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## THE RELATIONSHIP BETWEEN MODIFIABLE LIFESTYLE FACTORS AND MUSCLE LIPID DEPOTS IN OLDER ADULTS

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THE RELATIONSHIP BETWEEN MODIFIABLE LIFESTYLE FACTORS AND  
MUSCLE LIPID DEPOTS IN OLDER ADULTS

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DISSERTATION

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A dissertation submitted in partial fulfillment of the  
requirements for the degree of Doctor of Philosophy in the  
College of Medicine at the University of Kentucky

By

Maja Redzic, MS

Lexington, KY

Co-Director: Dr. D. Travis Thomas, Professor of Clinical Sciences  
and Dr. Geza Bruckner, Professor of Clinical Sciences

Lexington, KY

2016

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## ABSTRACT OF DISSERTATION

### THE RELATIONSHIP BETWEEN MODIFIABLE LIFESTYLE FACTORS AND MUSCLE LIPID DEPOTS IN OLDER ADULTS

Muscle health declines with age, influencing both muscle strength and performance. Modifiable lifestyle factors may mitigate muscle dysfunction by altering muscle lipid depots. Vitamin D status, dietary intake, and physical activity each play a role in muscle health during aging. Low vitamin D status (measured as 25(OH)D) is prevalent among older adults and has been associated with functional limitations and impaired physical performance. Previously, 25(OH)D concentrations were inversely associated with extramyocellular lipid (EMCL) and our lab observed a significant positive relationship between 25(OH)D concentrations and IMCL. Studies have shown mixed results regarding the effects dietary fat intake interventions have on muscle lipid depots. It is well established that exercise contributes to improved muscle health, but to date studies have not examined the relationship between daily physical activity levels, muscle lipid depots, and local muscle tissue hemodynamics. We used novel non-invasive technology to measure gastrocnemius muscle lipid depots (Proton Magnetic Resonance Spectroscopy) and monitor local muscle tissue hemodynamics during a foot plantar flexion exercise (Near Infrared/Diffuse Correlation Spectroscopy). The first aim was to further elucidate the relationship between 25(OH)D concentrations, physical function, and muscle lipid depots. We also examined 25(OH)D concentrations and muscle lipid depots differences in older adults with varied body mass index (BMI) and physical activity levels. We observed a negative relationship between 25(OH)D concentrations and EMCL. In females, greater 25(OH)D concentrations and lower EMCL were significant predictors of faster Four Square Step Test times, independent of BMI and age. Greater EMCL and IMCL content were observed with increased BMI, but statistical significance was not reached. The second aim was to retrospectively examine the relationship between dietary fat intake and muscle lipid depots in participants enrolled in a double-blinded placebo-controlled trial. We also investigated the relationship between physical activity levels and local muscle tissue hemodynamics. Increased dietary fat intake ratio of polyunsaturated to

saturated fatty acids (PUFA:SFA) and study intervention (7 days of aerobic training) were the best predictors of lower IMCL content, independent of age, BMI, and physical activity. Compared with individuals with lower physical activity levels, individuals that reported greater physical activity had lower relative blood flow and relative oxygen consumption during and after a foot plantar flexion exercise. Together these findings suggest that increased physical activity, consumption of greater PUFA:SFA, and maintenance of sufficient 25(OH)D concentrations may have favorable effects on muscle lipid depots and contribute to the preservation of muscle health.

**KEYWORDS:** vitamin D, dietary fat intake, physical activity, intramyocellular lipid, extramyocellular lipid

Maja Redzic

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November 29, 2016

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THE RELATIONSHIP BETWEEN MODIFIABLE LIFESTYLE FACTORS AND  
MUSCLE LIPID DEPOTS IN OLDER ADULTS

By

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## LIST OF ABBREVIATIONS

1-alpha, 25 dihydroxy vitamin D (1,25(OH)<sub>2</sub>D<sub>3</sub>, calcitriol); 25-hydroxyvitamin D (25(OH)D, calidiol); 25-hydroxyvitamin D 24 hydroxylase (24-OHase); 25-hydroxyvitamin D3-1-hydroxylase (1 alpha-OHase, CYP27B1); analysis of variance (ANOVA); blood flow (BF); blood oxygen saturation (StO<sub>2</sub>); body mass index (BMI); bone mineral density (BMD); cardiovascular disease (CVD); Center for Disease Control (CDC); centimeter (cm); cerebrovascular accidents (CVA); cholecalciferol (vitamin D<sub>3</sub>); coefficient of variation (CV); computed tomography (CT); counts per minute (CPM); cytosine (C); day (d); deoxy-hemoglobin (Hb); diacylglycerol (DAG); DAG-O-acyltransferase (DGAT); diffuse correlation spectroscopy (DCS); dual-energy X-ray absorptiometry (DXA); echo time (TE); electrocardiogram (EKG); ergocalciferol (vitamin D<sub>2</sub>); extramyocellular lipid (EMCL); fat-free mass (FFM); Four Square Step Test (FSST); General Education Development (GED); Graded Exercise Test (GXT); guanine (G); high fat diet (HFD); high performance liquid chromatography (HPLC); Hounsfield Units (HU); insulin resistance (IR); intramyocellular lipid (IMCL); kilograms (kg); meter (m); milliampere (mA); milliliter (mL); milliseconds (ms); millimeter of mercury (mmHg); mineral-free lean (MFL); minutes (min); magnetic resonance imaging (MRI); magnetic resonance spectroscopy (MRS); maximal voluntary contractions (MVC); metabolic equivalents (MET); monoacylglycerol (MAG); monoacylglycerol acyltransferase (MGAT); monounsaturated fatty acids (MUFA); nanograms (ng); near-infrared spectroscopy (NIRS); Nutrition Data System for Research (NDS-R); oxygen consumption rate (VO<sub>2</sub>); tissue oxygen saturation (StO<sub>2</sub>); oxy-hemoglobin (HbO<sub>2</sub>); parathyroid hormone (PTH); parts per million (ppm); perilipin

(PLIN1-5); peripheral artery disease (PAD); Physical Activity Scale for the Elderly (PASE); phosphocreatine recovery (PCR); polyunsaturated fatty acids (PUFA); positron emission tomography (PET); proton MR spectroscopy (1H-MRS); pro-vitamin D3 (7-dehydrocholesterol); radio frequency (RF); rate of perceived exertion (RPE); repeated measures analysis of variance (RMANOVA); Recommended Dietary Allowance (RDA); relative tissue blood flow (rBF); relative tissue oxygen consumption (rVO<sub>2</sub>); relative tissue oxygen extraction fraction (rOEF); repetition time (TR); Research Electronic Data Capture tool (REDCap); retinoid-X receptor (RXR); saturated fatty acid (SFA); second (s); socioeconomic status (SES); standard deviation (SD); Statistical Package for the Social Sciences (SPSS); total hemoglobin concentration (THC); triacylglycerol (TAG); ultraviolet (UV); vector magnitude (VM); vitamin D binding protein (VDBP); vitamin D receptor (VDR); week (wk)

## Chapter 1: Background

### 1.1 Vitamin D: Overview

Vitamin D is a fat-soluble secosteroid, traditionally associated with bone health [1, 2]. Vitamin D has existed on the Earth for at least 500 million years and caused a 270-year scientific inquiry for the cure of rickets, a disease first recognized in the 1650s [2]. The rickets epidemic emerged during the Industrial Revolution, in temperate zones, where the pollution from factories blocked the sun's ultraviolet rays (UV) [2]. By the late 19th and early 20th century, rickets, the incomplete calcification of the epiphyseal closure in children [3], affected more than 80% of children living in United States' and European cities [4]. While some cases of rickets are related to hereditary syndromes, renal disease, or use of medication, outbreaks of rickets predominantly stem from nutritional insufficiency [5]. Such nutritional insufficiency occurs when metabolites of vitamin D are deficient and less commonly can result from dietary deficiency of calcium or phosphorus.

In 1921 Hess and Unger reported UV radiation was an effective treatment for rickets [2]. Today rickets remains rare in developed nations due to increased awareness and consumption of vitamin D fortified foods. However, low vitamin D status in adults is prevalent and has become a worldwide epidemic. Analogous to rickets in children, failure of osteoid to calcify in adults is termed osteomalacia. Vitamin D is not only important in optimal bone health, reducing the risk of osteomalacia and osteoporosis in adults [1], but low levels have also been implicated in many acute and chronic diseases such as cancer, diabetes, and cardiovascular diseases [6, 7]. More recently, maintaining sufficient vitamin D

status has been associated with improved musculoskeletal health [8] affecting muscle strength and size [7]. Age-related muscle mass decreases have been associated with increased risk for fractures and falls [9]. Concurrently, the high prevalence of vitamin D deficiency and insufficiency in the growing aged population leaves older adults susceptible to decreased muscle function and subsequently increased risk for falls. Therefore, it is imperative to effectively measure, assess, and understand the role vitamin D plays on skeletal muscle in older adults.

