The Relationship Between Adiponectin Levels and Appendicular Lean Mass in Postmenopausal Women

Thesis

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By

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Abstract

Menopause is associated with a rapid change in body composition characterized by an increase in fat mass and a decrease in lean mass. Loss of skeletal muscle mass is associated with increased morbidity, mortality, and reduced quality of life. Adiponectin is a protein secreted from adipocytes that may play an important role in modulating energy metabolism by increasing mitochondrial structure and function in skeletal muscle. To assess the relationship of skeletal muscle mass and adiponectin in postmenopausal women, we conducted secondary analysis from data collected in five clinical studies at The Ohio State University. Ninety-five postmenopausal women were evaluated for body composition by dual energy x-ray absorptiometry and total plasma or serum adiponectin levels by enzyme-linked immunoabsorbent assay or electrochemilluminescence. Increases in adiponectin levels were associated with increased appendicular lean mass when adjusted for body mass index in postmenopausal women without diabetes. This relationship between adiponectin and lean body mass suggests that increasing adiponectin levels through dietary or pharmacological approaches may aid in preventing skeletal muscle loss in healthy, postmenopausal women.
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Major Field: Human Nutrition
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<tr>
<td>ACC1</td>
<td>Acetyl CoA Carboxylase 1</td>
</tr>
<tr>
<td>ALM</td>
<td>Appendicular Lean Mass</td>
</tr>
<tr>
<td>ALM/BMI</td>
<td>Appendicular Lean Mass adjusted for Body Mass Index</td>
</tr>
<tr>
<td>AMPK</td>
<td>AMP-activated protein kinase</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>CRP</td>
<td>C-Reactive Protein</td>
</tr>
<tr>
<td>CT</td>
<td>Computed Tomography</td>
</tr>
<tr>
<td>DEXA</td>
<td>Dual X-Ray Absorptiometry</td>
</tr>
<tr>
<td>FNIH</td>
<td>Foundation for the National Institute of Health</td>
</tr>
<tr>
<td>IL-6</td>
<td>Interleukin-6</td>
</tr>
<tr>
<td>MetS</td>
<td>Metabolic Syndrome</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>p38 MAPK</td>
<td>p38 Mitogen-Activated Protein Kinase</td>
</tr>
<tr>
<td>PAES</td>
<td>Physical Activity and Education Services</td>
</tr>
<tr>
<td>T2DM</td>
<td>Type 2 Diabetes</td>
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Chapter 1: Introduction

Menopause is associated with a rapid change in body composition characterized by an increase in trunk fat mass and a decrease in lean mass [1]. Sarcopenia refers to a loss of muscle mass associated with muscle weakness that develops with aging [2]. Close to half of individuals 80 years or older are affected by sarcopenia [3] and it results in adverse health effects such as increased risk for disability, frailty, and mortality [4]. The three components that characterize sarcopenia are a loss of muscle mass, a decrease in muscle strength, and a decrease in muscle function [5]. Although there are no standard diagnostic criteria to determine sarcopenia, recent guidelines have been proposed so sarcopenia can be diagnosed and treated. Identifying biomarkers to predict for sarcopenia could aid in the prevention of muscle loss.

Adiponectin is a protein secreted primarily from subcutaneous adipocytes that exerts a variety of biological effects on the body [6]. Adiponectin has been shown to improve insulin sensitivity, reduce inflammation, and be anti-atherogenic [7]. Adiponectin may also play an important role in modulating energy metabolism in skeletal muscle. Skeletal muscle has two adiponectin receptors, AdipoR1 and AdipoR2. Binding of adiponectin to these receptors induces signal transduction pathways that promote mitochondrial biogenesis and oxidative myofiber formation, which are important markers of functional
skeletal muscle [8, 9]. We propose that understanding the relationship between adiponectin and lean muscle mass may aid in designing ways to prevent sarcopenia.

**Aims**

1. Examine the relationship between adiponectin and appendicular lean mass in postmenopausal women.

2. Examine whether age, diabetic status, dietary protein, dietary vitamin D, serum/plasma linoleic acid, and interleukin-6 will modify any associations between adiponectin and appendicular lean mass in postmenopausal women.

**Hypotheses**

1. Increased levels of adiponectin will predict for increases in appendicular lean mass in postmenopausal women.

2. When the covariates (age, diabetic status, dietary protein, dietary vitamin D, and interleukin-6) are accounted for, the association between adiponectin and appendicular lean mass in postmenopausal women will be stronger.
Chapter 2: Literature Review

2.1 Menopausal Changes in Women

Menopause is associated with a rapid change in body composition characterized by an increase in trunk fat mass and a decrease in lean mass [1]. Perimenopause is associated with a change in body composition from gynoid to android fat distribution [10]. This increase in visceral adipose, intra-abdominal fat that surrounds the inner organs, coincides with an increased risk for metabolic syndrome and chronic diseases. The rates of abdominal obesity were 65.5% for women 40 – 59 years old and 73.8% for women over 60 in the United States in 2008 [10]. Hormonal changes, aging, psychosocial factors, genetics, changes in physical activity, and changes in eating patterns all contribute to the weight gain that occurs with menopause [10]. This weight gain occurs with a concomitant decrease in muscle mass. Muscle mass decreases after menopause at a rate of about 3% per year with a cumulative total loss of muscle mass of 30 – 50% by age 80 [11].

There are a variety of methods used to assess these changes in body composition. Computed tomography (CT) and magnetic resonance imaging (MRI) are the gold standards for measuring body composition. However, due to their high cost and the
high radiation exposure associated with CT, other methods are often used for assessing body composition. Dual energy x-ray absorptiometry, DEXA, has been shown to accurately measure body composition [5]. The machine is readily available, less expensive than MRI and CT, and emits less radiation than CT [12]. DEXA may be used as a valid method to assess skeletal muscle mass in postmenopausal women 50 – 79 [5]. DEXA can accurately measure fat mass and fat free mass although it may slightly overestimate lean mass and underestimate fat mass in obese subjects [12].

2.2 Sarcopenia

Sarcopenia is the loss of muscle mass that occurs with aging. Besides aging, the loss of muscle mass is affected by hormonal changes, chronic diseases, decreases in physical activity, and changes in dietary pattern [13]. About 50% of total body mass is lean muscle in young adults and this decreases to about 25% in older adults [4]. This decrease in lean muscle mass is accompanied by an increase in intramuscular triglyceride accumulation in muscle, which reduces muscle quality and may impair muscle function [14]. Additionally, mitochondrial dysfunction associated with aging results in increased reactive oxygen species. An increase in reactive oxygen species can cause a state of chronic low-grade inflammation. Biomarkers of inflammation are significantly associated with sarcopenia [14].

Regeneration of skeletal muscle mass is important in delaying the onset sarcopenia. Muscle cells, or myofibers, are regenerated via satellite cells that differentiate into myoblasts when activated to repair damaged myofibers. As people age this regeneration
process slows and the ability to repair damaged muscle decreases resulting in scar tissue, inflammation, and intramuscular triglyceride accumulation. This delayed healing process may be due to a decrease in the number of satellite cells or a failure of the aged satellite cells to respond normally to the muscle damage. As a result, decreases in muscle mass and strength may occur [15].

There are currently no clinical diagnostic criteria to identify sarcopenia. Muscle mass, muscle strength, and muscle function are used to assess for sarcopenia. Muscle mass is frequently reported by appendicular muscle mass which is the summation of arm and leg lean muscle mass. Appendicular muscle has been found to be the best predictor for skeletal muscle mass when compared to total body muscle mass [5] because over 75% of skeletal muscle is found in the appendicular regions and this is the primary muscle involved in walking and physical activity [14, 16, 17].

A variety of definitions have been proposed to assess for sarcopenia. The Foundation for the National Institute of Health (FNIH) Sarcopenia Project was founded to propose universal clinical cutoff values that can be used to assess sarcopenia. First, the FNIH has proposed low lean mass to be defined as an appendicular muscle mass standardized to body mass index of less than 0.512 in women and less than 0.789 in men [18]. These cutoff values identify when lean mass is associated with a higher likelihood of muscle weakness and physical disability [18]. Second, the FNIH has proposed decreased muscle function, or mobility impairment, to be defined as a gait speed of less than 0.8 meters/second (m/s). Mobility impairment is important for the assessment of sarcopenia
because of its association with muscle weakness, frailty, and reduced quality of life [18]. Third, the FNIH has proposed muscle weakness to be defined as a maximal grip strength of less than 16 kg in women and less than 26 kg in men. Intermediate levels of weakness have been proposed to be a grip strength of less than 20 kg in women and less than 32 kg in men. These values have been associated with an increased risk for mobility impairment associated with disability and mortality [19]. For analyses of my thesis, we have data for muscle mass but not muscle function or muscle strength.

2.3 Adiponectin

Adiponectin is a hormone secreted primarily from subcutaneous adipocytes. Adiponectin is protective against cardiovascular disease and type 2 diabetes mellitus, T2DM [7]. Adiponectin acts as an anti-inflammatory and increases insulin sensitivity [7]. Increasing levels of total adiposity [20] and visceral adiposity [21] are associated with decreasing levels of adiponectin.

