71	940		

Repeated Measures ANOVA Dietary Covariate Analysis (Folate)

Effect	Repeated Measures Analysis of Variance (LignanDat Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	*21041046*	*1*	*21041046*	*581.4759*	*0.000000*
Sex	1807	1	1807	0.0499	0.823835
group	72358	1	72358	1.9996	0.161706
Sex*group	65366	1	65366	1.8064	0.183217
Error	2569176	71	36186		
TIME	7080	1	7080	0.7183	0.399555
TIME*Sex	3529	1	3529	0.3580	0.551536
TIME*group	2616	1	2616	0.2654	0.608066
TIME*Sex*group	2592	1	2592	0.2630	0.609862
Error	699881	71	9857		

Repeated Measures ANOVA Dietary Covariate Analysis (Vitamin D)

Effect	Repeated Measures Analysis of Variance (LignanD Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	*4986397*	*1*	*4986397*	*193.3367*	*0.000000*
Sex	4356	1	4356	0.1689	0.682331
group	22993	1	22993	0.8915	0.348274
Sex*group	1385	1	1385	0.0537	0.817441
Error	1831179	71	25791		
TIME	14920	1	14920	1.8339	0.179957
TIME*Sex	19456	1	19456	2.3915	0.126440
TIME*group	4154	1	4154	0.5106	0.477223
TIME*Sex*group	3447	1	3447	0.4237	0.517211
Error	577617	71	8135		

Repeated Measures ANOVA Dietary Covariate Analysis (Vitamin E)

Effect	Repeated Measures Analysis of Variance (LignanDa Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	*16749.76*	*1*	*16749.76*	*696.8201*	*0.000000*
Sex	15.56	1	15.56	0.6474	0.423732
group	67.74	1	67.74	2.8182	0.097597
Sex*group	51.24	1	51.24	2.1318	0.148682
Error	1706.66	71	24.04		
TIME	19.97	1	19.97	2.4716	0.120383
TIME*Sex	12.41	1	12.41	1.5360	0.219302
TIME*group	1.60	1	1.60	0.1983	0.657484
TIME*Sex*group	10.46	1	10.46	1.2947	0.259003
Error	573.78	71	8.08		

Repeated Measures ANOVA Dietary Covariate Analysis (Iron)

Effect	Repeated Measures Analysis of Variance (LignanDa Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	*27654.23*	*1*	*27654.23*	*644.8001*	*0.000000*
Sex	81.63	1	81.63	1.9034	0.172030
group	52.57	1	52.57	1.2258	0.271956
Sex*group	119.97	1	119.97	2.7973	0.098826
Error	3045.05	71	42.89		
TIME	*67.56*	*1*	*67.56*	*4.5346*	*0.036684*
TIME*Sex	0.53	1	0.53	0.0355	0.851011
TIME*group	1.91	1	1.91	0.1279	0.721671
TIME*Sex*group	11.00	1	11.00	0.7379	0.393212
Error	1057.88	71	14.90		

Repeated Measures ANOVA Dietary Covariate Analysis (Zinc)

Effect	Repeated Measures Analysis of Variance (LignanDa Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	*21978.40*	*1*	*21978.40*	*452.4697*	*0.000000*
Sex	30.38	1	30.38	0.6255	0.431644
group	57.10	1	57.10	1.1756	0.281925
Sex*group	165.00	1	165.00	3.3968	0.069496
Error	3448.78	71	48.57		
TIME	8.62	1	8.62	0.6589	0.419656
TIME*Sex	0.05	1	0.05	0.0037	0.951605
TIME*group	1.71	1	1.71	0.1310	0.718448
TIME*Sex*group	0.22	1	0.22	0.0171	0.896344
Error	929.17	71	13.09		

ANOVA Covariate Analysis (Kilocalories of energy expenditure)

Effect	Univariate Tests of Significance for kcal/wk (kcalwk[a Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	*77899681*	*1*	*77899681*	*193.7851*	*0.000000*
Group	1148364	1	1148364	2.8567	0.094455
Error	36179113	90	401990		

Repeated Measures ANOVA for IL-6 Experiment I

Effect	Repeated Measures Analysis of Variance (LignanDa Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	*6793.760*	*1*	*6793.760*	*124.4023*	*0.000000*
Sex	118.334	1	118.334	2.1668	0.144938
group	6.573	1	6.573	0.1204	0.729561
Sex*group	49.660	1	49.660	0.9093	0.343163
Error	4368.896	80	54.611		
TIME	132.418	3	44.139	1.9527	0.121773
TIME*Sex	26.471	3	8.824	0.3903	0.760059
TIME*group	136.696	3	45.565	2.0158	0.112354
TIME*Sex*group	32.326	3	10.775	0.4767	0.698802
Error	5425.079	240	22.604		