#### 1.1.1 Vitamin D Measurements

In the human body, vitamin D exists in two main forms that differ in side chain structure. One of the forms is cholecalciferol (vitamin D<sub>3</sub>) which is mainly synthesized endogenously through skin exposure to UVB radiation. In addition, small amounts of vitamin D<sub>3</sub> can also be obtained from dietary animal sources such as egg yolk and oily fish [2]. Ergocalciferol (vitamin D<sub>2</sub>) is the other form that is found in plants and yeast. Traditionally, vitamin D<sub>2</sub> and D<sub>3</sub> were thought to be equipotent; however, human studies have shown that raising 25-hydroxy vitamin D (25(OH)D) concentrations with vitamin D<sub>3</sub> is more than 2-fold effective [2, 10]. Vitamin D<sub>2</sub> is less effective in raising 25(OH)D concentrations because of the differences in binding affinities for vitamin D binding protein (VDBP) between vitamin D<sub>2</sub> and D<sub>3</sub>. Vitamin D<sub>2</sub> has a lower binding affinity to VDBP resulting in a shorter circulating half-life and increased circulating clearance rate than that of vitamin D<sub>3</sub> [10]. It is vital to note that hepatic 25-hydroxylase has a higher affinity



for vitamin D<sub>3</sub>, converting it to 25(OH)D<sub>3</sub> five times faster than the rate of conversion from vitamin D<sub>2</sub> to 25(OH)D<sub>2</sub>.

The vitamin D metabolite, 25(OH)D, is a diagnostic measure used to evaluate vitamin D status. Since the mid-1970s high performance liquid chromatography (HPLC) has been the gold standard for 25(OH)D assay as it provides the ability to quantify both forms (D<sub>2</sub> and D<sub>3</sub>) [11]. Although 25(OH)D is the inactive form of vitamin D, it is the major circulating metabolite with a half-life of 2-3wks [12]. The highest concentration of 25(OH)D can be found in plasma and 25(OH)D is stored in the adipose and muscle tissues. Eighty-five percent of the inactive form 25(OH)D and the active form 1,25-dihydroxyvitamin D (1,25(OH)D) is bound to VDBP, 10-15% is bound to albumin, and 0.03% is free or unbound [13]. Although the majority of vitamin D is bound, the unbound portion of 1,25(OH)D, or active form, is able to exert biological activity, following the principle of the free hormone hypothesis [14]. The free hormone hypothesis states that unbound hormones are available to exert biological activity while hormones bound to proteins are relatively inactive. VDBP concentrations can be altered with pathological conditions and vary between individuals, this in turn can affect circulated 25(OH)D concentrations [14-17]. Researchers suggest that VDBP should be measured and assessed as VDBP concentrations could be a confounding variable when interpreting 25(OH)D concentrations [14].

The biologically active form of vitamin D is calcitriol, 1,25(OH)D, which is primarily produced in the proximal tubules of the nephron in the kidneys. Calcitriol is an inappropriate measure of vitamin D status due to its short half-life

of 4-6 hours and the circulated form that is 1,000 fold less than 25(OH)D [11]. Although 1,25(OH)D is an inappropriate measure of vitamin D status in healthy individuals, there are disease states in which it may be appropriate to measure 1,25(OH)D. For example, patients with sarcoidosis, exhibit increased 1,25(OH)D concentrations as the immune cells produce calcitriol resulting in vitamin D toxicity and hypercalcemia. During chronic renal failure the disorder or the absence of 1-alpha-hydroxylase, enzyme located in the kidneys responsible for the conversion of 25(OH)D to 1,25(OH)D, results in reduced 1,25(OH)D concentrations [18]. Disease states can affect vitamin D measures; therefore, it is important to fully understand vitamin D metabolism and consider which vitamin D metabolites are necessary to assess.

#### 1.1.2 Vitamin D Metabolism

Vitamin D can be synthesized cutaneously through skin exposure to UVB rays or through dietary intake of natural and fortified foods or supplements. Pro-vitamin D3 (7-dehydrocholesterol) is located in the epidermal skin layer and in response to heat-induced UVB radiation undergoes isomerization to form pre-vitamin D3. Pre-vitamin D3 can 1) bind to the VDBP in the bloodstream and be transported to the liver where it undergoes hydroxylation or 2) be converted to tachysterol and lumisterol [2]. Excess exposure to UVB radiation results in the continuous production of the inactive compounds, tachysterol and lumisterol. Further, the inert compounds act as a reservoir and therefore can be converted back into pre-vitamin D3. This mechanism safeguards from sun-induced vitamin D toxicity. Conversely, vitamin D derived from natural foods, fortified foods and/or

supplements (vitamin D2 and D3) can be incorporated into chylomicrons, released into the lymphatic system, and then bound to VDBP in venous circulation. A small portion of the dietary vitamin D is extracted by adipose tissue and muscle; however, the remainder of dietary vitamin D and the most of the endogenously synthesized vitamin D is transported to the liver [2].

In the liver, pre-vitamin D is hydroxylated by the enzyme 25-hydroxylase (also known as cytochrome p450) and converted to 25(OH)D (calcidiol). Next, 25(OH)D is 1-hydroxylated by 25-hydroxyvitamin D 1-hydroxylase (1 alpha-OHase), located primarily in the proximal tubules of the kidney, resulting in the active form 1,25(OH)D (calcitriol) [2, 19]. Although primarily the enzyme, 1 alpha-OHase, plays a role in the kidneys and converts calcidiol to the bioactive form calcitriol, the enzyme is also present in various tissue cells such as in keratinocytes, immune cells, myocytes, and osteoblasts which allows for vitamin D signaling at a local, tissue specific level [20].

Serum parathyroid hormone (PTH) plays an important role in regulating the conversion of calcidiol to calcitriol. Calcium-sensing receptors located on chief cells in the parathyroid glands are stimulated during low serum calcium concentrations, to release PTH. Conversely, when calcium concentrations are high, reverse inhibition of parathyroid gland receptors occurs [21]. In addition, in the kidney, PTH can increase the activity of 1 alpha-OHase, which in turn increases the production of calcitriol. Calcitriol can act in the small intestine by binding to the retinoid-X receptor (RXR) to increase the transcription of epithelial calcium transporter proteins and calbindin. Calcium transporters and binding

proteins increase calcium absorption, raising serum calcium concentrations [2]. Further, PTH can bind to the PTH1 receptor located on osteoblasts. Upon prolonged PTH exposure, RANKL (located on the osteoblasts) binds to RANK (located on the osteoclasts) to stimulate bone resorption resulting in the release of calcium from the bone into the serum [22]. Conversely, intermittent exposure of PTH and higher calcitriol concentrations cause osteoblast proliferation, increases in bone mineralization, and decreased serum calcium concentrations.

During high 25(OH)D concentrations, a negative feedback mechanism inhibits 25-hydroxylase decreasing further production of 25(OH)D in the liver [2]. Further, high 1,25(OH)D concentrations can inhibit 1 alpha-OHase reducing calcitriol synthesis in the kidney. Also, 1,25(OH)D can be degraded by 24-OHase to 1,24,25(OH)D (calcitroic acid) which undergoes biliary excretion.

### 1.1.3 Vitamin D Sources

Most notably, vitamin D can be obtained through UVB ray exposure. It is recommended that exposing the arms, legs, face, or back without sunscreen for 5-30 minutes, at least 2 times a week between the hours of 10:00AM and 3:00PM, would provide adequate vitamin D synthesis [23]. As a result of 5-10 minutes of UVB exposure, 3,000IU of vitamin D3 can be cutaneously synthesized [24]. Individuals residing at latitudes above 35°N or below 35°S, during the months of November-March, synthesize minimal vitamin D through UVB ray exposure [25].

The Recommended Dietary Allowance (RDA) for individuals between 1-70 years of age is 600IU/d and for individuals older than 70 is 800IU/d [23, 26]. The

higher levels for older adults is recommended as decreases in intestinal absorption occur with aging [27]. Natural foods containing higher vitamin D levels include oily fish such as salmon (500-1000IU/3.5oz serving) and UVB-exposed mushrooms (~100IU/3.5oz serving) [24, 25]. To a lesser extent, foods such as beef liver and egg yolks also contain vitamin D. Fortified foods in the United States including milk, juice, and some cheeses and yogurts typically contain 100IU/serving [23, 25, 28].