Adiponectin exists in several different forms: In adipocytes, adiponectin protein is a monomer. In circulation, adiponectin undergoes oligomerization to form trimmers (low molecular weight), hexamers (medium molecular weight), or multimers (high molecular weight). In circulation, adiponectin primarily exists in full-length form and constitutes about 0.01% of plasma protein [22, 23]. Full-length adiponectin can also be proteolytically cleaved to form globular adiponectin which constitutes less than 1% of circulating adiponectin [23]. The high molecular weight form is the most biologically active form; however, adiponectin receptor R1 (AdipoR1) on skeletal muscle has a higher
affinity for globular adiponectin [23]. The concentration of the high molecular weight form may be more strongly, inversely associated with insulin resistance and metabolic syndrome than total adiponectin levels [22, 24]. For this analysis, total adiponectin values were measured. There are no normative values for adiponectin concentrations in humans. Adiponectin concentrations in the blood usually vary between 0.5 and 30 µg/mL [25]. However, adiponectin concentrations vary greatly depending on the population being studied and the method used to determine adiponectin concentrations. Factors affecting adiponectin levels are described in Section 2.4.

Adiponectin plays an important role in lipid and glucose metabolism. Adiponectin receptors exist on skeletal muscle and the liver. Skeletal muscle contains two adiponectin receptors, AdipoR1 and AdipoR2 [6]. AdipoR1 is the primary adiponectin receptor on skeletal muscle and is most responsive to globular adiponectin [26]. AdipoR2 is the primary receptor on the liver and is most responsive to full-length adiponectin [26]. Adiponectin activates AdipoR1 on skeletal muscle and promotes Ca^{2+} influx. This Ca^{2+} influx activates Calmodulin-dependent protein kinase kinase-B, CaMKKB. CaMKKB, along with liver kinase B1, LKB1 promote full activation of AMP-activated protein kinase, AMPK. AMPK activates SIRT1 and PGC-1α [8]. PGC-1α expression and activation increases which promotes mitochondrial biogenesis, oxidative metabolism, and formation of myofibers [8]. AMPK phosphorylates acetyl CoA carboxylase 1, ACC1, inhibiting conversion of acetyl CoA to malonyl CoA. Inhibition of carnitine palmitoyl transferase 1, CPT-1, by malonyl CoA is decreased [26]. Fatty acids are transported into the mitochondria and fatty acid oxidation can increase [27].
Adiponectin is important for increased mitochondrial biogenesis in skeletal muscle. The binding of adiponectin to adiponectin receptors activates p38 mitogen-activated protein kinase, p38 MAPK. Activation of p38 MAPK induces mitochondrial biogenesis via increased gene expression and activity of PGC-1α [28]. Adiponectin has been found to suppress MAPK phosphatase-1, MKP1, which inhibits p38 MAPK. Therefore, inhibition of MKP1 allows for expression of PGC-1α and mitochondrial biogenesis in skeletal muscle [28].

Skeletal muscle is one of the primary organs involved in glucose uptake and metabolism [29]. Insulin, secreted from the pancreas, is important in regulation of blood glucose levels. Increases in fasting blood glucose levels can lead to the development of T2DM. Adiponectin acts as an insulin sensitizer. Adiponectin stimulates translocation of the GLUT4 receptor to increase glucose uptake in skeletal muscle [26]. Activation of AMPK inhibits hepatic gluconeogenesis and improves insulin sensitivity via the down regulation of glucose-6-phosphotase and phosphoenolpyruvate carboxykinase [23]. AMPK inactivates glycogen synthase to slow the formation of glycogen [26]. Higher adiponectin levels are associated with a lower risk for T2DM [30]. Thiazolidinediones, TZDs, are used to treat those with impaired insulin sensitivity and alter adiponectin levels. Rosiglitazone is a TZD that activates PPARγ and improves insulin sensitivity. Among African Americans with T2DM, treatment with rosiglitazone is associated with increased adiponectin levels [31].

Adiponectin plays a role in the prevention of cardiovascular disease. It decreases the
expression of adhesion molecules that play a role in atherosclerosis [32]. Adiponectin inhibits NF-κB, a transcription factor that regulates production of cytokines and other inflammatory molecules. Among postmenopausal women (n=27), adiponectin gene expression is positively associated with IκB-α mRNA in adipose tissue [33]. IκB-α inhibits activation of NF-κB and subsequent increases in proinflammatory molecules. Thus, adiponectin slows the progression of atherosclerosis and low-grade inflammation that can alter metabolic processes.

2.4 Factors that Affect Adiponectin Levels

2.4.1 Age, Body Mass Index, and Gender

Adiponectin levels vary depending on a person’s age, body mass index (BMI), and gender. Among healthy adults 45 – 70 years old, total adiponectin levels are positively associated with age. Adults 59 years or older have higher adiponectin levels than those under 59 after adjusting for BMI. Whites compared to non-whites have significantly higher adiponectin levels. Individuals with BMI levels in the normal range have significantly higher adiponectin levels than overweight and obese adults. [34]

Varying associations between adiponectin and muscle mass have been reported depending on the population investigated. Among males over 54 years old (n = 73) with chronic heart failure there is an inverse, negative association between adiponectin levels and arm lean mass and muscle strength [16]. Among healthy males (n = 20) there is no significant association between adiponectin levels and arm lean mass and muscle strength [16]. Among healthy premenopausal women (n = 66) there is no significant association
between adiponectin and total lean mass and appendicular lean mass [35]. Among South Korean women (n = 162) there is an inverse, negative correlation between adiponectin and total lean body mass and arm lean body mass [36]. Among South Korean men (n = 152) there is no significant correlation between adiponectin and total lean mass but there is a significant, negative correlation between adiponectin and arm lean body mass [36]. Differences in age, gender, and overall health status appear to affect adiponectin levels and the relationship between adiponectin and lean mass.

2.4.2 Breast Cancer History

A history of breast cancer may alter a person’s energy metabolism affecting skeletal muscle mass and adiponectin concentrations. Postmenopausal women (n = 2281) with low adiponectin levels are at increased risk for breast cancer when adjusted for cofounders such as BMI and age [22, 37]. Among breast cancer survivors, higher adiponectin levels are associated with a 61% decreased risk of breast cancer related death [38]. No data are available to compare adiponectin levels of breast cancer survivors and age-matched controls.

2.4.3 Central Obesity

Central obesity has a variety of metabolic effects on the body that affect adiponectin concentrations. On average, women (n=69) experience a 10.94% increase in trunk adipose mass during the menopausal transition [21]. Visceral fat is more significantly related to metabolic syndrome, insulin resistance, and T2DM than subcutaneous fat [39]. Increased visceral fat is associated with decreased adiponectin concentrations. The
inverse association between central obesity and adiponectin levels is stronger than the association between total fat mass or BMI to adiponectin concentrations [39]. At least a 10% decrease in weight is needed before adiponectin levels significantly increase in humans [24].

Increases in central obesity may affect the interactions between adipose tissue and muscle. Adipose tissue and muscle secrete a variety of adipokines and myokines, respectively. These proteins are important for signaling between adipose and muscle cells that control metabolic processes. Adipokines and myokines play an important role in the modulation of the ratio of lean-to-fat mass. When this ratio becomes imbalanced, the secretions of cytokines are affected. For example, myostatin secretions from myocytes are increased in extremely obese women compared to lean healthy subjects, which inhibits muscle growth [40]. Some cytokines, such as IL-6, are released from both myocytes and adipocytes and can induce different signaling pathways depending on location of secretion [41].

**2.4.4 Hormone Replacement Therapy**

The use of hormone replacement therapy affects the body composition of women. Perimenopause is associated with fluctuating changes in hormone levels. Decreases in estrogen, progesterone, and progestin levels increase abdominal adipose accumulation [42]. Postmenopausal women (n = 76) taking hormone replacement therapy have significantly higher adiponectin levels than women who do not [32]. Hormone therapy suppresses weight gain in post-menopausal women, supporting the hypothesis that
adiponectin levels would be higher in post-menopausal women on hormone therapy [42]. However, results are mixed with other studies showing no significant difference in adiponectin levels for those on hormone therapy [42, 43].

2.4.5 Linoleic Acid

Linoleic Acid, 18:2n6, is an essential omega-6 fatty acid. Linoleic acid plays an important role in cellular membrane structure and function, regulation of gene expression, and eicosanoid synthesis. Linoleic acid is metabolized via desaturation and elongation reactions to form arachidonic acid, a precursor for leukotrienes, prostaglandins, and thromboxanes. Traditional food sources rich in linoleic acid include vegetable oils such as safflower, soybean, grape seed, and corn oil as well as in many nuts and seeds. Total lean mass significantly increased in healthy individuals (n=39) when overfed muffins that contained high-linoleic sunflower oil of about 40 grams per day for seven weeks. The increase in lean mass was nearly three times greater compared to when the individuals were overfed with muffins that contained palm oil [44]. In postmenopausal women with T2DM, 8 grams a day of high-linoleic safflower oil for 16 weeks significantly increased total lean body mass by about 1.6% [45].

2.4.6 Leptin

Like adiponectin, leptin is an adipokine secreted primarily from adipose. Leptin sends signals to the hypothalamus to suppress appetite. Increased leptin levels are associated with increasing BMI and waist circumference [46]. Leptin acts on both adipose and skeletal tissue to increase lipid oxidation and glucose transport. In skeletal muscle,
administration of leptin decreases myofibril protein synthesis [47]. In elderly overweight and obese women, leptin is an independent predictor of adiponectin gene expression [33].