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Repeated Measures ANOVA for TNF-α Experiment I

Effect	Repeated Measures Analysis of Variance (LignanDa Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	*2029.566*	*1*	*2029.566*	*142.4178*	*0.000000*
Sex	5.917	1	5.917	0.4152	0.521173
group	2.960	1	2.960	0.2077	0.649832
Sex*group	5.257	1	5.257	0.3689	0.545313
Error	1140.063	80	14.251		
TIME	18.753	3	6.251	1.0553	0.368804
TIME*Sex	2.317	3	0.772	0.1304	0.941953
TIME*group	5.748	3	1.916	0.3235	0.808391
TIME*Sex*group	43.189	3	14.396	2.4304	0.065861
Error	1421.620	240	5.923		

Repeated Measures ANOVA for Metabolic Syndrome Score Experiment I

Effect	Repeated Measures Analysis of Variance (LignanDa Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	*3.22573*	*1*	*3.225730*	*5.15842*	*0.025573*
Sex	0.00237	1	0.002368	0.00379	0.951067
group	*2.57710*	*1*	*2.577102*	*4.12117*	*0.045370*
Sex*group	0.01736	1	0.017363	0.02777	0.868043
Error	55.02926	88	0.625332		
TIME	*1.80607*	*1*	*1.806071*	*20.59772*	*0.000018*
TIME*Sex	0.00203	1	0.002025	0.02310	0.879548
TIME*group	0.15728	1	0.157278	1.79371	0.183924
TIME*Sex*group	*0.39142*	*1*	*0.391423*	*4.46407*	*0.037447*
Error	7.71611	88	0.087683		

Repeated Measures ANOVA for TAG Experiment I

Effect	Repeated Measures Analysis of Variance (LignanDa Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	*821.7824*	*1*	*821.7824*	*209.4249*	*0.000000*
Sex	0.0415	1	0.0415	0.0106	0.918282
group	*19.0743*	*1*	*19.0743*	*4.8609*	*0.030141*
Sex*group	1.5208	1	1.5208	0.3876	0.535235
Error	337.4636	86	3.9240		
TIME	0.0443	3	0.0148	0.0823	0.969599
TIME*Sex	0.1132	3	0.0377	0.2101	0.889327
TIME*group	1.1728	3	0.3909	2.1776	0.091102
TIME*Sex*group	*1.8688*	*3*	*0.6229*	*3.4699*	*0.016749*
Error	46.3182	258	0.1795		

Repeated Measures ANOVA for HDL Experiment I

Effect	Repeated Measures Analysis of Variance (LignanDa Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	*690.5694*	*1*	*690.5694*	*1516.386*	*0.000000*
Sex	*6.6576*	*1*	*6.6576*	*14.619*	*0.000248*
group	*1.8180*	*1*	*1.8180*	*3.992*	*0.048876*
Sex*group	0.1244	1	0.1244	0.273	0.602576
Error	39.1648	86	0.4554		
TIME	*1.3701*	*3*	*0.4567*	*29.206*	*0.000000*
TIME*Sex	0.0378	3	0.0126	0.805	0.491912
TIME*group	0.0465	3	0.0155	0.992	0.397148
TIME*Sex*group	0.0624	3	0.0208	1.331	0.264868
Error	4.0343	258	0.0156		

Repeated Measures ANOVA for glucose Experiment I

Effect	Repeated Measures Analysis of Variance (LignanDa Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	*10201.94*	*1*	*10201.94*	*10000.66*	*0.000000*
Sex	*11.25*	*1*	*11.25*	*11.03*	*0.001319*
group	0.00	1	0.00	0.00	0.976381
Sex*group	0.00	1	0.00	0.00	0.974471
Error	87.73	86	1.02		
TIME	0.13	3	0.04	0.48	0.710603
TIME*Sex	0.16	3	0.05	0.57	0.635442
TIME*group	0.18	3	0.06	0.65	0.582756
TIME*Sex*group	0.04	3	0.01	0.14	0.933282
Error	24.33	258	0.09		

Repeated Measure ANOVA for abdominal fat Experiment I

Effect	Repeated Measures Analysis of Variance (LignanData[. Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	*802599650*	*1*	*802599650*	*588.9708*	*0.000000*
Sex	*18096264*	*1*	*18096264*	*13.2796*	*0.000488*
group	1525881	1	1525881	1.1197	0.293327
Sex*group	577875	1	577875	0.4241	0.516882
Error	103566380	76	1362716		
TIME	16037	1	16037	0.4524	0.503225
TIME*Sex	13768	1	13768	0.3884	0.535002
TIME*group	51900	1	51900	1.4642	0.230016
TIME*Sex*group	44454	1	44454	1.2541	0.266292
Error	2693920	76	35446		