Dietary vitamin D supplements can be consumed to increase and maintain 25(OH)D concentrations. Current clinical guidelines for treatment of vitamin D deficiency is to supplement with vitamin D for 8wks (50,000IU/wk or 6,000IU/d), to achieve a concentration of 25(OH)D>30 nanograms/milliliter (ng/mL), followed by maintenance therapy of 1,500–2,000IU/d [27]. Heaney et al. [29] observed a 20ng/mL and 30ng/mL increase in 25(OH)D in healthy overweight participants after administering 50,000IU/wk for 4 and 6wks, respectively. In addition, Ekwaru et al. [29] recommended 2-3 times greater vitamin D supplementation for obese individuals and 1.5 times greater for overweight individuals relative to normal weight individuals. These differences in vitamin D supplementation recommendations are a result of genetic and modifiable lifestyle factors that contribute to vitamin D status.

#### 1.1.4 Genetic Polymorphisms in VDBP that Modulate Vitamin D Status

Genetic polymorphisms are the genetic variations that occur between individuals. VDBP variants are due to the polymorphisms in the VDBP gene guanine and cytosine (GC) and are defined as GC1F, GC1S, and GC2 [30].

Genetic polymorphism in VDBP effect vitamin D status; therefore, previous research has investigated the role of physiologic factors on VDBP and subsequently on vitamin D status in pathologic conditions.

Pathologic conditions such diabetes and cancer may alter VDBP and 25(OH)D concentrations. In Type I diabetic patients with nephropathy, an increase in urinary loss of VDBP contributes to lower 25(OH)D concentrations [31]. Conversely, the role of VDBP in 25(OH)D concentrations and cancer risk is still emerging. VDBP has not been directly linked with increased risk of cancer, but as 25(OH)D is modulated by VDBP researchers suggest that examination of both 25(OH)D and VDBP are necessary to understand the association observed between vitamin D status and cancer risk [14, 32].

Physiological factors such as sex and race affect VDBP and 25(OH)D concentrations. Studies suggest that VDBP concentrations are greater in females than males potentially due to the effect of estrogen stimulating VDBP synthesis [14]. Although estrogen decreases with menopause, Kaeslin et al. [33] observed greater 25(OH)D concentrations in postmenopausal women compared with aged-matched males potential due to higher VDBP concentrations. Also studies have shown VDBP genetic polymorphisms are associated with race and could alter VDBP levels and binding affinity [14, 15]. The functional significance of these genetic polymorphisms are not yet completely understood, but VDBP can alter circulating 25(OH)D concentrations and should be considered as a potential confounding factor in the interpretation of 25(OH)D concentrations.

One study included a cohort of African American (n=1,181) and White (n=904) middle-aged adults [15]. The authors observed that African Americans had consistently lower 25(OH)D concentrations and lower VDBP compared with Whites; however, the estimated bioavailable 25(OH)D was similar. The bioavailable or free 25(OH)D is the circulating vitamin D that is not bound to VDBP. These data suggest that low 25(OH)D concentrations combined with low VDBP may not indicate vitamin D deficiency. Additionally, differences in race and protein variants have been observed. African Americans and Asians are more likely to carry the GC1F VDBP. GC1F has the highest affinity for 25(OH)D and is associated with low VDBP. Conversely, Whites are more likely to carry GC1S and GC2. GC2 has a lower affinity for 25(OH)D and is associated with higher VDBP concentrations [14]. Therefore, 25(OH)D concentrations are in part due to the genetic variation in VDBP, in which lower VDBP with greater binding affinity may be a genetic trait to protect African Americans from vitamin D deficiency [15].

Lutsey et al. [34] provided another example of genetic variance influencing pathology between races. This study examined the association between 25(OH)D, VDBP, and heart failure among Whites and African Americans (total n=12,215). The authors observed that lower 25(OH)D concentrations were independently associated with increased risk of developing heart failure but only in White individuals. However, in both White and African American individuals, who were genetically predisposed to higher VDBP, lower 25(OH)D was associated with greater risk of heart failure. With higher VDBP a greater portion

of 25(OH)D was bound and subsequently less vitamin D was bioavailable contributing to increased cardiovascular risk.

A more recent study by Alzaman et al. [17] examined the effects of vitamin D supplementation (2,000IU/d or 4,000IU/d for 16wks) on bound and free 25(OH)D between African American (n=57) and White (n=151) adults with pre- or well-controlled diabetes. Prior to supplementation, the authors observed that African Americans had lower total 25(OH)D but similar free 25(OH)D concentrations compared with Whites. In both races, an increase in free and bound 25(OH)D post-supplementation was observed. Further, lower VDBP concentrations were observed in African Americans when a monoclonal antibody immunoassay, a more widely used technique to measure VDBP was employed. However, this technique was scrutinized as falsely lowering VDBP values in African Americans due to the differentiation against a VDBP variant that is more common in African Americans. In light of this, the authors applied a polyclonal antibody technique, which resulted in no significant racial differences between VDBP concentrations. In addition, Nielson et al. [16] examined these techniques with a larger sample size (African American n=101; White n= 919). The authors confirmed that previous findings of racial differences in VDBP concentration may be attributed to the monoclonal assay bias as no racial differences exist when alternative methods were employed. Due to discrepancies between methodologies used, further investigation is warranted to assess if racial differences in VDBP concentrations truly exist.



Together these studies provide evidence to the importance of VDBP genetic polymorphisms and the effects of both pathological and physiological factors on VDBP. Disease states can alter the production of VDBP which modulated 25(OH)D concentrations. As low 25(OH)D have been associated with multiple diseases, the combination of examining both 25(OH)D and VDBP is important to the understanding of the effects of vitamin D on disease risk. Further, difference among opposite sexes exists, in which higher VDBP concentrations are typically observed in females compared with males. Racial differences in VDBP variants between African Americans (GC1F) and Whites (GC1S and GC2) exist. African Americans have lower VDBP but higher binding affinity to 25(OH)D compared with Whites and as a result the bioavailability of 25(OH)D concentrations is similar between races. Due the racial variability in the VDBP gene, it is crucial that the techniques used to assess VDBP and free and bound 25(OH)D are taken into consideration.

#### 1.1.5 Factors that Modulate Vitamin D Status

Considerable amount of the vitamin D requirements can be met through sensible UVB exposure, whereas very few foods naturally contain vitamin D and fortified foods typically only contain 100IU/serving [28]. Multiple factors play a role in the ability to synthesize vitamin D cutaneously. Factors such as time of day, season of the year, and latitude play an important role since the zenith angle of the sun changes with Earth's rotation. The solar zenith angle becomes more oblique early or late in the day. Likewise, the angle is more oblique during the months of November-March and below 35°S or above 35°N [25]. At a more

oblique zenith angle the photons must travel longer distances through the ozone layer which make UVB rays more susceptible to absorption by the ozone, thus decreasing the amount of photons reaching the Earth's surface [25]. In addition, the UVB rays that are not absorbed in the ozone layer can be absorbed by air pollution or clouds, reducing UVB energy by 50-60% [23].

The skin is comprised of the superficial thinner layer, epidermis, and the deeper layer, dermis. UVB rays (290-315nm) penetrate the epidermal layer, while UVA rays are longer in wavelength (320-400nm) and penetrate deeper into the dermis. Greatest 7-dehydrocholesterol concentrations are located in the epidermal layer of the skin and absorb UVB light at 290-320nm, thus UVB radiation is effective at converting 7-dehydrocholesterol to pre-vitamin D<sub>3</sub>.

Other factors such as sunscreen and sunblock use and skin melanin content can reduce the skin's ability to produce vitamin D [28]. When applied as directed, sunscreen with a sun protection factor as low as 8 can reduce 25(OH)D by approximately 20ng/mL [2]. Melanin is derived from tyrosine and is responsible for the pigmentation of human skin, pupils, and hair color. The natural selection hypothesis states that lighter skin color, at higher latitudes with lower UVB radiation, evolved to optimize and facilitate vitamin D production [35]. The Fitzpatrick scale can be used to estimate the response of various skin types to UVB exposure ranging from Type I (score 0-6, always burns and never tans) to Type VI (score 35-36, never burns or tans) [36]. Individuals with greater scores on the Fitzpatrick scale have more melanin, which can act as a natural

sunscreen. Individuals with skin Type I are 6-times more efficient at synthesizing vitamin D from UVB rays compared to individuals with a skin Type VI.