2.4.7 Inflammatory Markers: IL-6 and C-Reactive Protein

Chronic low-grade inflammation contributes to the loss of muscle mass seen with sarcopenia. Cytokines secreted as a result of inflammation exert catabolic effects on muscle mass and muscle strength [48]. Interleukin-6, IL-6, is a marker of inflammation that is secreted from both adipose and skeletal muscle. IL-6 levels increase with increasing adiposity [47]. Administration of IL-6 in rats causes catabolism of muscle [49]. Among 70 – 79 year old men and women (n = 3075) increased IL-6 levels are associated with decreased muscle mass and muscle strength [48]. C-reactive protein (CRP), also a marker of inflammation, is synthesized by the liver in response to increases in IL-6. Increasing levels of adiponectin have been associated with decreasing levels of high-sensitive CRP [33]. High levels of CRP have been shown in women with a BMI ≥ 30 kg/m² [33]. However, not all women with a high BMI have elevated levels of CRP [33]. Increased levels of IL-6 and CRP in men and women (n = 986) are associated with a 2 to 3 fold increased risk of losing over 40% of their muscle strength [49].

2.4.7 Dietary Protein and Dietary Vitamin D Intake

Deficiencies in protein intake and vitamin D are thought to affect the development of sarcopenia. The recommended dietary intake (RDA) for protein in adults is 0.8 grams per kilogram of body weight per day (g/kg/day). Approximately 27 – 41% of adult women do not meet the RDA for dietary protein [50]. Difficulty chewing and
swallowing, the high cost of protein rich foods, and perceived intolerances or aversions to certain foods may contribute decreased protein intake that occurs with aging [50, 51]. However, moderately increasing protein intake in adults may help promote protein anabolism and prevent protein catabolism [50]. Additionally, low vitamin D intake in older people may contribute to sarcopenia [52]. Binding of vitamin D to vitamin D receptors on skeletal muscle increases muscle mass, strength, and function [53]. As people age, adipose mass accumulates in muscle [54] and deficiencies in vitamin D can result in atrophy of type II muscle fibers and further increase intramuscular adipose tissue [55]. Lipid accumulation in skeletal muscle can impair insulin-signaling transduction (prevents Glut4 translocation and impair insulin receptor substrate, IRS-1) and is associated with insulin resistance [27]. Current treatment methods for sarcopenia include increased protein intake (up to 1.2 g/kg/day) and vitamin D supplementation [52] as studies have shown that increasing protein in the diet can enhance muscle mass, strength, and function [4, 52, 56] and supplementing the diet with vitamin D can improve muscle strength and function [34, 35[53]].
Chapter 3: Study Design and Methodology

I hypothesize that adiponectin has the potential to be used a biomarker to predict for sarcopenia; to test this hypothesis the relationship between appendicular lean mass and adiponectin will be analyzed in postmenopausal women. In addition, the relationship between adiponectin and trunk adipose mass will be analyzed. This secondary analysis will be performed on data collected from five randomized control trials conducted at The Ohio State University between 2004 and 2015 [45, 57, 58]. All data investigated for this thesis is baseline data, before the women underwent the study interventions. Several covariates that could affect the relationship between appendicular lean mass and adiponectin, e.g., age, diabetic status, dietary protein, dietary vitamin D, serum/plasma linoleic acid levels, and IL-6 will also be investigated.

3.1 Study I

Healthy women, some of whom were breast cancer survivors, were recruited between 2011 and 2013 for Study I [57]. For this analysis only postmenopausal women were included. Women were excluded from the study if they had a history of any other prior cancers besides breast cancer, chronic obstructive pulmonary disease, symptomatic ischemic heart disease, alcohol/drug abuse, immune-related conditions, or major inflammatory diseases. Women were excluded if they were on lipid lowering medications, angiotensin type I receptor blockers, or medications with major
immunological or endocrinological consequences. Further details about the study can be found in the paper published by Kiecolt-Glaser et al [57].

3.2 Study II
Postmenopausal, married women without any chronic health problems, between ages 21 and 65 were recruited for Study II between 2011 and 2013 [58]. Individuals were excluded for smoking, alcohol, or drug abuse, HbA1C > 6.5, anemia, and prescription medication excluding levothyroxine. Individuals with a BMI < 25 were excluded if they vigorously exercised more than 2 hours per week and individuals with a BMI ≥ 25 were excluded if they vigorously exercised more than 5 hours per week. Further details about the study can be found in the paper published by Kiecolt-Glaser et al [58].

3.3 Study III
Overweight or obese (BMI ≥ 25 kg/m²), postmenopausal women with T2DM (HbA1c ≥ 6.5% and ≤ 14%) between the ages of 18 and 70 were recruited from 2004-2007 for Study III. Subjects were excluded for substance and tobacco use, renal disease, abnormal kidney function, gastrointestinal diseases and disorders, impaired cognitive function, insulin use, hormone replacement therapy, use of a pacemaker or defibrillator. Further details about the study can be found in the paper published by Norris et al [45].

3.4 Study IV
Postmenopausal women ages 30-70 years with a BMI between 25 and 45 kg/m² and a diagnosis of T2DM, with a HbA1c ≤ 9% were recruited between 2008 and 2011 for Study IV. Individuals were excluded from the study if they had abnormal liver, kidney,
gastrointestinal, myocardial or cognitive functions. In addition they were excluded for use of insulin, hormone replacement therapies, over the counter medications that could affect endpoint measurements, or substance abuse (personal communication, Dr. Martha Belury).

3.5 Study V
Postmenopausal women aged 50 to 69 were enrolled between 2014 and 2015 for Study V. Subjects were overweight or obese (BMI \( \geq 25 \) and \( \leq 55 \) kg/m\(^2\)) and had a waist circumference \( > 88 \) cm. The women met at least one of the following metabolic syndrome criteria: elevated triglycerides (>150 mg/dl), reduced HDL-C (<50mg/dl), elevated blood pressure (>130mm Hg systolic or >85 mm Hg diastolic), or elevated blood glucose (>100 mg/dl and <126 mg/dl). Individuals were excluded if they had T2DM, unstable weight, were taking medications that could affect body composition, or had recently used hormone replacement therapy. In addition, individuals were excluded from the study if they had abnormal liver, kidney, gastrointestinal, myocardial or cognitive functions or had history of substance abuse (personal communication, Dr. Martha Belury).

3.6 Location of Studies
All studies visits occurred at the Ohio State University; Visits for Studies I-IV occurred at the Clinical Research Center at The Ohio State University. Visits for Study V visits occurred at the Human Performance Laboratory at The Ohio State University.

3.7 Biochemical Assays
Cytokine and adipocytokine analyses were performed on blood samples collected after a minimum 10 hour fast. The samples were centrifuged, and the plasma or serum was extracted and then stored at -70°C or -80°C until analyses were performed. Total adiponectin levels were measured. In Studies I and II, IL-6 and adiponectin were measured via electrochemiluminescence method using Meso Scale Discovery kits, and the Meso Scale Discovery Sector Imager 2400. Samples values were extrapolated using a four parameter logistic fit [58]. In the Study III, leptin, IL-6, CRP, and adiponectin were analyzed by the CRC with kits from Linco Research Inc, St Charles, MO. In Studies IV and V, adiponectin and IL-6 were analyzed via Enzyme-Linked Immunosorbent Assay (ELISA) according to the manufacturer’s instructions (Millipore Corporation, St. Charles, MO 63304 and Temecula, CA 92590).

3.8 Body Composition

Body composition was assessed via dual energy x-ray absorptiometry (DEXA). DEXAs used at the Clinical Research Center (CRC) included a Lunar iDEXA and a Lunar DPX-NT (GE Healthcare, Fairfield, CT). The DEXA machine used in the Human Performance Laboratory was a Lunar iDEXA (Lunar Corp, Madison, WI). To assess the validity of the DEXA machine used, two scans were performed on a subset of the subjects to determine the coefficient of variance. The coefficient of variance for trunk adipose mass is unavailable for Studies I and II. The coefficient of variance for trunk adipose mass in Studies III, IV, and V were 1.83% (n=12), 1.16% (n=2), and 0.72% (n=11).

3.9 Dietary Analysis
Dietary intake was assessed by 24-hour recalls in Studies I and II and 3-day food records in Studies III, IV, and V. For Study I subjects were contacted 1 – 3 times and asked about their diet in the previous 24 hours. For Study II subjects were contacted 1 – 2 times and asked about their diet in the previous 24 hours. The data are an average of the 24-hour recalls. For Studies III, IV, and V subjects completed 3-day food records and study investigators clarified any ambiguous descriptions at the next study visit. Dietary records were analyzed using the Nutrition Data System for Research (University of Minnesota, NCC Food and Nutrient Database).