Repeated Measures ANOVA for systolic blood pressure Experiment I

Effect	Repeated Measures Analysis of Variance (LignanD Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	*2385178*	*1*	*2385178*	*7574.705*	*0.000000*
Sex	641	1	641	2.034	0.158152
group	469	1	469	1.491	0.226124
Sex*group	97	1	97	0.309	0.580128
Error	22357	71	315		
TIME	27	1	27	0.413	0.522645
TIME*Sex	1	1	1	0.019	0.891102
TIME*group	18	1	18	0.278	0.599379
TIME*Sex*group	1	1	1	0.009	0.925280
Error	4644	71	65		

Repeated Measures ANOVA for diastolic blood pressure Experiment I

Effect	Repeated Measures Analysis of Variance (LignanDa Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	*964127.3*	*1*	*964127.3*	*8856.655*	*0.000000*
Sex	404.9	1	404.9	3.719	0.057780
group	336.0	1	336.0	3.086	0.083272
Sex*group	113.4	1	113.4	1.041	0.310958
Error	7729.0	71	108.9		
TIME	47.7	1	47.7	2.078	0.153877
TIME*Sex	42.3	1	42.3	1.841	0.179109
TIME*group	28.0	1	28.0	1.218	0.273523
TIME*Sex*group	*94.9*	*1*	*94.9*	*4.131*	*0.045836*
Error	1631.5	71	23.0		

Repeated Measures ANOVA for leukocytes Experiment I

Effect	Repeated Measures Analysis of Variance (LignanDa Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	*10222.19*	*1*	*10222.19*	*1789.578*	*0.000000*
Sex	0.00	1	0.00	0.000	0.999440
group	5.37	1	5.37	0.940	0.335127
Sex*group	0.31	1	0.31	0.054	0.817013
Error	462.68	81	5.71		
TIME	0.83	3	0.28	0.785	0.503050
TIME*Sex	0.67	3	0.22	0.639	0.590540
TIME*group	1.44	3	0.48	1.367	0.253620
TIME*Sex*group	1.16	3	0.39	1.104	0.348263
Error	85.37	243	0.35		

Repeated Measures ANOVA for lymphocytes Experiment I

Effect	Repeated Measures Analysis of Variance (LignanDa Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	*1080.339*	*1*	*1080.339*	*1177.094*	*0.000000*
Sex	0.057	1	0.057	0.062	0.804458
group	*5.133*	*1*	*5.133*	*5.592*	*0.020430*
Sex*group	2.900	1	2.900	3.159	0.079245
Error	74.342	81	0.918		
TIME	0.038	3	0.013	0.356	0.784634
TIME*Sex	0.010	3	0.003	0.098	0.961305
TIME*group	0.018	3	0.006	0.168	0.917876
TIME*Sex*group	0.090	3	0.030	0.848	0.468942
Error	8.633	243	0.036		

Repeated Measures ANOVA for monocytes Experiment I

Effect	Repeated Measures Analysis of Variance (LignanDa Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	*69.05821*	*1*	*69.05821*	*1214.795*	*0.000000*
Sex	0.01141	1	0.01141	0.201	0.655402
group	0.03074	1	0.03074	0.541	0.464249
Sex*group	0.00032	1	0.00032	0.006	0.940132
Error	4.60486	81	0.05685		
TIME	0.01021	3	0.00340	0.912	0.435975
TIME*Sex	0.01214	3	0.00405	1.083	0.356773
TIME*group	0.00171	3	0.00057	0.153	0.927894
TIME*Sex*group	0.01700	3	0.00567	1.518	0.210534
Error	0.90750	243	0.00373		

Repeated Measures ANOVA for eosinophils Experiment I

Effect	Repeated Measures Analysis of Variance (LignanDa Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	*10.46650*	*1*	*10.46650*	*142.5905*	*0.000000*
Sex	0.06046	1	0.06046	0.8237	0.366797
group	0.16423	1	0.16423	2.2373	0.138600
Sex*group	0.03053	1	0.03053	0.4159	0.520816
Error	5.94560	81	0.07340		
TIME	0.01101	3	0.00367	1.0012	0.392956
TIME*Sex	*0.03802*	*3*	*0.01267*	*3.4577*	*0.017107*
TIME*group	0.00054	3	0.00018	0.0492	0.985518
TIME*Sex*group	0.01027	3	0.00342	0.9343	0.424700
Error	0.89069	243	0.00367		