With an increase in aging, atrophic skin changes occur and the thickness of the epidermal skin layer is reduced. 7-dehydrocholesterol is located in the epidermis and concentrations decrease with aging due to the thinning of the epidermal skin layer. In older adults, 7-dehydrocholesterol concentrations are two-fold lower compared with younger adults. With the reduction in pro-vitamin D concentrations, the ability to cutaneously form pre-vitamin D<sub>3</sub> is decreased, subsequently leading to an overall decrease in 25(OH)D concentration [28]. Past studies have suggested that intestinal absorption of dietary vitamin D decreases with age; however, recent studies observed only minor structural changes to the small intestines that do not significantly contribute to altered vitamin D absorption [27].

Due to the lipophilic nature of vitamin D<sub>3</sub>, adiposity also plays a role in the bioavailability of circulating 25(OH)D. The volumetric dilution and greater sequestration into the adipose tissue hypotheses have been attributed to the following observed associations [37-42]. In one meta-analysis, the prevalence of vitamin D deficiency was 24-35% greater in the overweight and obese individuals compared with the normal weight individuals [43]. Previously, our lab examined the role body composition and BMI play in 25(OH)D response after a 6-month vitamin D supplementation regimen (4,000IU/d) in athletes [40]. We observed a trend between lower fat mass and a lower decrease in 25(OH)D concentrations. Also a significant negative correlation between BMI and 25(OH)D change in the

supplement group was noted. Interestingly, BMI ranged from 18.5 to 26 kilograms/meters<sup>2</sup> (kg/m), with only 2 of 32 participants classified as overweight, suggesting that diminished 25(OH)D concentrations post-supplementation extended beyond overweight and obese individuals and included individuals in the normal BMI category. A recent systematic review [39] examined the effect of weight loss on 25(OH)D. The authors pooled randomized and non-randomized trials and observed a small, significant increase in 25(OH)D concentrations with weight-loss; however, a dose-response relationship was not detected. These studies suggest that increased adiposity lowers 25(OH)D concentrations, potentially increasing the risk of vitamin D deficiency or insufficiency in individuals with greater adiposity.

#### 1.1.6 Controversies in Vitamin D Recommendations

To date, RDA requirements for vitamin D, UVB exposure, and 25(OH)D concentrations for optimal health remain controversial. Although the RDA requirements are set at 600-800IU/d for adults [23], many scientists have stated that this amount is inadequate and should be increased to at least 800-1,000IU/d [25]. The Endocrine Society suggests levels of 1,500-2,000IU/d may be necessary in order to raise 25(OH)D concentrations above 30ng/mL [27]. Furthermore, the National Institutes of Health Office of Dietary Supplements states that no vitamin D synthesis through sun exposure can occur without increased risk of skin cancer [23]. Conversely, due to the short time exposure to UVB radiation required to acquire adequate vitamin D levels, researchers

suggest that sensible sun exposure would lead to minimal risk in the development of skin cancer [28, 44].

Similar to dietary intake and UVB exposure recommendations, a controversy exists about defining 25(OH)D cut-offs for deficiency, insufficiency, and sufficiency. Vitamin D deficiency has been set at 25(OH)D < 20ng/mL, a cut-off that is considered inadequate for bone health [24, 26, 27]. Vitamin D insufficiency is typically considered as 20-29ng/mL; however, some researchers suggest that the insufficiency range should be expanded to a concentration threshold of 32ng/mL as it has been established that this concentration increases intestinal calcium transport by 45-65% [6, 14, 24, 44-46]. Some researchers identifying sufficient vitamin D status starting at 30ng/ml [23], 32ng/mL [14], or more recently 40ng/mL [27, 28, 47, 48]. These concentrations are considered beneficial beyond maintaining optimal bone health and potentially promoting muscle health.

Similarly, the upper limit for what is considered healthy is surrounded by controversy. The National Institutes of Health [23] identifies a 25(OH)D concentrations of >50ng/mL as being associated with potentially adverse effects. Conversely, the Endocrine Society practice guidelines suggest that concentrations up to 150ng/mL are safe and do not induce hypercalcemia [27]. The tolerable upper intake level set by the National Institutes of Health is 4,000IU/d for individuals older than 9 years of age [18]. Excessive vitamin D intake can result in increased calcium levels, which may lead to an increased risk of kidney stones, tissue calcification, and damage to the heart and blood vessels.

However, individuals supplemented with 10,000IU/d for a period of 5-months reported no adverse events [23]. Further, Drincic et al. [41] suggested that normal weight individuals need 4,000IU/d and 5,000IU/d to sustain a 25(OH)D concentration of 32ng/mL and 40ng/mL, respectively.

The recommendations for dietary intake, UVB exposure, and cut-off parameters for adequate vitamin D status vary between governmental recommendations and the recommendations of researchers. Due to these controversies, the prevalence of vitamin D deficiency reported in populations such as in older adults varies, as there is no standardized definition of vitamin D deficiency across all studies. Further, in studies examining the beneficial effects of vitamin D supplementation on health, varying vitamin D supplementation dosages are used leading to inconclusive data. The need for standardized definitions and cut-offs across the scientific community and governmental regulating bodies is both topical and essential for effectively addressing the issue of increased prevalence of vitamin D deficiency. For the purpose of this dissertation vitamin D status will be defined as deficiency set at  $25(\text{OH})\text{D} < 20\text{ng/mL}$ , insufficiency set at  $20\text{-}30\text{ng/mL}$ , and sufficiency  $25(\text{OH})\text{D} \geq 30\text{ng/mL}$ .

#### 1.1.7 Vitamin D Status in Older Adults

Vitamin D deficiency and insufficiency is a worldwide epidemic and there is an especially high prevalence of low vitamin D status in older adults [24]. In part, this high prevalence can be attributed to cutaneous decreases in vitamin D synthesis due to decreased concentration of 7-dehydrocholesterol in the

epidermal skin layer. Likewise, disease states and physiological changes can alter vitamin D pharmacokinetics (the effect the body has on vitamin D) and pharmacodynamics (the effect vitamin D has on the body) [46]. One physiological example is the decrease of calcitriol production due to decreasing renal function observed with aging. Studies suggest that 40 to 100% of older community-dwelling adults are vitamin D deficient [24, 49, 50]. In part, this wide range of vitamin D deficiency is due to diverse cut-off parameters set for vitamin D deficiency and heterogeneity of both physiological and lifestyle factors in the older population.

It is justified to distinguish between community-dwelling and institutionalized older adults. Typically, community-dwelling adults are younger, more independent, and have a lower prevalence of vitamin D deficiency primarily due to increased time spent outdoors [49, 51]. A decline in bone and muscle mass occur with aging, which can result in increased nursing home admission and hospitalizations [50]. With institutionalization, a decrease in mobility and time spent outdoors further contributes to lower vitamin D status. The concern of low vitamin D status in older adults has been aimed at maintaining adequate vitamin D and calcium levels to reduce the risk of falls and bone fractures [52]. However, bone and muscle tissues are functionally linked. Vitamin D can exert its influence beyond the bone by acting on skeletal muscle, potentially preserving muscle strength and function during aging, leading to an overall decrease in risk of falls and fractures [49].

### 1.1.8 Muscle Health and Aging Physiology

Sarcopenia, defined as the age-related loss of muscle mass, is greatest after 50 years of age, with rates of muscle mass decline at 12-15% per decade. Muscle loss affects men and women differently, with men experiencing greater loss than women potential due to decreases in testosterone with aging [53]. The progressive muscle wasting with aging decreases physical function and increases the risk of falls leading to a loss of independence and an increase in morbidity and mortality [9, 49]. As the population of 65 year olds is expected to double in the next 30 years, interest in combating sarcopenia and improving quality of life are rising [54].

Loss of muscle mass leading to decreased muscle strength and function is a multifaceted issue. Histologically, decreases in the amount and size of mitochondria and a decline in the number of Type I (slow twitch) and Type II (fast twitch) muscle fibers is seen with aging. Both increased inflammation and lipotoxicity along with decreased neuronal stimulation, muscle protein synthesis, and capillary blood flow occur with aging further contributing to sarcopenia [9]. Studies have shown that increased ectopic fat depots in muscle are associated with reduced strength and performance [55, 56]. These factors combined limit the ability of the muscle to generate power with age and compromise muscle function, which lead to a decrease in quality of life. It is evident that modifiable lifestyle factors such as aerobic and resistance training and nutrition can play a significant role in preserving or limiting the progression of muscle mass loss [57].