3.10 Fatty Acid Analysis

A portion of collected blood was aliquoted for plasma, serum, or erythrocyte fatty acid analysis. Specifically, this thesis investigated linoleic acid. Lipids from plasma or serum were extracted with chloroform:methanol (2:1 v:v) and methylated into fatty acid methyl esters. Lipids from erythrocytes were extracted and methylated with boron trifluoride into fatty acid methyl esters. All fatty acid methyl esters were analyzed by gas chromatography using a 30-m Omegawax capillary column (Supelco Chromotography Products) and helium as a carrier gas. Retention times were compared to authentic standards.

3.11 Statistical Analysis

Descriptive data of the cohorts are expressed as means ± SD. Pearson correlations were used to determine the relationship among adiponectin, IL-6, appendicular lean mass (ALM), trunk adipose mass, and various subject characteristics. ANOVA and Tukey’s multiple comparisons were used to determine differences among studies. A linear-mixed
effect model was used to investigate the relationship between adiponectin and ALM/BMI for tests of group differences and interactions. Explanatory variables were added into the model as fixed effects including age, IL-6, protein, and vitamin D intake. IL-6 values were right-skewed, therefore, all analyses for IL-6 used natural-log transformed values to better approximate normality of residuals. Of primary interest for the models were the effects of adiponectin, T2DM status, and IL-6 on ALM/BMI and trunk adipose mass. The interaction among adiponectin, T2DM, and IL-6 were also investigated. A random effect for the different studies was included in the model. Data were analyzed using SPSS software (IBM Version 22).
Chapter 4: Results
### Table 4.1 Comparison of Demographics of Subjects by Study

<table>
<thead>
<tr>
<th></th>
<th>Study I (n = 44)</th>
<th>Study II (n = 3)</th>
<th>Study III (n = 11)</th>
<th>Study IV (n = 21)</th>
<th>Study V (n = 16)</th>
<th>Total (n = 95)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>55 (7.00)</td>
<td>51 (1.00)</td>
<td>59 (8.16)</td>
<td>58 (6.55)</td>
<td>59 (5.12)</td>
<td>57 (6.83)</td>
<td>0.107</td>
</tr>
<tr>
<td>Range</td>
<td>43-75</td>
<td>50-52</td>
<td>47-70</td>
<td>49-70</td>
<td>52-69</td>
<td>43-75</td>
<td></td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>28.2 (5.21)</td>
<td>23.8 (4.19)</td>
<td>31.0 (3.87)</td>
<td>35.2 (5.35)</td>
<td>31.1 (4.34)</td>
<td>30.4 (5.69)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>BMI &lt; 25, N (%)</td>
<td>12 (27%)</td>
<td>2 (67%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>14 (15%)</td>
<td></td>
</tr>
<tr>
<td>BMI 25-29.99, N (%)</td>
<td>18 (41%)</td>
<td>1 (33%)</td>
<td>6 (55%)</td>
<td>4 (19%)</td>
<td>7 (44%)</td>
<td>36 (28%)</td>
<td></td>
</tr>
<tr>
<td>BMI &gt; 30, N (%)</td>
<td>14 (32%)</td>
<td>0 (0%)</td>
<td>5 (45%)</td>
<td>17 (81%)</td>
<td>9 (56%)</td>
<td>45 (47%)</td>
<td></td>
</tr>
<tr>
<td><strong>Race, N (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>36 (82%)</td>
<td>2 (66%)</td>
<td>8 (73%)</td>
<td>20 (95%)</td>
<td>13 (81%)</td>
<td>79 (83%)</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>7 (16%)</td>
<td>1 (33%)</td>
<td>2 (18%)</td>
<td>1 (5%)</td>
<td>3 (19%)</td>
<td>14 (15%)</td>
<td></td>
</tr>
<tr>
<td>American Indian/</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (9%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (1%)</td>
<td></td>
</tr>
<tr>
<td>Alaskan Native</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pacific</td>
<td>1 (2%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (1%)</td>
<td></td>
</tr>
<tr>
<td><strong>T2DM, N (%)</strong></td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>11 (100%)</td>
<td>21 (100%)</td>
<td>0 (0%)</td>
<td>32 (34%)</td>
<td></td>
</tr>
<tr>
<td><strong>History of Cancer, N (%)</strong></td>
<td>34 (77%)</td>
<td>0 (0%)</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>34 (74%)</td>
<td>n=46</td>
</tr>
<tr>
<td><strong>Hormone Replacement Therapy, N (%)</strong></td>
<td>0 (0%)</td>
<td>1 (33%)</td>
<td>0 (0%)</td>
<td>1 (5%)</td>
<td>0 (0%)</td>
<td>2 (2%)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>Years Since Menopause</strong></td>
<td><strong>Mean (SD)</strong></td>
<td>n/a</td>
<td>11.6 (11.2)</td>
<td>12.7 (10.1)</td>
<td>9.3 (8.8)</td>
<td>11.2 (9.8)</td>
<td>0.592</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>n/a</td>
<td>1-37</td>
<td>0-35</td>
<td>2-37</td>
<td>n=46</td>
<td></td>
</tr>
</tbody>
</table>

Values are Mean (SD) unless otherwise noted.
* Missing 2 values for FACT
Table 4.1 Comparison of Demographics of Subjects by Study

Demographic descriptions of the subjects are categorized by study and the summation of all five studies. Years since menopause is not available for I and II and information about previous cancer history is not available for Studies III, IV, and V. Data is expressed as mean (standard deviation) or number (% of total number).

4.1 Demographics of the Studies

Differences in demographics among the studies are reported in Table 4.1. All subjects were postmenopausal women with an average of 11.2 years since their last menstrual cycle. Majority of the women in all studies were Caucasian and the overall average age for all the women was 57 years. There was a significant difference in BMI among studies (p < 0.001). Study I and II included women with a BMI in the normal category (<25 kg/m²) while study III, IV, and V did not. All of the women in Study III and IV had T2DM while none of the women in Study I, II, or V did. There was a significant difference in use of hormone replacement therapy among the studies (p < 0.001); 2 subjects were on hormone replacement therapy while in the studies. 77% of the women from Study I had a history of breast cancer. None of the subjects had cancer while in the studies.
# Table 4.2: Comparison of baseline body composition measurements by study.

Baseline body composition measurements of the subjects are categorized by each study and the summation of all five studies. Appendicular lean mass, total lean mass, and trunk adipose tissue were measured by DEXA. Values are expressed as mean (SD). One-way ANOVA was used to determine differences between studies. Data are considered statistically significant at P<0.05.

<table>
<thead>
<tr>
<th>Study</th>
<th>Study II</th>
<th>Study III</th>
<th>Study IV</th>
<th>Study V</th>
<th>Total</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 44)</td>
<td>(n = 11)</td>
<td>(n = 21)</td>
<td>(n = 16)</td>
<td>(n = 95)</td>
<td></td>
</tr>
<tr>
<td>Appendicular Lean Mass/BMI</td>
<td>0.66 (0.090)</td>
<td>0.58 (0.077)</td>
<td>0.57 (0.075)</td>
<td>0.64 (0.070)</td>
<td>0.63 (0.095)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Range</td>
<td>0.53-0.99</td>
<td>0.45-0.68</td>
<td>0.45-0.72</td>
<td>0.50-0.78</td>
<td>0.45-0.99</td>
<td></td>
</tr>
<tr>
<td>Total Lean Mass/BMI</td>
<td>1.50 (0.21)</td>
<td>1.36 (0.14)</td>
<td>1.29 (0.18)</td>
<td>1.38 (0.17)</td>
<td>1.43 (0.22)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Trunk Adipose Mass (kg)</td>
<td>16.13 (5.92)</td>
<td>19.72 (3.05)</td>
<td>25.10 (6.85)</td>
<td>19.01 (4.66)</td>
<td>18.86 (6.71)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>
Table 4.3: Comparison of baseline blood measurements by study.

<table>
<thead>
<tr>
<th>Blood Measurements</th>
<th>Study I (n = 44)</th>
<th>Study II (n = 3)</th>
<th>Study III (n = 11)</th>
<th>Study IV (n = 21)</th>
<th>Study V (n = 16)</th>
<th>Total (n =95)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiponectin (μg/ml)*</td>
<td>17.74 (7.28)</td>
<td>16.22 (5.69)</td>
<td>6.65 (4.59)</td>
<td>10.75 (8.57)</td>
<td>13.12 (5.77)</td>
<td>14.23 (7.91)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>IL-6 (pg/ml)**</td>
<td>2.99 (3.68)</td>
<td>0.7 (0.87)</td>
<td>2.26 (1.66)</td>
<td>17.98 (7.31)</td>
<td>13.99 (4.33)</td>
<td>7.32 (7.93)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CRP (mg/l)***</td>
<td>1.74 (1.39)</td>
<td>1.08 (0.89)</td>
<td>0.99 (1.50)</td>
<td>n/a</td>
<td>n/a</td>
<td>1.56 (1.41)</td>
<td>0.254</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>33.12 (26.14)</td>
<td>19.91 (18.62)</td>
<td>22.37 (12.75)</td>
<td>n/a</td>
<td>n/a</td>
<td>30.40 (24.09)</td>
<td>0.313</td>
</tr>
<tr>
<td>Linoleic Acid (plasma/serum, % of total fatty acid content)#</td>
<td>30.68 (3.35)</td>
<td>37.07 (1.86)</td>
<td>26.58 (11.37)</td>
<td>27.00 (3.60)</td>
<td>n/a</td>
<td>29.43 (4.18)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Linoleic Acid (erythrocytes, % of total fatty acid content)</td>
<td>10.79 (1.2)</td>
<td>12.32 (0.77)</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>10.88 (1.23)</td>
<td>0.036</td>
</tr>
</tbody>
</table>

Values are Mean (SD) unless otherwise noted.
*Missing 4 values for Study IV
**Missing 4 values for Study IV, 4 for Study V, deleted 1 outlier in Study III
***Deleted 1 outlier in Study I
#Missing 1 value for Study I, 2 values for Study III
Table 4.3: Comparison of baseline blood measurements by study.