Repeated Measures ANOVA for basophils Experiment I

Effect	Repeated Measures Analysis of Variance (LignanDa Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	*0.110143*	*1*	*0.110143*	*29.52393*	*0.000001*
Sex	0.004608	1	0.004608	1.23516	0.269694
group	0.006639	1	0.006639	1.77955	0.185944
Sex*group	0.000594	1	0.000594	0.15920	0.690943
Error	0.302182	81	0.003731		
TIME	*0.012059*	*3*	*0.004020*	*6.31467*	*0.000385*
TIME*Sex	0.002159	3	0.000720	1.13032	0.337417
TIME*group	0.001362	3	0.000454	0.71300	0.545081
TIME*Sex*group	0.002477	3	0.000826	1.29714	0.276019
Error	0.154688	243	0.000637		

Appendix H ANOVA Tables from Flaxseed Oil Study

ANOVA Covariate Analysis (Age)

Effect	Univariate Tests of Significance for Age (FlaxOilDat Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	217692.4	1	217692.4	6117.976	0.000000
Group	6.1	1	6.1	0.172	0.680436
Error	1743.5	49	35.6		

ANOVA Covariate Analysis (Height)

Effect	Univariate Tests of Significance for Height (FlaxOil Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	1456246	1	1456246	16596.42	0.000000
Group	0	1	0	0.00	0.985898
Error	4299	49	88		

ANOVA Covariate Analysis (Body Mass)

Effect	Univariate Tests of Significance for Wtpre (FlaxOilD Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	299419.0	1	299419.0	1239.985	0.000000
Group	117.3	1	117.3	0.486	0.489096
Error	11832.0	49	241.5		

ANOVA Covariate Analysis (Chest Press Muscle Strength)

Effect	Univariate Tests of Significance for CPpre (FlaxOilD Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	689864.3	1	689864.3	173.8606	0.000000
Group	364.3	1	364.3	0.0918	0.763178
Error	194427.9	49	3967.9		

ANOVA Covariate Analysis (Leg Press Muscle Strength)

Effect	Univariate Tests of Significance for LPpre (FlaxOil Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	*4544575*	*1*	*4544575*	*411.6505*	*0.000000*
Group	449	1	449	0.0407	0.840929
Error	540954	49	11040		

ANOVA Covariate Analysis (Lean Tissue Mass)

Effect	Univariate Tests of Significance for Leanpre (FlaxOilData.sta) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	*1.368660E+11*	*1*	*1.368660E+11*	*1086.121*	*0.000000*
Group	1.497729E+07	1	1.497729E+07	0.119	0.731756
Error	6.174668E+09	49	1.260136E+08		

Repeated Measures ANOVA Dietary Covariate Analysis (Kilocalories)

Effect	Repeated Measures Analysis of Variance (Spreadsheet Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	*293520369*	*1*	*293520369*	*532.0016*	*0.000000*
Sex	1196238	1	1196238	2.1682	0.147560
Group	1523857	1	1523857	2.7620	0.103186
Sex*Group	953567	1	953567	1.7283	0.195004
Error	25931231	47	551728		
TIME	341510	1	341510	3.3959	0.071670
TIME*Sex	57642	1	57642	0.5732	0.452775
TIME*Group	8664	1	8664	0.0862	0.770412
TIME*Sex*Group	19158	1	19158	0.1905	0.664493
Error	4726550	47	100565		

Repeated Measures ANOVA Dietary Covariate Analysis (Total Fat)

Effect	Repeated Measures Analysis of Variance (Spreadsh Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	*483182.1*	*1*	*483182.1*	*312.1157*	*0.000000*
Sex	743.0	1	743.0	0.4799	0.491864
Group	813.7	1	813.7	0.5256	0.472054
Sex*Group	752.2	1	752.2	0.4859	0.489213
Error	72760.1	47	1548.1		
TIME	*811.3*	*1*	*811.3*	*4.3706*	*0.041999*
TIME*Sex	96.3	1	96.3	0.5190	0.474836
TIME*Group	105.0	1	105.0	0.5655	0.455791
TIME*Sex*Group	27.5	1	27.5	0.1480	0.702213
Error	8724.6	47	185.6		

Repeated Measures ANOVA Dietary Covariate Analysis (MUFA)

Effect	Repeated Measures Analysis of Variance (Spreadsh Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	*76022.75*	*1*	*76022.75*	*275.0662*	*0.000000*
Sex	163.91	1	163.91	0.5930	0.445096
Group	40.75	1	40.75	0.1474	0.702724
Sex*Group	328.36	1	328.36	1.1881	0.281278
Error	12989.85	47	276.38		
TIME	*174.94*	*1*	*174.94*	*5.5399*	*0.022821*
TIME*Sex	37.79	1	37.79	1.1966	0.279580
TIME*Group	10.83	1	10.83	0.3431	0.560849
TIME*Sex*Group	0.67	1	0.67	0.0211	0.885157
Error	1484.21	47	31.58		