### 1.1.9 The Role of Vitamin D in Muscle Physiology during Aging

Generally, low vitamin D status has been associated with functional limitations and poor physical performance in older adults [38, 49, 50]. However, randomized controlled trials and observational studies have yielded mixed results. A recent systematic review and meta-analysis concluded that vitamin D supplementation had limited influence on muscle strength and mobility preservation in older adults [49]. However, studies included in the analysis widely varied in type of vitamin D supplementation (D2 or D3), dosage of vitamin D, and the combination of vitamin D supplementation with calcium. In addition, functional performance and strength tests varied between assessed interventions. Therefore, the authors suggested that the small sample size among individual trials and heterogeneity of studies preclude the ability to form a fixed conclusion [49]. A 3-year longitudinal study reported that deficient vitamin D status ( $25(\text{OH})\text{D} < 20\text{ng/mL}$ ) was associated with a 2-fold increased risk of muscle mass loss compared with sufficient vitamin D status ( $25(\text{OH})\text{D} > 30\text{ng/mL}$ ) in older adults [58]. In a sample of 20 older adults between the ages of 65-85, our lab observed a significant positive relationship between higher  $25(\text{OH})\text{D}$  concentrations and faster Four-Square-Step Test times (FSST); however we did not see a relationship between vitamin D status and other physical performance (gait speed, timed up and go, and 5-times sit-to-stand) or strength measures [59]. An observational study that included over 2,000 older adults suggested that vitamin D deficiency was associated with weaker handgrip strength and lower performance measures (gait speed, 6-minute walk, and 5-times sit-to-stand)

when compared with individuals with 25(OH)D > 40 ng/mL [60]. Higher concentrations of 25(OH)D were also associated with better lower extremity function (8-foot walk and sit-to-stand test) in both inactive and active adults 60 years of age or older [61]. Lower-extremity function improved from 3.9 to 5.6% from the lowest to the highest 25(OH)D quintile. Further this study suggests that it may be advantageous to reach and maintain 25(OH)D concentrations between 36.5-40 ng/mL as these levels were associated with better lower-extremity function. Other research also suggests that 25(OH)D concentrations greater than 40 ng/mL may be beneficial for muscle health [27, 28, 47]. Shuler et al. [47] reviewed the role vitamin D plays in athletes, the authors' suggest that decreased risk of stress fractures occurs at 40-50 ng/mL and potential athletic performance enhancement occurs at 50-60 ng/mL. In addition, due to variability in assays used to assess 25(OH)D, Hossein-nezhad et al. [28] suggests that maintenance of 25(OH)D between 40-60 ng/mL is ideal. Similarly, the Endocrine Society suggests that maintenance of 40 ng/mL ensures that the individuals' true 25(OH)D concentrations is greater than 30 ng/mL [27]. The Endocrine Society also notes that PTH levels begin to plateau in adults with a 25(OH)D concentration between 30-40 ng/mL, this normalization of PTH indirectly implies that this 25(OH)D concentration can be used to define insufficiency (25(OH)D < 30 ng/mL). Together these studies suggest that 25(OH)D ≥ 30 ng/mL should be considered as sufficient, but a 25(OH)D > 40 ng/mL ensures that sufficient status is met and may be beneficial for muscle health.

The effects of maintaining sufficient vitamin D status on physical function in older adults is under investigation. A 5-year prospective study observed that higher vitamin D levels with adequate physical activity may prevent functional decline [38]. This study followed 615 community-dwelling adults over the age of 50 and observed that higher vitamin D and physical activity levels were associated with decreased gains in body fat and decreased decline of muscle mass. Likewise, during exercise, higher vitamin D levels were associated with increased fat oxidation, further contributing to overall improved muscle health [62, 63]. Sinha et al. [63] examined the effects of vitamin D supplementation (20,000IU of vitamin D3 every other day for 10-12wks) on skeletal mitochondrial oxidative function in severely deficient adults (n=12, 25(OH)D<6ng/mL, age: 18-50) that presented to a primary care facility with fatigue or muscle cramps. The authors' observed an improvement in mitochondrial oxidative function as serum 25(OH)D was negatively associated with phosphocreatine recovery half-time ( $T_{1/2}$  PCR). Magnetic resonance spectroscopy was used to quantify the  $T_{1/2}$  PCR, the time for PCR to decrease below 50%. PCR is typically used as an index to measure mitochondrial oxidative capacity. PC is readily and rapidly broken down by creatine kinase into creatine and phosphate. The phosphate is then donated to adenosine diphosphate (ADP) to form adenosine triphosphate (ATP) which is used to fuel the cell for short intense activity (<10s). The resynthesis of PC post-exercise is inversely proportional to the rate of oxygen consumption, suggesting that PC recovery could be used as an index of oxidative ATP production and a measure of mitochondrial function [64]. Further higher 25(OH)D concentrations

were positively associated with improvements in fatigue and myopathy symptoms. These studies suggest that there may be synergistic interaction in which vitamin D and exercise combined are multiplicative and not simply additive [53] and provide a protective effect for muscle decline. In combination, these findings may suggest that vitamin D supplementation, which increases and maintains adequate 25(OH)D concentrations, may result in muscle function improvements for those who have a baseline vitamin D deficiency.

Over the past several years researchers have been investigating the direct and indirect effects vitamin D has on muscle tissue. Vitamin D can act on the skeletal muscle through genomic and non-genomic pathways involved in cellular metabolism and function. The genomic pathway is slow-acting; whereas the non-genomic pathway is fast-acting (seconds to minutes). Vitamin D receptors (VDR) and CYP27B1 (gene which codes for the protein 1 alpha-OHase) are both expressed in adult muscle cells allowing for circulating and locally converted calcitriol to act on the muscle [65, 66]. VDR is a ligand-dependent transcription factor. Once VDR is bound by calcitriol it can translocate to the nucleus and play a role in the genomic pathway. In the nucleus, VDR forms a heterodimer with the RXR and binds to the vitamin D response elements in skeletal muscle's DNA, thereby activating gene transcription and expression [66-68]. It is thought that binding of calcitriol to non-genomic VDR promotes the phosphorylation of intracellular proteins and formation of second messengers, which upregulate intracellular calcium and increase pathways involved in muscle protein synthesis [66-68]. Vitamin D regulates muscle contractions through controlling calcium

handling within the muscle cell [2]. During a muscle contraction nerve stimulation cause a depolarization of the sarcolemma, which leads to calcium release from the sarcoplasmic reticulum. Calcitriol can act on calcium binding proteins such as calmodulin, located on the sarcolemma and sarcoplasmic reticulum, to regulate calcium flux [66].

Unfortunately, these genomic and non-genomic pathways may be less efficient with aging as skeletal muscle VDR expression was negatively associated with age in muscle tissue of female orthopedic patients [69]. These findings were independent of biopsy location or 25(OH)D concentrations. There was no relationship reported between VDR expression and vitamin D status, which may be due to the small sample size (n=20) and primarily low 25(OH)D concentrations (<20ng/ml). This cross-sectional trial was not able to address whether VDR expression could be increased by vitamin D supplementation in vitamin D deficient individuals. As vitamin D and VDR play an important role in muscle strength and performance observational and randomized controlled trials have been completed to understand the mechanistic role of vitamin D and VDR in muscle health [68].

One study observed that treatment with calcitriol in human myoblasts increased VDR gene expression in a dose-dependent manner [70]. Vitamin D supplementation increased VDR concentration and cross-sectional muscle fiber size in human cells [65, 71]. In C2C12 mice myoblasts, similar results were seen as treatment with calcitriol increased protein synthesis [66]. Furthermore, as part of a randomized double-blind placebo-controlled trial, vitamin D supplementation

over 4-months had a persistent 20% increase in VDR expression and muscle fiber size in women 65 years of age or older [65, 66].

## 1.2 Musculoskeletal Lipid Droplets Characteristics

Musculoskeletal lipid droplets can be classified as intramyocellular (IMCL) or extramyocellular (EMCL) lipids. IMCL is fat that is stored within the muscle cell, typically in close proximity to the mitochondria [72, 73]. Conversely, extramyocellular lipid is adipose tissue accumulation outside of the muscle cell. This adipose tissue accumulation infiltrates the interstitial layers of the muscle [74].