Baseline measurements of the subjects are categorized by each study and the summation of all five studies. Adipokines and cytokines were measured from plasma or serum. Data on CRP and leptin were unavailable for Studies IV and V. Erythrocytes were not collected for fatty acid analysis in Study III and IV. Fatty acid analysis is not currently available for Study V. Values are expressed as mean (SD). One-way ANOVA was used to determine differences between studies. Data are considered statistically significant at P<0.05.
Table 4.4: Comparison of baseline dietary measurements by study.

<table>
<thead>
<tr>
<th></th>
<th>Study I (n = 44)</th>
<th>Study II (n = 3)</th>
<th>Study III (n = 11)</th>
<th>Study IV (n = 21)</th>
<th>Study V (n = 16)</th>
<th>Total (n = 92)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dietary Intake</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dietary Protein Intake (g/kg/day)</td>
<td>0.99 (0.32)</td>
<td>1.17 (0.62)</td>
<td>0.93 (0.25)</td>
<td>0.86 (0.24)</td>
<td>0.95 (0.27)</td>
<td>0.95 (0.30)</td>
<td>0.420</td>
</tr>
<tr>
<td>Dietary Vitamin D (μg)</td>
<td>3.63 (1.96)</td>
<td>11.30 (11.87)</td>
<td>5.20 (4.77)</td>
<td>4.62 (2.32)</td>
<td>3.03 (1.53)</td>
<td>4.15 (3.24)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Linoleic Acid (% of kcal)</td>
<td>7.09 (2.72)</td>
<td>9.00 (3.89)</td>
<td>6.06 (2.17)</td>
<td>6.88 (2.86)</td>
<td>7.17 (1.86)</td>
<td>7.01 (2.38)</td>
<td>0.428</td>
</tr>
</tbody>
</table>

Values are Mean (SD) unless otherwise noted.

*Missing 1 value for Study III, 2 for Study IV
Table 4.4: Comparison of baseline dietary measurements by study.

Baseline dietary measurements of the subjects are categorized by each study and the summation of all five studies. Dietary intake was collected by 24-hour recalls for Studies I and II and from 3-day food records for Studies III, IV, and V were analyzed using NDSR. Data on dietary supplement use are not available. Values are expressed as mean (SD). One-way ANOVA was used to determine differences between studies. Data are considered statistically significant at P<0.05.

4.2 Baseline Measurements of Studies

In this cohort, there were significant differences in all body composition measurements between studies (Table 4.2, p < 0.001). The average ALM/BMI was 0.63 with a range of 0.45 – 0.99, the average TLM/BMI was 1.43, and the average trunk adipose mass was 18.86 kg. There were significant differences in adiponectin and IL-6 levels among the studies (Table 4.3, p<0.001). There were significant differences in adiponectin and IL-6 levels between studies (Table 4.3, p<0.001). The average adiponectin level was 14.23 μg/ml and average IL-6 level was 7.32 pg/ml. CRP and leptin averages for Studies I, II, III were 1.56 mg/l and 30.40 ng/ml, respectively. The average linoleic acid as a percentage of total fatty acids in the plasma/serum was 29.43%. The average linoleic acid as a percentage of total fatty acids in erythrocytes was 10.88% and significantly different among studies (p =0.036). Average protein intake was 0.95 g/kg/day and 17.3% of kilocalories (not shown) and average vitamin D intake was 4.15 μg/day, which was significantly different among studies (Table 4.4). According to food records, on average
7.01% of total calories consumed were linoleic acid.
Figure 4.1: Correlation between ALM/BMI and TLM/BMI

A Pearson correlation was conducted to compare appendicular lean mass and total lean mass, both divided by BMI. Values are a ratio of lean mass measured in kilograms divided by BMI (kg/m²). Data are considered statistically significant at P<0.05.
4.3 Appendicular and Total Lean Mass

Appendicular lean mass is used to assess loss of muscle mass. ALM/BMI is used instead of TLM/BMI to measure low lean muscle mass because the appendicular regions contain over 75% of total body skeletal muscle which is important for body movement and function [16]. We compared appendicular lean mass to total lean mass to see if they are as strongly correlated as the literature suggests. There is a significant positive correlation between ALM/BMI and TLM/BMI ($R^2 = 0.890 \ p < 0.001$).
<table>
<thead>
<tr>
<th></th>
<th>Biochemical Markers</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adiponectin (µg/ml)</td>
<td>IL-6</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>-0.072</td>
<td>0.149</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>-0.301**</td>
<td>0.569**</td>
<td></td>
</tr>
<tr>
<td>T2DM</td>
<td>-0.431**</td>
<td>0.415**</td>
<td></td>
</tr>
<tr>
<td>History of Cancer</td>
<td>-0.034</td>
<td>0.013</td>
<td></td>
</tr>
<tr>
<td>Hormone Replacement Therapy</td>
<td>-0.163</td>
<td>-0.093</td>
<td></td>
</tr>
<tr>
<td>Years Since Menopause</td>
<td>0.040</td>
<td>0.197</td>
<td></td>
</tr>
<tr>
<td>Appendicular Lean Mass</td>
<td>0.271**</td>
<td>-0.379**</td>
<td></td>
</tr>
<tr>
<td>Trunk Adipose Mass (kg)</td>
<td>-0.362**</td>
<td>0.542**</td>
<td></td>
</tr>
<tr>
<td>Protein (g/kg/day)</td>
<td>-0.280**</td>
<td>0.151</td>
<td></td>
</tr>
<tr>
<td>Dietary Vitamin D (µg)</td>
<td>-0.054</td>
<td>-0.104</td>
<td></td>
</tr>
<tr>
<td>Serum/Plasma Linoleic Acid</td>
<td>0.459**</td>
<td>-0.269*</td>
<td></td>
</tr>
</tbody>
</table>

**P < 0.01  
*P < 0.05

**Table 4.5: Correlation of biochemical markers and baseline characteristics and measurements.**

Pearson correlations were performed on adiponectin and IL-6 compared to baseline demographics and baseline study measurements. All studies and available data points are combined. Data are considered statistically significant at P<0.05. P values are indicated as either <0.05 or <0.01.
Table 4.6: Correlation of body composition and baseline characteristics and measurements.

Pearson correlations were performed on appendicular lean mass and trunk adipose mass compared to baseline demographics and baseline study measurements. All studies and available data points are combined. Data are considered statistically significant at P<0.05. P values are indicated as either <0.05 or <0.01.
4.4 Relationship among biomarkers, body composition, and various subject characteristics.

The relationships among the different biomarkers and body composition components investigated are very interrelated. Correlations were investigated in order to understand how the subject characteristics are related to the outcomes of interest. According to Table 4.5, adiponectin, important for lipid and glucose metabolism, was positively correlated with ALM/BMI (p < 0.01) and serum/plasma linoleic acid (p < 0.01). Conversely, IL-6, a marker of inflammation, was negatively correlated with ALM/BMI (p < 0.01) and serum/plasma linoleic acid (p < 0.05). Adiponectin was negatively correlated with BMI, trunk fat and dietary protein (p < 0.01) while IL-6 was positively correlated to BMI and trunk fat (p < 0.01). According to Table 4.6, ALM was negatively correlated with BMI, IL-6 (p < 0.01) and positively correlated with adiponectin (p < 0.01), leptin (p < 0.05), and serum/plasma linoleic acid (p < 0.01). Trunk adipose mass was negatively correlated with adiponectin, dietary protein and serum/plasma linoleic acid (p < 0.01) while positively correlated with trunk fat, BMI (p < 0.01), IL-6 (p < 0.01), CRP (p < 0.05) and leptin (p < 0.01). Individuals with T2DM have significantly lower ALM/BM and adiponectin levels and significantly higher trunk adipose mass and IL-6 values than those without T2DM (p < 0.01).
### Number of Subjects According to ALM/BMI

<table>
<thead>
<tr>
<th></th>
<th>Study I</th>
<th>Study II</th>
<th>Study III</th>
<th>Study IV*</th>
<th>Study V</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low ALM/BMI (&lt;0.512)</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>Normal ALM/BMI (≥ 0.512)</td>
<td>44</td>
<td>3</td>
<td>8</td>
<td>16</td>
<td>15</td>
<td>86</td>
</tr>
</tbody>
</table>

### Adiponectin Levels according to ALM/BMI, Mean (SD)

<table>
<thead>
<tr>
<th></th>
<th>Study I</th>
<th>Study II</th>
<th>Study III</th>
<th>Study IV*</th>
<th>Study V</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low (&lt;0.512)</td>
<td>---</td>
<td>---</td>
<td>8.14</td>
<td>16.55</td>
<td>20.89</td>
<td>13.94 (7.24)</td>
</tr>
<tr>
<td>Normal (≥ 0.512)</td>
<td>17.73</td>
<td>16.22</td>
<td>6.09</td>
<td>8.97</td>
<td>12.60</td>
<td>14.26 (7.84)</td>
</tr>
</tbody>
</table>

*p = 0.54  p = 0.13  p = 0.91

*Four excluded from analysis because no adiponectin values, 3 Normal ALM/BMI, 1 Low ALM/BMI
Adiponectin levels are (µg/ml)

---

**Table 4.7: Adiponectin Levels compared to categorized ALM/BMI.**

The number of subjects with low ALM/BMI or normal ALM/BMI was quantified by each study and summation of all five studies. ANOVA was performed to find differences in means based on low or normal ALM/BMI. Adiponectin levels are expressed as µg/ml. Data are considered statistically significant at P<0.05.
4.5 No differences in adiponectin levels based on ALM/BMI.