Repeated Measures ANOVA Dietary Covariate Analysis (omega-6)

Effect	Repeated Measures Analysis of Variance (Spreadsh Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	*27846.72*	*1*	*27846.72*	*294.8411*	*0.000000*
Sex	2.93	1	2.93	0.0310	0.860887
Group	150.28	1	150.28	1.5911	0.213390
Sex*Group	16.67	1	16.67	0.1765	0.676277
Error	4438.99	47	94.45		
TIME	30.77	1	30.77	1.9803	0.165941
TIME*Sex	0.01	1	0.01	0.0004	0.984814
TIME*Group	5.41	1	5.41	0.3485	0.557818
TIME*Sex*Group	11.70	1	11.70	0.7533	0.389851
Error	730.20	47	15.54		

Repeated Measures ANOVA Dietary Covariate Analysis (omega-3)

Effect	Repeated Measures Analysis of Variance (Spreadsh Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	*254.7075*	*1*	*254.7075*	*277.3071*	*0.000000*
Sex	0.0620	1	0.0620	0.0675	0.796214
Group	2.6041	1	2.6041	2.8351	0.098855
Sex*Group	0.3729	1	0.3729	0.4060	0.527093
Error	43.1697	47	0.9185		
TIME	0.0683	1	0.0683	0.3283	0.569386
TIME*Sex	0.0000	1	0.0000	0.0000	0.994657
TIME*Group	0.3512	1	0.3512	1.6878	0.200229
TIME*Sex*Group	0.0708	1	0.0708	0.3404	0.562365
Error	9.7809	47	0.2081		

Repeated Measures ANOVA Dietary Covariate Analysis (Carbohydrate)

Effect	Repeated Measures Analysis of Variance (Spreads Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	*4354979*	*1*	*4354979*	*584.4422*	*0.000000*
Sex	14238	1	14238	1.9108	0.173414
Group	*53070*	*1*	*53070*	*7.1220*	*0.010417*
Sex*Group	*38605*	*1*	*38605*	*5.1808*	*0.027437*
Error	350221	47	7452		
TIME	3239	1	3239	1.3258	0.255382
TIME*Sex	2106	1	2106	0.8622	0.357868
TIME*Group	1	1	1	0.0002	0.987772
TIME*Sex*Group	157	1	157	0.0643	0.800968
Error	114821	47	2443		

Repeated Measures ANOVA Dietary Covariate Analysis (Protein)

Effect	Repeated Measures Analysis of Variance (Spreadsh Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	*439712.1*	*1*	*439712.1*	*368.5872*	*0.000000*
Sex	1304.8	1	1304.8	1.0937	0.300998
Group	1479.8	1	1479.8	1.2405	0.271044
Sex*Group	789.7	1	789.7	0.6620	0.419972
Error	56069.4	47	1193.0		
TIME	*764.7*	*1*	*764.7*	*4.3268*	*0.042949*
TIME*Sex	60.8	1	60.8	0.3439	0.560376
TIME*Group	0.0	1	0.0	0.0002	0.988566
TIME*Sex*Group	142.6	1	142.6	0.8072	0.373520
Error	8302.4	47	176.6		

Repeated Measures ANOVA Dietary Covariate Analysis (Vitamin A)

Effect	Repeated Measures Analysis of Variance (Dietary_DATA.sta) Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	*1.532153E+10*	*1*	*1.532153E+10*	*138.0926*	*0.000000*
Sex	1.703036E+08	1	1.703036E+08	1.5349	0.221524
Group	3.172259E+08	1	3.172259E+08	2.8592	0.097478
Sex*Group	1.930192E+08	1	1.930192E+08	1.7397	0.193570
Error	5.214700E+09	47	1.109511E+08		
TIME	4.109510E+07	1	4.109510E+07	1.4938	0.227727
TIME*Sex	2.279455E+07	1	2.279455E+07	0.8286	0.367334
TIME*Group	3.935854E+06	1	3.935854E+06	0.1431	0.706956
TIME*Sex*Group	1.174513E+06	1	1.174513E+06	0.0427	0.837198
Error	1.293020E+09	47	2.751107E+07		

Repeated Measures ANOVA Dietary Covariate Analysis (Vitamin B6)