### 1.2.1 Intramyocellular Lipid Characteristics

Skeletal muscle lipid depots serve as a dynamic energy substrate. There exists a high degree of IMCL heterogeneity including size, cellular location, and type of proteins located on the lipid droplet phospholipid monolayer [73]. IMCL can range between 0.3 $\mu$ m and 1.5 $\mu$ m in diameter, with larger sized and greater amount of IMCL droplets in Type I (oxidative slow twitch) compared with Type II (fast twitch) muscle fibers [75]. IMCL is located in-between a series of sarcomeres, known as myofibrils, and is typically located adjacent to the mitochondria [72]. Although lipid droplet motion during muscle contraction remains somewhat uncharacterized, researchers assume that due to the restrictions within the cell very limited movement occurs [73].

Primarily, IMCL is comprised of triacylglycerol (TAG) and diacylglycerol (DAG) and multiple enzymes play a role in lipid depot formation. Monoacylglycerol (MAG) can be acylated to form DAG with subsequent acylation

to form TAG. Both monoacylglycerol acyltransferase (MGAT) and DAG-O-acyltransferase (DGAT) play a role in the MAG-DAG-TAG pathway. Furthermore, two isoforms of DGAT enzymes are located within the muscle (DGAT1 and DGAT2). DGAT1 and DGAT2 are located in the endoplasmic reticulum, but DGAT2 is also located on the mitochondrial membrane and may also be located on the lipid droplet monolayer. DGAT2 is thought to be involved in the formation of TAG [73]. These lipid droplets are synthesized in the endoplasmic reticulum, however the precise mechanism by which the lipid droplets are formed is still under investigation.

Coat proteins are embedded throughout the phospholipid monolayer [73, 76]. Five proteins comprise the major protein family, perilipin (PLIN 1-5) [73, 76]. PLIN 2-5 are localized in the skeletal muscle [73, 76]. Specifically, PLIN2 aids in lipid storage [73], PLIN3 plays a role in fat oxidation [73], and PLIN4 assists with packaging of newly synthesized lipids [73, 76]. PLIN5 plays a role in the interaction between the mitochondria and the lipid monolayer [73]. It has been observed that endurance training increases PLIN5 expression [73, 76]. In skeletal muscle, PLIN5 increases IMCL oxidative gene expression and thereby may regulate substrate oxidation during exercise [73].

### 1.2.2 Factors Playing a Role in IMCL Turnover

Over the past decade, researchers have shown that IMCL depots are extremely dynamic [72]. Increased IMCL content has been associated with aging, insulin resistance, Type II diabetes, and obesity [72, 77]. Conversely, increased IMCL content has also been observed in athletes with high endurance leading to

the term “athletes paradox” [77]. In addition to an athletic population, obese individuals after an aerobic exercise intervention experienced higher IMCL with improved insulin sensitivity and greater oxidative capacity [74, 77, 78]. Due to the paradoxical association of IMCL with insulin sensitivity and resistance, IMCL has garnered prodigious interest from the scientific community.

Many factors play a role in IMCL accumulation, including age, adiposity, diet, and exercise. Although aging has been associated with higher IMCL and increased insulin resistance, this relationship is multifactorial. For example, de la Maza et al. [79] compared healthy younger and older men and observed that a greater IMCL content was associated with increased abdominal fat and insulin resistance, independent of age. Choi et al. [80] compared normal weight and obese older adults and observed that the obese group had a greater percentage of Type I muscle fibers and a 2-fold greater number and total area of the lipid droplets. Increased IMCL was associated with lower force, power, and slower contractions in the muscle fibers. Conversely, acute (7 consecutive days) [74] and chronic (16wk) [77] aerobic exercise interventions in older adults were shown to improve insulin sensitivity, increase IMCL content [74, 77], and improve oxidative capacity [77]. Our lab previously observed a positive relationship between vitamin D status and IMCL in older adults, independent of physical activity and BMI [59]. Also in C2C12 myotubes, calcitriol had a 1.5-2.0 fold increase in expression of IMCL packaging proteins PLIN2 and PLIN3 [81]. In combination, these data suggest that vitamin D and exercise may play a positive



role in IMCL accrual, which can be oxidized during times of increased muscle energy expenditure.

### 1.2.3 Extramyocellular Lipid Characteristic and Function

EMCL are visible adipocytes located within the muscle tissue and between both muscle groups and muscle fibers [74, 82]. The physiological function of EMCL still remains largely unreported and unknown [74, 83]. However, greater EMCL accumulation has been observed with aging, greater adiposity, impaired insulin sensitivity, and reduced physical function [58, 74, 84].

Hogrel et al. [85] observed that individuals' 70-80 years of age had greater EMCL compared with younger healthy individuals. Furthermore, it was observed that EMCL was greater in older overweight men compared with older normal weight women. Likewise, with greater BMI levels greater EMCL levels were also observed [84]. Velan et al. [84] observed that overweight young adult males had greater EMCL compared with normal weight males. Correspondingly, de la Maza et al. [79] observed similar results in which greater central obesity was associated with greater EMCL and insulin resistance. It is essential to note that Haus et al. [74] observed a decrease in EMCL and insulin resistance after 7 consecutive days of aerobic training in obese, insulin resistant men.

Previous work has observed that lower 25(OH)D concentrations were associated with greater EMCL in both the young and elderly. Tagliafico et al. [55] observed that in the elderly vitamin D deficiency was associated with reduced physical performance (balance and gait) and increased EMCL of the thigh. Similarly, in young healthy women, vitamin D insufficiency was associated with

increased EMCL [86]. Previously our lab found no relationship between EMCL, 25(OH)D, BMI or muscle strength and performance measures [59]. These results may differ from aforementioned findings due differences in subject characteristics, sample size, vitamin D status, and techniques used to quantify EMCL.

### 1.3 Diet and Muscle Lipid

The interest in how dietary intake plays a role in preserving muscle health is essential in maintaining muscle strength and function, which decreases with aging [57]. As muscle lipid depots can affect muscle function, the role of diet on IMCL and EMCL changes has garnered much interest. Initially it was thought that through increased caloric intake, there was an increase in fatty acid uptake, adding to the lipid overflow and leading to fat storage in the muscle [30]. With concurrent low mitochondrial lipid oxidation and lipid turnover, muscle lipid was implicated in increased insulin resistance [77]. However, the accretion of muscle lipid (IMCL) congruent with greater mitochondrial oxidation and lipid turnover can lead to improved insulin sensitivity. Recent data suggests that multiple lifestyle factors can play a role in muscle lipid metabolism.

Dietary interventions may play a key role in reducing lipid overflow and fatty acid processing in the skeletal muscle. Previous data suggests that there are inter-individual gene expression changes in response to a high fat diet (HFD). Studies have observed increased fat oxidation in lean subjects but not in obese subjects. In order to further explore this relationship Kakehi et al., [87] examined the effect of a HFD in healthy non-obese men. The 3-day HFD consisted of 60%

fat (~45% saturated fatty acid (SFA), ~30% monounsaturated fatty acid (MUFA), ~25% polyunsaturated fatty acid (PUFA)), 20% carbohydrates, and 20% protein. The authors examined high responders, those with large increases in IMCL, versus, low responders, those with small increased in IMCL. The authors observed that the high responders had upregulated gene expression related to fatty acid oxidation. A short-term HFD may result in free fatty acid influx, which exceeds the expenditure in the skeletal muscle leading to IMCL accumulation and increased insulin resistance. The upregulated fatty acid oxidation gene expression may suggest a compensatory mechanism to decrease lipid accumulation.

Many studies suggest that the type of fat SFA, PUFA, or MUFA is an important factor in lipid metabolism [88, 89]. In a single-blinded randomized crossover trial, older insulin resistant men consumed either a high SFA, MUFA, or PUFA diet [89]. The authors observed that compared with a high SFA diet, a high PUFA diet was associated with reduced muscle uptake of fatty acids and an increase in IMCL turnover. Furthermore, a higher MUFA and PUFA diet may result in lower uptake of lipids into the skeletal muscle, as PUFA and MUFA rich chylomicrons may be removed from the circulation via hepatic lipase instead of lipoprotein lipase.

St-Onge et al. [90] examined the effects of 25d crossover controlled feeding trial. Participants (19-65YO) consumed a low fat diet (14.2% from MUFA, 8.5% from SFA, and 5.2% from PUFA), a HFD (15.9% from MUFA, 11.4% from SFA, and 5.8% from PUFA), and a high PUFA diet (15.3% from MUFA, 8.5%

from SFA, and 9.7% from PUFA). The authors hypothesized that a higher PUFA diet would result in lower IMCL accumulation compared with a HFD, as unsaturated fatty acids are more readily oxidized compared with SFA. However, they observed a decrease in IMCL after a low fat diet and an increase in IMCL in both HFD interventions. The authors concluded that IMCL content is not modulated by the dietary fat type but the total fat intake. Further, other researchers have noted that the amount of IMCL is not as important as the content and turnover of the IMCL [88, 89].