In total, 9 individuals had low ALM/BMI and 86 individuals had normal ALM/BMI. No women from Study I and II had low ALM/BMI. With relatively few individuals having clinically low ALM/BMI, a goal of this thesis was to determine if adiponectin can be used as an early indicator for loss of muscle mass. There was no significant difference in adiponectin levels between those with low or normal ALM/BMI both overall (p=0.914) and across studies (Study III p=0.539, Study IV p=0.125).
A Pearson correlation was performed to compare adiponectin and ALM/BMI in Study I ($R^2 = 0.079 \ p = 0.064$). Adiponectin values are expressed as $\mu g/ml$ and ALM/BMI is a ratio of ALM measured in kilograms divided by BMI (kilograms/meters$^2$). Data are considered statistically significant at $P<0.05$. 

Figure 4.2: Correlation Between Adiponectin and ALM/BMI in Study I.
Figure 4.3: Correlation Between Adiponectin and ALM/BMI in Study II.

A Pearson correlation was performed to compare adiponectin and ALM/BMI in Study II ($R^2 = 0.780\ p =0.310$). Adiponectin values are expressed as $\mu g/ml$ and ALM/BMI is a ratio of ALM measured in kilograms divided by BMI (kilograms/meters$^2$). Data are considered statistically significant at $P<0.05$. 
Figure 4.4: Correlation Between Adiponectin and ALM/BMI in Study III.

A Pearson correlation was performed to compare adiponectin and ALM/BMI in Study III ($R^2 = 0.079 \ p = 0.404$) Adiponectin values are expressed as $\mu g/ml$ and ALM/BMI is a ratio of ALM measured in kilograms divided by BMI (kilograms/meters$^2$). Data are considered statistically significant at $P<0.05$. 
Figure 4.5: Correlation Between Adiponectin and ALM/BMI in Study IV.

A Pearson correlation was performed to compare adiponectin and ALM/BMI in Study IV (R^2 = 0.114 p = 0.186) Adiponectin values are expressed as µg/ml and ALM/BMI is a ratio of ALM measured in kilograms divided by BMI (kilograms/meters^2). Data are considered statistically significant at P<0.05.
Figure 4.6: Correlation Between Adiponectin and ALM/BMI in Study V.

A Pearson correlation was performed to compare adiponectin and ALM/BMI in Study V \((R^2 = 0.002 \ p = 0.959)\) adiponectin values are expressed as \(\mu g/ml\) and ALM/BMI is a ratio of ALM measured in kilograms divided by BMI (kilograms/meters\(^2\)). Data are considered statistically significant at \(P<0.05\).
Figure 4.7: Correlation Between Adiponectin and ALM/BMI across all studies.

Pearson correlation to compare adiponectin and ALM/BMI across all studies ($R^2 = 0.073$, $p = 0.009$). Adiponectin values are expressed as $\mu$g/ml and ALM/BMI is a ratio of ALM measured in kilograms divided by BMI (kilograms/meters$^2$). Data are considered statistically significant at $P<0.05$. 
4.6 Correlations between adiponectin and ALM/BMI.

There are currently no good biomarkers to assess for low muscle mass as measured by ALM/BMI. We investigated the relationship between adiponectin and ALM/BMI for use as a potential biomarker. There was a trend towards a significant correlation between adiponectin and ALM/BMI in the postmenopausal women from Study I (Figure 4.2: $R^2 = 0.079$ p = 0.064) unadjusted for any other covariates (n=44). There was no significant correlation between adiponectin and ALM/BMI in the postmenopausal women when unadjusted for any covariates from Study II (Figure 4.3: $R^2 = 0.780$ p =0.310 n=3), Study III (Figure 4.4: $R^2 = 0.079$ p = 0.404 n=11), Study IV (Figure 4.5: $R^2 = 0.114$ p = 0.186 n=17), or Study V (Figure 4.6: $R^2 = 0.002$ p = 0.959, n=16). When all studies were combined there was a very weak, but statistically significant correlation between adiponectin and ALM/BMI (Figure 4.7: $R^2 = 0.073$ p = 0.009 n=92), unadjusted for any other covariates.
Table 4.8: Fixed effects of adiponectin, T2DM, IL-6 and their interactions in a linear mixed-effect model of ALM/BMI

<table>
<thead>
<tr>
<th></th>
<th>Model 1 Regression Coefficient</th>
<th>P value</th>
<th>Model 2 - Age Regression Coefficient</th>
<th>P value</th>
<th>Model 3 - IL-6 Regression Coefficient</th>
<th>P value</th>
<th>Model 4 - Dietary Protein and Vitamin D Regression Coefficient</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ALM/BMI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>0.198</td>
<td>0.002</td>
<td>0.193</td>
<td>0.001</td>
<td>0.315</td>
<td>0.002</td>
<td>0.114</td>
</tr>
<tr>
<td>T2DM</td>
<td>-0.090</td>
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<td>-0.090</td>
<td>0.000</td>
<td>0.021</td>
<td>0.001</td>
<td>-0.092</td>
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<td>IL-6</td>
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<td>---</td>
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<td>0.005</td>
</tr>
<tr>
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<td>0.007</td>
<td>0.017</td>
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<td>IL-6</td>
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<td>-0.003</td>
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<td>0.006</td>
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<td>0.004</td>
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<td>---</td>
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<td>0.024</td>
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</table>

All IL-6 values are transformed to natural log
Adiponectin is measured in μg/ml, IL-6 (pg/ml), vitamin D (mcg), age (years), protein (g/kg/day)
Table 4.8: Fixed effects of adiponectin, T2DM, IL-6 and their interactions in a linear mixed-effect model of ALM/BMI.

A linear-mixed effect model was used to analyze the relationship of adiponectin, T2DM, and IL-6 to ALM/BMI. The interactions among adiponectin, T2DM, and IL-6 were also investigated. Explanatory variables were added into the model as fixed effects, which included age in model 2, IL-6 in model 3, and dietary protein and dietary vitamin D in model 4. These models are not additive. IL-6 values were right-skewed; therefore, all analyses for IL-6 used natural-log transformed values to better approximate normality of residuals. A random effect for the different studies was included in the model. Adiponectin is measured in \( \mu g/ml \), IL-6 in pg/ml, vitamin D in mcg, age in years, and protein in g/kg/day. Data are considered statistically significant at P<0.05.

4.7 Interactions between adiponectin and T2DM affect the relationship between adiponectin and ALM/BMI.

Analysis was conducted to determine if adiponectin, T2DM, and IL-6 predict for changes in ALM/BMI. Adiponectin was not a significant predictor of ALM/BMI when no other variables were included in the model (p = 0.198). T2DM status affected ALM/BMI. The mean difference in ALM/BMI between those with and without T2DM was significant (p < 0.001) such that those with T2DM on average have an ALM/BMI 0.090 lower than those without T2DM. There was a significant negative relationship between IL-6 and ALM/BMI (p = 0.003). Every 1 pg/ml increase in natural log of IL-6 resulted in a 0.025 decrease in ALM/BMI. When converted to IL-6, every 1 pg/ml IL-6 resulted in a 0.004
decrease in ALM/BMI. A significant adiponectin-by-T2DM interaction was found for ALM/BMI, for those without T2DM every 1 ug/ml increase in adiponectin results in a 0.003 increase in ALM/BMI (p = 0.025). This remained true with models 2, 3 and 4. For those with T2DM, adiponectin did not significantly predict for ALM/BMI (p = 0.162). For the model that included IL-6, every 1 μg /ml increase in adiponectin resulted in a 0.003 increase in ALM/BMI (p = 0.047). For those with T2DM, adiponectin was not a significant predictor of ALM/BMI (p = 0.162). There was no significant interaction between IL-6 and T2DM in the model for ALM/BMI (p = 0.671). A significant adiponectin-by-IL-6 interaction was found for ALM/BMI (p = 0.005). The positive relationship between adiponectin and ALM/BMI was attenuated by IL-6. There was not a significant three-way interaction between adiponectin, T2DM, and IL-6 (p = 0.200, data not shown in table).
Table 4.9: Fixed effects of adiponectin, T2DM, IL-6 and their interactions in a linear mixed-effect model of trunk adipose mass.