Effect	Repeated Measures Analysis of Variance (Dietary_D Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	*344.0281*	*1*	*344.0281*	*395.5691*	*0.000000*
Sex	0.9745	1	0.9745	1.1205	0.295222
Group	1.8439	1	1.8439	2.1201	0.152022
Sex*Group	0.8470	1	0.8470	0.9739	0.328775
Error	40.8761	47	0.8697		
TIME	0.1303	1	0.1303	0.7744	0.383337
TIME*Sex	0.0254	1	0.0254	0.1508	0.699538
TIME*Group	0.0000	1	0.0000	0.0001	0.992637
TIME*Sex*Group	0.0148	1	0.0148	0.0881	0.767876
Error	7.9071	47	0.1682		

Repeated Measures ANOVA Dietary Covariate Analysis (Vitamin C)

Effect	Repeated Measures Analysis of Variance (Dietary_ Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	*1499583*	*1*	*1499583*	*318.1337*	*0.000000*
Sex	1320	1	1320	0.2801	0.599104
Group	*25204*	*1*	*25204*	*5.3469*	*0.025188*
Sex*Group	4988	1	4988	1.0582	0.308898
Error	221543	47	4714		
TIME	709	1	709	0.3980	0.531201
TIME*Sex	24	1	24	0.0132	0.908885
TIME*Group	94	1	94	0.0530	0.818902
TIME*Sex*Group	324	1	324	0.1816	0.671925
Error	83727	47	1781		

Repeated Measures ANOVA Dietary Covariate Analysis (Vitamin D)

Effect	Repeated Measures Analysis of Variance (Dietary_ Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	*2887388*	*1*	*2887388*	*111.2755*	*0.000000*
Sex	258	1	258	0.0099	0.920973
Group	9447	1	9447	0.3641	0.549155
Sex*Group	22355	1	22355	0.8615	0.358056
Error	1219561	47	25948		
TIME	15175	1	15175	3.9092	0.053906
TIME*Sex	*18442*	*1*	*18442*	*4.7508*	*0.034330*
TIME*Group	422	1	422	0.1087	0.743051
TIME*Sex*Group	7761	1	7761	1.9994	0.163957
Error	182451	47	3882		

Repeated Measures ANOVA Dietary Covariate Analysis (Vitamin E)

Effect	Repeated Measures Analysis of Variance (Dietary_D Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	*10079.38*	*1*	*10079.38*	*341.5758*	*0.000000*
Sex	27.75	1	27.75	0.9403	0.337161
Group	67.85	1	67.85	2.2994	0.136121
Sex*Group	48.40	1	48.40	1.6401	0.206595
Error	1386.90	47	29.51		
TIME	14.01	1	14.01	3.8650	0.055227
TIME*Sex	1.17	1	1.17	0.3228	0.572635
TIME*Group	0.00	1	0.00	0.0000	0.998924
TIME*Sex*Group	2.10	1	2.10	0.5802	0.450044
Error	170.42	47	3.63		

Repeated Measures ANOVA Dietary Covariate Analysis (Folate)

Effect	Repeated Measures Analysis of Variance (Dietary_D Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	*13824738*	*1*	*13824738*	*420.7932*	*0.000000*
Sex	309	1	309	0.0094	0.923099
Group	124916	1	124916	3.8022	0.057166
Sex*Group	41539	1	41539	1.2643	0.266541
Error	1544138	47	32854		
TIME	20726	1	20726	3.7245	0.059668
TIME*Sex	580	1	580	0.1042	0.748231
TIME*Group	20	1	20	0.0036	0.952338
TIME*Sex*Group	1594	1	1594	0.2865	0.594989
Error	261542	47	5565		

Repeated Measures ANOVA Dietary Covariate Analysis (Iron)

Effect	Repeated Measures Analysis of Variance (Dietary_D Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	*17489.40*	*1*	*17489.40*	*409.4284*	*0.000000*
Sex	85.94	1	85.94	2.0118	0.162673
Group	36.53	1	36.53	0.8553	0.359789
Sex*Group	32.14	1	32.14	0.7525	0.390096
Error	2007.68	47	42.72		
TIME	18.62	1	18.62	2.7835	0.101889
TIME*Sex	0.53	1	0.53	0.0786	0.780399
TIME*Group	0.90	1	0.90	0.1348	0.715198
TIME*Sex*Group	0.02	1	0.02	0.0031	0.955615
Error	314.46	47	6.69		

Repeated Measures ANOVA Dietary Covariate Analysis (Zinc)