Animal models have suggested that the specific types of fatty acids exert different influences on IMCL. Although, both unsaturated fatty acids and SFA may lead to IMCL accretion, unsaturated fatty acids are preferentially oxidized over SFA [88]. Overwhelmingly, cell and animal models have suggested that SFA and trans-fats reduce insulin sensitivity, while PUFA improve insulin sensitivity [88, 91]. Specifically, in cell studies, SFA such as palmitate, the most prevalent free fatty acid in the plasma, alongside with stearate and arachidate can be stored as IMCL and induce DAG and ceramide synthesis, inhibiting AKT which results in downregulation of GLUT4. Together these actions lead to a reduction in insulin sensitivity and overall decreases in glucose uptake. Conversely, rodents fed a HFD enriched with n-3 PUFA, had a protective effect against insulin resistance development. Specifically, eicosapentaenoic acid and docosahexaenoic acid may increase lipid turnover by upregulating genes involved in lipid oxidation [88]. Mice fed a HFD enriched with n-3 PUFAs for 10wks had a decrease in ceramide and long chain Acyl-CoAs implicated in

increased insulin resistance [92]. The authors suggested that an n-3 PUFA diet effects muscle lipid content by decreasing the bioactive lipid mediators, which negatively influence insulin signaling. Although the authors did not report any difference in mitochondrial oxidative capacity, they suggested that mitochondrial membrane fluidity increases with an n-3 PUFA, while a high SFA diet can cause rigidity in the membrane. Therefore, it is feasible that n-3 PUFAs would promote an overall greater mitochondrial oxidation leading to greater lipid turnover.

To a lesser extent studies have examined the relationship between diet and EMCL. Higher EMCL content was seen with obesity and has been associated with insulin resistance. It remains unclear if EMCL is a marker of insulin resistance or plays a role in contributing to insulin resistance. Haus et al. [74], completed a combined intervention of a low-glycemic index diet and exercise. The authors' observed a decrease in EMCL, increase in IMCL, and improved insulin sensitivity, but attributed these findings to the exercise regimen and not the dietary changes. Studies have shown that a decrease in total caloric intake and weight loss can lead to significant decreases in EMCL. However, weight loss in older adults who may already be frail or have lower BMI may not be a desirable option and instead physical activity should be considered [30].

#### 1.4 Physical Activity and Muscle Lipid

Physical activity and exercise are related but differ in their definition. Physical activity is defined as voluntary or involuntary body movement produced by the muscle that results in energy expenditure, whereas exercise is defined as a subset of physical activity that is structured, planned, and repetitive and used

for the purpose to gain or maintain strength and aerobic capacity [93]. Exercise has been implicated in both IMCL and EMCL. Multiple exercise interventions in both young and older individuals have been conducted over the years to further understand the effects of exercise on muscle lipid depots. One study recruited both obese men and women, 60-75 years of age for a 16wk exercise intervention [77]. The participants completed a 4-5d/wk, 45-minute session at a moderate intensity (75% of heart rate maximum). Post-exercise, 21% increase in IMCL and a decrease in both DAG and ceramides was observed. In addition, oxidative capacity and capillary density improved. An 8wk randomized controlled trial examined the effects on IMCL and skeletal muscle oxidative phosphorylation [94]. Obese adolescence completed 20-minutes of stationary bicycling at 60-80% heart rate reserved (difference between resting heart rate and maximum heart rate) followed by 25-minutes of combined stretching and resistance training. The authors observed an improvement in overall fitness and power, and consistent with increased resting energy expenditure and lower respiratory quotient, an increase in IMCL. These data suggest that there is a greater reliance on lipid oxidation after an exercise intervention. Haus et al. [74] examined the effects of a shorter, 7 consecutive day, exercise regimen (60 minutes/d, ~80-85% of heart rate maximum) on insulin resistance, obese men. The authors observed a decrease in EMCL and an increase in IMCL with improved insulin sensitivity suggesting a potential change in lipid oxidation and mobilization to support muscle demands.

Unlike the aforementioned studies, Rouffet et al. [95] examined the immediate muscle lipid changes in healthy, normal-weight to overweight males with a combined short-term dietary intervention and exercise test. In this sample, the authors observed that overall higher IMCL stores were associated with greater VO<sub>2</sub> max. A significant decrease in IMCL was observed after a 2-hour exercise (treadmill walking at 50% VO<sub>2</sub> max) leading to conclude that IMCL is consumed and used during aerobic exercise. IMCL levels also increased after a 3d fat depletion-repletion diet (depletion equated to an intake of 26% fat, 58% carbohydrate, and 16% protein for 2.5d followed by a repletion phase of 58% fat, 24% carbohydrate, and 18% protein intake) compared with a high fat diet (58% fat, 24% carbohydrate, and 18% protein) alone. These studies provide evidence for the dynamic storage and mobilization of IMCL with short and long-term exercise interventions.

### 1.5 Scope of Dissertation

Aging is associated with changes in musculoskeletal health. Both muscle strength and performance are reduced with age and muscle lipid metabolism is altered. Vitamin D status, diet, and exercise all play a role in muscle lipid metabolism and contribute to overall health and quality of life. Low vitamin D status has been associated with increased EMCL and previously our lab observed a positive linear relationship between 25(OH)D concentrations and IMCL. However, there is a need to further examine how 25(OH)D and muscle lipid depots related with physical function in a healthy older cohort. Furthermore, studies have examined the effect of short-term dietary interventions on muscle

lipid depot changes, but to date no research has been presented examining the relationship of daily dietary habits and muscle lipid depots in older adults.

Preceding studies have examined the effect of structured exercise interventions on muscle lipid; however, we will focus on how physical activity relates to muscle lipid depots.

Specific Aim 1. To examine the relationship between 25(OH)D concentrations and muscle lipid depots (IMCL and EMCL) in older adults. Multiple factors contribute to this relationship including age, adiposity, and physical activity. This chapter will discuss and compare the relationship between vitamin D status and muscle lipid depots among older adults with different levels of physical activity and adiposity.

Specific Aim 2. To elucidate the relationship between dietary intake and physical activity on local muscle tissue hemodynamics and muscle lipid in older adults. This chapter will discuss how dietary intake, specifically fat intake, relates with muscle lipid depots. Further, structured exercise is known to improved global and local tissue hemodynamics and this chapter will focus on the potential relationship between physical activity levels and local muscle tissue hemodynamics.



## Chapter 2: Methods and Materials

### 2.1 Novel Techniques

The skeletal muscle is involved in both mechanical and metabolic function [96]. From a mechanical perspective, skeletal muscle is important for body movement and posture control, which contribute to overall health and functional independence. Metabolically, skeletal muscle plays an important role in glucose homeostasis, fuel oxidation, and strongly influences basal metabolic rate [96]. Due to its important mechanical and metabolic function, various techniques have been developed to quantify and assess skeletal muscle. Throughout history, skeletal muscle has been difficult to quantify in vivo [97]. Traditionally skeletal muscle was quantified through indirect measures such as urinary creatinine excretion [98]. Recently, more sophisticated techniques have been used such as Magnetic Resonance Imaging (MRI), computed tomography (CT), and Dual-energy X-ray absorptiometry (DXA) (Table 2.1).

These advanced techniques are especially important and are utilized to observe and analyze muscle decline with aging. MRI provides a participant friendly and non-invasive method to obtain high resolution MR images. Our lab used MRI and MR spectroscopy (MRS) to examine both EMCL and IMCL in adults 60 years of age or older. Further, our lab used non-invasive instrument that combines Near-Infrared Spectroscopy and Diffuse Correlation Spectroscopy (NIRS/DCS) to monitor blood flow and oxygen consumption during a dynamic foot plantar flexion exercise protocol. The combined techniques, MRI and

NIRS/DCS, allow us to examine muscle lipid and hemodynamics, and use these variables as an index to monitor local muscle tissue metabolism.