<table>
<thead>
<tr>
<th>Trunk Adipose Mass</th>
<th>Model 1 Regression Coefficient</th>
<th>P value</th>
<th>Model 2 - Age Regression Coefficient</th>
<th>P value</th>
<th>Model 3 - IL-6 Regression Coefficient</th>
<th>P value</th>
<th>Model 4 - Dietary Protein and Vitamin D Regression Coefficient</th>
<th>P value</th>
</tr>
</thead>
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<td>0.018</td>
<td>-0.189</td>
<td>0.019</td>
<td>-0.106</td>
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<td>-0.196</td>
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</tr>
<tr>
<td>T2DM</td>
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<td>6.555</td>
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<td>4.858</td>
<td>0.001</td>
<td>5.517</td>
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<td>IL-6</td>
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<td>Adiponectin * T2DM</td>
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<td>0.633</td>
<td>0.091</td>
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<td>0.193</td>
<td>0.958</td>
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<td>Adiponectin</td>
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<td>3.435</td>
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<td>---</td>
<td>0.884</td>
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</table>

All IL-6 values are transformed to natural log
Trunk adipose mass measured in kg, adiponectin (μg/ml), IL-6 (pg/ml), protein (g/kg/d), age (years), vitamin D (mcg)
Table 4.9: Fixed effects of adiponectin, T2DM, IL-6 and their interactions in a linear mixed-effect model of trunk adipose mass.

A linear-mixed effect model was used to analyze the relationship of adiponectin, T2DM, and IL-6 to trunk adipose mass. The interactions among adiponectin, T2DM, and IL-6 were also investigated. Explanatory variables were added into the model as fixed effects, which included age in model 2, IL-6 in model 3, and dietary protein and dietary vitamin D in model 4. These models are not additive. IL-6 values were right-skewed; therefore, all analyses for IL-6 used natural-log transformed values to better approximate normality of residuals. A random effect for the different studies was included in the model.

Adiponectin is measured in ug/ml, IL-6 in pg/ml, vitamin D in mcg, age in years, and protein in g/kg/day. Data are considered statistically significant at P<0.05.

4.8 Adiponectin and IL-6 predict for changes in trunk adipose mass.

Adiponectin and IL-6 were investigated to predict for changes in trunk adipose mass, which is implicated in obesity and adverse metabolic conditions. Adiponectin is a significant predictor for decreased trunk adipose mass (p = 0.018). Every 1 μg/ml increase in adiponectin resulted in a 0.190 kg decrease in trunk adipose mass. It remains significant when age is included in the model (p = 0.019) or dietary protein and vitamin D (p = 0.005) but not IL-6 (p = 0.210). T2DM is not a significant predictor for trunk adipose mass (p = 0.160). IL-6 is a significant predictor for trunk adipose mass (p < 0.001) and remains significant when age or dietary protein and vitamin D are added to the model. Every 1 pg/ml increase in IL-6 resulted in a 0.356 increase in trunk adipose mass.
(p = 0.004, data not shown in table). There was no significant interaction between adiponectin and T2DM in the model for trunk adipose mass (p = 0.633). In addition, there was no significant interaction between IL-6 and T2DM in the model for trunk adipose mass (p = 0.303). Finally, no significant interaction between adiponectin and IL-6 was found for predicting trunk adipose mass (p = 0.803).
A linear-mixed effect model was used to analyze the interaction of adiponectin and T2DM and predicting for ALM/BMI. A random effect for the different studies was included in the model. Data are considered statistically significant at P<0.05.

4.9 Adiponectin significantly predicts for ALM/BMI in postmenopausal women without T2DM but not for postmenopausal with T2DM.

A significant adiponectin-by-T2DM interaction was found for ALM/BMI. For those without T2DM, every 1 μg /ml increase in adiponectin results in a 0.003 increase in ALM/BMI (p = 0.025). For those with T2DM, adiponectin was not a significant predictor of ALM/BMI (p = 0.162).
<table>
<thead>
<tr>
<th></th>
<th><strong>ALM/BMI</strong></th>
<th></th>
<th><strong>Trunk Adipose Mass</strong></th>
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</thead>
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<td>Regression Coefficient</td>
<td>P value</td>
<td>Regression Coefficient</td>
</tr>
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<td>Linoleic Acid</td>
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<td>Linoleic Acid</td>
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<tr>
<td>T2DM</td>
<td>0.032</td>
<td>0.738</td>
<td>1.661</td>
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</tbody>
</table>

Table 4.10: Fixed effects of linoleic acid, adiponectin, T2DM, and their interactions in a linear mixed-effect model of ALM/BMI and trunk adipose mass.

A linear-mixed effect model was used to analyze the relationship of plasma or serum linoleic acid, adiponectin, and T2DM, to ALM/BMI or trunk adipose mass. The association between linoleic acid and body composition components was also investigated when taking into account the interaction between adiponectin and T2DM. A random effect for the different studies was included in the model. Adiponectin is measured in μg/ml and plasma/serum linoleic acid was measured as a percent of total fatty acids. Data are considered statistically significant at P<0.05.

4.10 Linoleic acid predicts for increases in ALM/BMI and decreases in trunk adipose mass.

Increased intake of linoleic acid, an essential omega-6 fatty acid, has recently been shown
to increase total lean body mass in postmenopausal women with T2DM [45]. Therefore, we investigated the relationship between linoleic acid and ALM/BMI and trunk adipose mass. Plasma/serum linoleic acid is a significant predictor for increased ALM/BMI (p = 0.009). Every 1% increase in linoleic acid resulted in a 0.007 increase in ALM/BMI. Increased linoleic acid predicts for a decrease in trunk adipose mass (p = 0.014). Every 1% increase in linoleic acid resulted in a 0.465 kg decrease in trunk adipose mass. A significant adiponectin-by-T2DM interaction was found for ALM/BMI and trunk adipose mass. Linoleic acid remained a significant predictor for ALM/BMI and trunk adipose mass when this interaction was taken into account.
Chapter 5: Discussion

As women undergo menopause they experience dramatic changes in body composition characterized by an increase in fat mass and a decrease in muscle mass [1]. Sarcopenia is the decrease in muscle mass, strength, and function that occurs with aging [2]. This loss of muscle mass is associated with increased morbidity, mortality, and reduced quality of life [4]. Here we analyzed the relationship between different biomarkers and ALM/BMI or trunk adipose mass in postmenopausal women. While much of the literature discusses occurrence of sarcopenia in individuals over 60, the average in this cohort is 57 years old and therefore, a goal of this thesis was to determine if adiponectin can be used as an early indicator for loss of muscle mass. Appendicular lean muscle mass (the summation of arm and leg muscle mass) was adjusted for body mass index as indicated by the committee of the National Institute of Health Sarcopenia Project to account for differences in body size among individuals [18]. We found that ALM/BMI was strongly correlated with total lean mass adjusted for body mass index, TLM/BMI (Figure 1).

5.1 Demographic descriptions and baseline measurements were significantly different among cohorts.
Due to differences in the primary goals of each randomized control trial, we found a significant difference for BMI, ALM/BMI, trunk adipose mass, adiponectin, IL-6, dietary vitamin D, and plasma/serum linoleic acid among study cohorts. However, our findings may be affected by differences in the exclusion and inclusion criteria of the studies (e.g. BMI requirements, T2DM status) [45, 57, 58]. Due to incomplete data on muscle strength and muscle function, this thesis focuses only on one component of sarcopenia, low muscle mass. Here the prevalence of low muscle mass was 9% in the postmenopausal women (Table 4.7), which is consistent with reports from other groups [56]. We found that there were no women with low lean muscle mass in study I and II (Table 4.7) Women in these cohorts did not have T2DM and several (n=14) had BMIs in the normal range unlike women in Studies III, IV, and V (Table 4.1). Consistent with our findings, Davidson et al. found that age-associated reductions in appendicular lean mass are accelerated in women with T2DM compared to healthy controls [59]. Additionally, lower rates of sarcopenia are seen in healthier populations compared to populations with other comorbidities [56].

5.2 Adiponectin predicts for ALM/BMI in postmenopausal women without T2DM.

Adiponectin, which has receptors on skeletal muscle, has been shown to increase mitochondrial biogenesis, oxidative metabolism, and increase myofiber formation in skeletal muscle [9]. Thus, increasing adiponectin levels, either by lifestyle (e.g. increased physical activity, weight loss) or pharmacological approaches (e.g. adiponectin mimetics), may have a potential role in improving muscle mass. In addition, there are
currently no biomarkers to identify those at increased risk for sarcopenia. Therefore, understanding the relationship between adiponectin and muscle mass may allow it to serve as a biomarker for those at risk for sarcopenia.

We found that adiponectin was significantly correlated with ALM/BMI when the studies were combined and analyzed with a Pearson correlation (Figure 4.7). However, when the studies were combined in a linear mixed-effect model to account for differences among studies there was no correlation between adiponectin and ALM/BMI (Table 4.8). After analyzing Pearson correlations for each study cohort individually, there was no correlation between adiponectin and ALM/BMI although there was a trend for a positive correlation in Study I (Figure 4.2). This suggests that differences in the study cohorts were affecting the relationship between adiponectin and ALM/BMI.