Effect	Repeated Measures Analysis of Variance (Dietary_D Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	*13204.07*	*1*	*13204.07*	*271.0451*	*0.000000*
Sex	25.83	1	25.83	0.5303	0.470113
Group	46.34	1	46.34	0.9513	0.334382
Sex*Group	34.10	1	34.10	0.6999	0.407052
Error	2289.62	47	48.72		
TIME	12.17	1	12.17	1.5938	0.213011
TIME*Sex	1.14	1	1.14	0.1492	0.701064
TIME*Group	1.75	1	1.75	0.2287	0.634734
TIME*Sex*Group	0.26	1	0.26	0.0346	0.853158
Error	359.01	47	7.64		

Repeated Measures ANOVA for IL-6 Experiment II

Effect	Repeated Measures Analysis of Variance (FlaxOilDa Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	*3718.049*	*1*	*3718.049*	*102.9713*	*0.000000*
Sex	*322.036*	*1*	*322.036*	*8.9188*	*0.004513*
Group	15.828	1	15.828	0.4384	0.511220
Sex*Group	1.863	1	1.863	0.0516	0.821313
Error	1660.951	46	36.108		
TIME	10.859	1	10.859	1.4206	0.239416
TIME*Sex	*63.712*	*1*	*63.712*	*8.3348*	*0.005907*
TIME*Group	0.518	1	0.518	0.0678	0.795774
TIME*Sex*Group	30.083	1	30.083	3.9354	0.053266
Error	351.630	46	7.644		

Repeated Measures ANOVA for TNF-α Experiment II

Effect	Repeated Measures Analysis of Variance (FlaxOilDa Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	*3793.233*	*1*	*3793.233*	*182.9232*	*0.000000*
Sex	*159.858*	*1*	*159.858*	*7.7089*	*0.007866*
Group	0.711	1	0.711	0.0343	0.853878
Sex*Group	0.090	1	0.090	0.0043	0.947701
Error	974.627	47	20.737		
TIME	0.042	1	0.042	0.0115	0.914898
TIME*Sex	*18.459*	*1*	*18.459*	*5.0287*	*0.029686*
TIME*Group	1.105	1	1.105	0.3010	0.585882
TIME*Sex*Group	10.690	1	10.690	2.9122	0.094512
Error	172.522	47	3.671		

Repeated Measures ANOVA for chest press strength Experiment II

Effect	Repeated Measures Analysis of Variance (FlaxOilD Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	*1705371*	*1*	*1705371*	*629.6481*	*0.000000*
Sex	*292783*	*1*	*292783*	*108.0999*	*0.000000*
Group	79	1	79	0.0291	0.865378
Sex*Group	6261	1	6261	2.3115	0.135264
Error	124589	46	2708		
TIME	*42886*	*1*	*42886*	*147.4087*	*0.000000*
TIME*Sex	*2212*	*1*	*2212*	*7.6042*	*0.008325*
TIME*Group	54	1	54	0.1868	0.667626
TIME*Sex*Group	12	1	12	0.0422	0.838108
Error	13383	46	291		

Repeated Measures ANOVA for leg press strength Experiment II

Effect	Repeated Measures Analysis of Variance (FlaxOilDa Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	*10010749*	*1*	*10010749*	*676.5601*	*0.000000*
Sex	*441988*	*1*	*441988*	*29.8710*	*0.000002*
Group	5244	1	5244	0.3544	0.554610
Sex*Group	15231	1	15231	1.0294	0.315725
Error	665844	45	14797		
TIME	*77194*	*1*	*77194*	*125.8535*	*0.000000*
TIME*Sex	23	1	23	0.0369	0.848543
TIME*Group	207	1	207	0.3376	0.564103
TIME*Sex*Group	63	1	63	0.1021	0.750781
Error	27601	45	613		

Repeated Measures ANOVA for elbow flexor muscle thickness Experiment II

Effect	Repeated Measures Analysis of Variance (FlaxOilDa Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	*1262.685*	*1*	*1262.685*	*1552.596*	*0.000000*
Sex	2.272	1	2.272	2.793	0.101314
Group	0.851	1	0.851	1.047	0.311480
Sex*Group	*4.090*	*1*	*4.090*	*5.029*	*0.029685*
Error	38.224	47	0.813		
TIME	*1.800*	*1*	*1.800*	*16.408*	*0.000190*
TIME*Sex	0.004	1	0.004	0.040	0.841423
TIME*Group	0.104	1	0.104	0.947	0.335410
TIME*Sex*Group	0.017	1	0.017	0.158	0.693111
Error	5.155	47	0.110		

Repeated Measures ANOVA for elbow extensor muscle thickness Experiment II

Effect	Repeated Measures Analysis of Variance (FlaxOilDa Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	*1900.048*	*1*	*1900.048*	*905.7070*	*0.000000*
Sex	6.011	1	6.011	2.8653	0.097129
Group	1.068	1	1.068	0.5091	0.479061
Sex*Group	0.259	1	0.259	0.1236	0.726698
Error	98.599	47	2.098		
TIME	*12.001*	*1*	*12.001*	*19.5538*	*0.000058*
TIME*Sex	1.627	1	1.627	2.6517	0.110128
TIME*Group	0.304	1	0.304	0.4952	0.485100
TIME*Sex*Group	1.169	1	1.169	1.9045	0.174101
Error	28.845	47	0.614		

Repeated Measures ANOVA for knee extensor muscle thickness Experiment II

Effect	Repeated Measures Analysis of Variance (FlaxOilDa Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	1350.284	1	1350.284	2068.717	0.000000
Sex	0.822	1	0.822	1.259	0.267478
Group	0.586	1	0.586	0.897	0.348385
Sex*Group	0.558	1	0.558	0.855	0.359904
Error	30.678	47	0.653		
TIME	2.358	1	2.358	16.988	0.000152
TIME*Sex	0.336	1	0.336	2.420	0.126533
TIME*Group	0.043	1	0.043	0.311	0.579466
TIME*Sex*Group	0.193	1	0.193	1.392	0.243945
Error	6.525	47	0.139		

Repeated Measures ANOVA for knee flexor muscle thickness Experiment II

Effect	Repeated Measures Analysis of Variance (FlaxOilDa Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	2806.580	1	2806.580	706.2000	0.000000
Sex	2.295	1	2.295	0.5775	0.451077
Group	0.882	1	0.882	0.2219	0.639802
Sex*Group	1.926	1	1.926	0.4845	0.489816
Error	186.787	47	3.974		
TIME	9.464	1	9.464	7.0824	0.010619
TIME*Sex	0.097	1	0.097	0.0729	0.788369
TIME*Group	0.538	1	0.538	0.4022	0.529018
TIME*Sex*Group	5.286	1	5.286	3.9558	0.052549
Error	62.807	47	1.336		

Repeated Measures ANOVA for lean tissue mass Experiment II

Effect	Repeated Measures Analysis of Variance (FlaxOilData.sta) Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	2.646469E+11	1	2.646469E+11	3413.016	0.000000
Sex	8.600813E+09	1	8.600813E+09	110.920	0.000000
Group	5.639441E+07	1	5.639441E+07	0.727	0.398088
Sex*Group	2.902381E+08	1	2.902381E+08	3.743	0.059059
Error	3.644402E+09	47	7.754048E+07		
TIME	1.132978E+07	1	1.132978E+07	8.579	0.005232
TIME*Sex	2.929585E+05	1	2.929585E+05	0.222	0.639830
TIME*Group	2.350983E+06	1	2.350983E+06	1.780	0.188559
TIME*Sex*Group	4.395054E+05	1	4.395054E+05	0.333	0.566773
Error	6.207114E+07	47	1.320662E+06		

Repeated Measures ANOVA for percent body fat Experiment II

Effect	Repeated Measures Analysis of Variance (FlaxOilDa Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	*86996.30*	*1*	*86996.30*	*1406.566*	*0.000000*
Sex	*3273.07*	*1*	*3273.07*	*52.919*	*0.000000*
Group	17.06	1	17.06	0.276	0.601918
Sex*Group	63.79	1	63.79	1.031	0.315020
Error	2906.96	47	61.85		
TIME	*15.51*	*1*	*15.51*	*12.516*	*0.000920*
TIME*Sex	0.19	1	0.19	0.156	0.694479
TIME*Group	1.05	1	1.05	0.845	0.362802
TIME*Sex*Group	0.62	1	0.62	0.502	0.481990
Error	58.23	47	1.24		

Repeated Measures ANOVA for fat mass Experiment II

Effect	Repeated Measures Analysis of Variance (FlaxOilData.sta) Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	*4.997567E+10*	*1*	*4.997567E+10*	*363.7163*	*0.000000*
Sex	4.183480E+08	1	4.183480E+08	3.0447	0.087538
Group	7.623470E+07	1	7.623470E+07	0.5548	0.460061
Sex*Group	2.038407E+08	1	2.038407E+08	1.4835	0.229303
Error	6.457936E+09	47	1.374029E+08		
TIME	1.783198E+06	1	1.783198E+06	0.3165	0.576413
TIME*Sex	3.303083E+06	1	3.303083E+06	0.5862	0.447720
TIME*Group	7.593750E+06	1	7.593750E+06	1.3477	0.251548
TIME*Sex*Group	1.518057E+06	1	1.518057E+06	0.2694	0.606162
Error	2.648317E+08	47	5.634717E+06		