One of the predominant differences between the studies was the presence or absence of women with T2DM. Individuals with T2DM have been shown to have significantly lower adiponectin levels than those without T2DM [60]. In addition, low adiponectin levels are associated with an increased risk of developing T2DM [61]. We found that those with T2DM had significantly lower ALM/BMI than those without T2DM (Table 4.8). These results are consistent with other studies demonstrating a lower ALM in people with T2DM than nondiabetic controls [59, 62]. Additionally, we found a significant interaction between adiponectin and T2DM in predicting for ALM/BMI (Table 4.8). Adiponectin did not predict for changes in ALM/BMI for those with T2DM,
but it did for those without T2DM (Figure 4.8). These results suggest that the changing environment implicated with T2DM (e.g. increased inflammation, changes in adipocyte secretion, insulin resistance) may make adiponectin a poor predictor of ALM/BMI in those with T2DM. For those without T2DM, adiponectin positively predicted for increased ALM/BMI (Figure 4.8). This is in contrast to Kosacka et al, who found a negative correlation between adiponectin and percent muscle mass in individuals without T2DM [63]. However, Kosacka et al used BIA to measure body composition while we used DEXA, a more accurate measure [64]. It is also possible that the relationship between adiponectin and ALM/BMI is different among postmenopausal women compared to men [63] as well as other populations. Bredella et al. found no significant association between adiponectin and ALM in premenopausal women [35]. Differences in body composition between men and women [65] and changes in body composition that occur during menopause [1] may explain these differences.

5.3 Inflammation predicts for decreases in ALM/BMI and increases in trunk adipose mass in postmenopausal women.

IL-6 is an inflammatory cytokine secreted from a variety of cells in response to stressors [41]. It has been shown to activate the ubiquitin-protease pathway, which is important for the degradation of proteins [66], as well as inhibit protein anabolism suggesting it may play an important role in accelerating muscle loss [67]. Obese individuals tend to have higher levels of circulating inflammatory cytokines [68] and this chronic low-grade inflammation is thought to contribute to a variety of adverse metabolic conditions.
including loss of muscle mass [67]. In this cohort we found that IL-6 did indeed predict for decreases in ALM/BMI (Table 4.8) as well as increases in trunk adipose mass (Table 4.9) supporting existing evidence of the role of IL-6 in changing body composition.

Inflammation is also associated with the pathogenesis of several chronic diseases, including T2DM [69]. Interestingly, we found that there was no significant interaction between IL-6 and T2DM in relation to ALM/BMI (Table 4.8). One reason for this is that is difficult to tease out the effects IL-6 and T2DM have on each other because they are interrelated. Chronic inflammation, as demonstrated by increased levels of IL-6, plays a role in the pathogenesis of T2DM [70]. High IL-6 levels are associated with twice the risk of developing T2DM [71]. In addition, those with T2DM have significantly higher IL-6 levels than those without T2DM [72].

5.4 Implications of obesity and T2DM on changes in muscle mass.

Approximately two-thirds of Americans are overweight or obese [46]. As people age, their physical activity often decreases [56]. This decrease in activity not only can lead to weight gain but also muscle wasting. Sarcopenic obesity, an increase in fat mass with a concomitant decrease in muscle mass, can result in increased disability, frailty, and morbidity despite the possibility that no changes in weight may be seen [4]. Thus as people age, a cyclical relationship begins to develop that exacerbates both obesity and muscle wasting. We measured trunk adipose mass as a marker of obesity. We found that increases in adiponectin predict for decreases in trunk adipose mass (Table
4.9). Trunk adipose mass is associated with an increased risk for metabolic syndrome, MetS [73]. MetS is a cluster of metabolic disturbances defined by meeting 3 out of the 5 following criteria in women: waist circumference greater than 88 cm, HDL < 50 mg/dl, triglycerides > 150 mg/dl, elevated fasting blood glucose (100 – 126 mg/dl), and blood pressure > 130/85 mmHg. Low adiponectin levels have been found to be correlated with all the factors of MetS [74]. Individuals with MetS are five times more likely to develop T2DM and are at increased risk for T2DM mortality [75]. We found a significant difference in ALM/BMI among cohorts (Table 4.2). The cohorts with diabetic women (Studies III, IV) had significantly lower ALM/BMI than Studies I and II, which did not have any diabetic women.

Obesity and T2DM are closely intertwined. Obesity alters cytokine excretion and exacerbates insulin resistance [70]. Insulin resistance can lead to the development of T2DM. Individuals with T2DM are more likely to be obese [69]. An increased inflammatory state impairs beta-cell function and is correlated with impaired insulin sensitivity [76]. Adiponectin has been shown to improve beta cell function [6] and replenishing adiponectin in adiponectin knockout mice improves insulin resistance [61]. Consistent with the literature [6, 60], adiponectin levels were lower in the women with T2DM. We found that postmenopausal women with T2DM have significantly lower ALM/BMI compared to those without T2DM (Table 4.8). The lower adiponectin levels seen in these women may exacerbate the adverse changes in body composition, characterized by an increase in adipose mass and decrease in muscle mass.
5.5 Dietary protein intake and dietary Vitamin D do not alter the relationship between adiponectin and ALM/BMI in postmenopausal women.

In this cohort, the relationship between adiponectin and ALM/BMI in those without T2DM did not change when the model was adjusted for dietary protein and vitamin D intake (Figure 4.8). The average dietary protein intake in these women (Table 4.4) exceeded the recommended dietary allowance (RDA) of 0.8g/kg/day of protein recommended for adults and therefore other factors including amount of protein consumed at each meal may be more important than total protein intake for conserving muscle mass. It may be more beneficial to consume 20-30 g of protein at every meal than to focus on consumption of 0.8 g/kg/day [77]. On the other hand, dietary vitamin D intake (Table 4.4) was substantially lower in this cohort than the RDA of 15-20 mcg. However, this data does not include supplemental vitamin D intake; therefore, we are unable to determine if these women were truly deficient in vitamin D.

5.6 Serum/plasma linoleic acid significantly predicts for changes in ALM/BMI and trunk adipose mass in postmenopausal women.

Serum/plasma linoleic acid was investigated as a covariate in this analysis because in previous studies it has been shown to significantly increase total lean mass [44, 45]. We found that serum/plasma linoleic acid was positively correlated with adiponectin and ALM/BMI while negatively correlated with IL-6 and trunk adipose mass (Table 4.5 and Table 4.6). Linoleic acid also predicted for increased ALM/BMI and this remained
significant when the interaction between adiponectin and T2DM was included (Table 4.10). While serum/plasma linoleic acid may not be easily used in clinical applications to identify low muscle mass, it may be a useful tool in research settings to predict for the occurrence of sarcopenia. Since linoleic acid also predicts for decreased trunk adipose mass, this suggests it may have a beneficial role in prevention of sarcopenic obesity.

**Study Significance and Implications**

The outcomes of this thesis are significant because they help elucidate the relationship between adiponectin, T2DM, ALM/BMI, and trunk adipose mass. Postmenopausal women with T2DM have significantly lower ALM/BMI compared to those without T2DM. In postmenopausal women without T2DM, adiponectin significantly predicts for increases in ALM/BMI. This remains true in models that control for age, IL-6, and protein and vitamin D intake. In contrast, in postmenopausal women with T2DM, adiponectin did not predict for changes in ALM/BMI. Low muscle mass, an important component of sarcopenia, has been implicated with increased morbidity, mortality, reduced quality of life, and decreased ability to perform activities of daily living [4]. There are currently no pharmacological interventions to prevent sarcopenia. Current prevention methods include increased protein intake (up to 1.2 g/kg/day), vitamin D supplementation, and increased physical activity (especially resistance training) [52]. This thesis suggests that increasing adiponectin levels in nondiabetic postmenopausal women may be beneficial in preserving muscle mass. It also opens the door to investigating the potential role of linoleic acid in increasing lean muscle mass.
Epilogue

Conclusions
This is the first study to demonstrate that the relationship between adiponectin and ALM/BMI is affected by T2DM status in postmenopausal women. In postmenopausal women without T2DM, adiponectin significantly predicts for increases in ALM/BMI. In contrast, in postmenopausal women with T2DM, adiponectin did not predict for changes in ALM/BMI. These results suggest that the changing environment implicated with T2DM (e.g. increased inflammation, changes in adipocyte secretion, insulin resistance) may make adiponectin a poor predictor of ALM/BMI in those with T2DM.

Limitations and Future Directions
There are several limitations to our study. First, this is a correlation study so no causations can be inferred from this data. The congruent measurement of changes in adiponectin levels over time and changes in lean mass should be investigated. Secondly, small cohort sizes limit the strength of the study. Third, no data were available for the other components of sarcopenia, muscle strength and muscle function. Because sarcopenia is defined as more than just mere muscle loss, future studies should examine
the relationship of adiponectin to all the components of sarcopenia. Fourth, dietary intake was from 3-day food records or 24-hour recalls and supplement use as well as sunlight exposure, which could significantly affect vitamin D levels, was not available. Furthermore, serum vitamin D samples would be more accurate of actual intake.

Finally, total adiponectin levels were analyzed. Globular adiponectin, which constitutes less than 1% of total adiponectin levels has been shown to have a stronger affinity to AdipoR1, the prominent receptor on skeletal muscle, and be the most biologically active form of adiponectin [6]. Measuring globular adiponectin specifically may be a better predictor for changes in ALM/BMI than total adiponectin.
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