### 2.1.1 MRI and Muscle Lipid Depots

MRI is a non-invasive imaging instrument that can produce detailed 3-dimensional images of the body including soft tissues, bones, and organs [99]. In order to produce these images MR uses the magnetic properties of atoms. The most abundant atom in the human body is hydrogen, which is commonly found in water and fat. Atoms have 3 types of motion, which include electrons spinning on their own axis, electrons orbiting the nucleus, and the nucleus spinning itself about its own axis. In the absence of an applied magnetic field hydrogen nuclei are randomly orientated, but when a strong static external magnetic field is applied most hydrogens align with the magnetic field. The MR produces a strong magnetic field which forces low-energy nuclei to align their axis of rotation parallel to the static external magnetic field ( $B_0$ ), whereas high-energy nuclei align anti-parallel to  $B_0$ . Only a couple of hydrogens out of every million are not aligned with the magnetic field; however due to the high number of hydrogen atoms in the body these non-aligned hydrogens allow for a detailed image. With a greater  $B_0$  (or higher Tesla) more hydrogens are aligned with the magnetic field, which results in an improved signal, image resolution, and overall image quality. It is important to note that each hydrogen nucleus spins on its own axis and the  $B_0$  produces a secondary spin or wobble, termed precession, to the hydrogen. Further, each nuclei has a gyromagnetic ratio, which is defined as the ratio of the magnetic momentum of an atom compared to its angular momentum

(the momentum an odd mass nuclei has in which the number of neutrons is slightly more or less than that of protons resulting in a net spin or angular momentum). These concepts are important as each nuclei has its own gyromagnetic ratio and when exposed to the same field strength will precess at different frequencies. For example hydrogen will precess at a different frequency than carbon, which allows researchers to focus imaging on hydrogen and ignore other atoms in the body.

In order to produce an MR image a short burst of energy in the form of electromagnetic radiation known as radio frequency (RF) pulse must be added towards the body part being imaged. When the RF pulse is applied, it allows for excitation to occur by forcing the unmatched hydrogens to spin at a particular frequency and direction, this is termed resonance. Both echo time (TE) and repetition time (TR) are the basic RF pulse sequence parameters measured in milliseconds (ms). TE is defined as the time from the application of the RF pulse to the peak of the signal in the coil and TR is the time between each excitation pulse. During the RF pulse, 3 gradient magnets, which are magnets inside the MR scanner with lower magnetic field strength, are turned on and off to alter the main magnetic field to create slices. Slices are area-specific images in which the body part can be observed in any of the 3 axis: sagittal (X), coronal (Y), and transverse (Z). When the RF pulse is off, hydrogens realign with the magnetic field and the energy absorbed from the RF pulse is released and the signal is received by the coil. Through a series of mathematical formulas, termed the Fourier transform, the data is transformed into an image.

The images, either T1- or T2-weighted, have a contrast gradient which emphasizes contrast characteristics of anatomical structures and allows researchers and physicians to examine different tissues. T1-weighted images are characterized by bright fat and fatty bone marrow and dark water with short TE and TR, whereas T2-weighted images are characterized by bright water and dark fat using longer TE and TR. Due to the better signal-to-noise ratio during scanning, T1-weighted images result in better anatomical delineation and are typically used. T1-weighted images depend on the differences between fat and water. If the TR is too long, both fat and water would return to  $B_0$  and the contrast between the two would be reduced, resulting in an image with poor differentiation of tissue. Thus, short-time TR parameters are set, which do not allow either water or fat to fully return to  $B_0$  after a RF pulse is applied, resulting in a high-contrast resolution image. In addition, due to the different resonance frequency between fat and water a chemical shift artifact occurs which produces white or dark bands on either side of the anatomical object. In order to reduce this artifact we use the same parameters to produce a fat saturated image. In this image we suppress the signal from fat. Saturation of the fat signal allows for better contrast-to-noise ratio and it is used to calibrate the non-fat suppressed image. Therefore, the two MR images, non-fat saturated and fat saturated, are used to provide a conventional MRI image of anatomical features.

Conventional MR imaging is used to assess structural images; however new functional imaging techniques, such as MRS, are used to assess metabolic changes in the body. MRS produces a spectrum that plots signal intensity versus

frequency rather than an anatomical image. The signals at specific chemical shifts (parts per million (ppm)) indicate different substrates in the body such as lipids. A single voxel is a point in 3-dimensional space positioned within the area of interest used to determine the relative amounts of each substrate. The advantage of using spectroscopy is that it allows for relative amounts of various metabolites, most notably lipids, to be measured.

A key advantage of administering a MR scan is that the MR does not emit ionizing radiation unlike X-rays and CT scans. Likewise, MR imaging of the brain, muscle, and ligaments/tendons results in higher quality images compared with other imaging technologies. Further, the MRS technique allows for metabolites in specific tissues to be assessed. Conversely, a major disadvantage of MR imaging is the high cost of the equipment and the need for highly trained personnel to operate the instrument. Due to the strong magnetic field that the MR emits, ferromagnetic items can be attracted into the MR scanner and are a risk to study personnel and the participant. Verbal screening and a hand-held metal detector can be used to ensure the participant has removed all ferromagnetic materials. Ferromagnetic implants and prosthetics are not compatible with the MRI. For example, pacemakers are an absolute contraindication to MR imaging; however, non-ferrous metallic implants may be imaged. Prior to scanning, details about body implants or any foreign body objects are documented and assessed to determine MR compatibility and safety. In addition, claustrophobic individuals may feel uncomfortable in an enclosed MRI; however new open MRI options are available although not all types of imaging are possible with an open MRI. During

the scan the electrical current in the wires of the 3 gradient magnets are opposed by the main magnetic field producing loud noise; therefore participants are provided with earplugs. Finally, the main cause of distorted images is motion artifact produced by the participant during scanning; therefore the individuals are asked to remain still for a prolonged period of time.

In our study, MRI and MRS were used to measure EMCL and IMCL content in the gastrocnemius (Figure 2.1). We chose to image the gastrocnemius for its superficial anatomical location, which allowed us to combine MR technology with NIRS/DCS used to assess hemodynamics in superficial tissues [100]. With aging, a decline in muscle function and an increase in risk of fall were observed [101, 102]. The gastrocnemius is key to gait stability in aging. Likewise, age alterations in muscle lipid occur and often play a role in declining muscle function. By examining EMCL and IMCL in the gastrocnemius we were able to further the understanding of functional muscle decline with aging.

Prior to scanning, all participants completed a MRI safety screening form (Appendix 1). Further, as dietary intake can influence muscle lipid content, participants were asked to refrain from eating overnight prior to scanning. Participants were positioned within a 3.0T TIM TRIO scanner (Siemens) in a feet first supine orientation with their lower leg (left or right), in a fixed dorsiflexed position, placed in a RF coil [59]. To minimize participant motion we taped the participant's foot in a dorsiflexed position to the coil. If repeat MRI scans were completed, a small oil-filled capsule was taped to the calf muscle to ensure same anatomical slices were acquired on the repeated visit. The capsule location was

recorded by measuring the distance from the most inferior point on the participant's heel to the middle portion of the capsule. In order to measure the anterior-posterior location we measured from the tibia to the middle of the capsule. At repeated scans the capsule was placed in the same location and served as a reference for slice positioning during imaging. T1-weighted images, non-fat and fat saturated, were completed and used to assess EMCL. 1H-MRS was used to quantify IMCL. In order to obtain IMCL data a single voxel placed in the gastrocnemius (TR/TE = 2,000/30 ms). In addition, an unsuppressed water spectrum of the same parameters in the single voxel was obtained (TR/TE = 2,000/30 ms). To achieve optimal IMCL and EMCL peak separation 1) the voxel was placed within the gastrocnemius avoiding EMCL and 2) automated gradient and manual adjustments of the gradient were performed. Total imaging time was approximately 35 to 45 minutes.

Data was transferred from Siemen's MR scanner to a Linux computer for quantification of IMCL and EMCL. LCModel (version 6.3, Stephen Provencher, Oakville, ON, Canada) was used to quantify IMCL using spectral fitting with water scaling and eddy current correction. LCModel was employed to estimate lipid peaks (1.5 ppm for EMCL CH<sub>2</sub>, 1.3 ppm for IMCL CH<sub>2</sub>, 1.1 ppm for EMCL CH<sub>3</sub>, 0.9 ppm for IMCL CH<sub>3</sub>) and creatine (2.8 and 3.0 ppm) (Figure 2.2). IMCL methylene protons (1.3 ppm CH<sub>2</sub>), which were used for statistical analysis. LCModel automatically scaled lipids and creatine to unsuppressed water peak (4.7 ppm). Sets of T1-weighted images (TR/TE = 650/22 ms) for high-resolution anatomy were used to assess EMCL. Gastrocnemius EMCL was quantified by

manually drawn regions of interest (Figure 2.3) using a fat segmentation program (FSL View, FMRIB Software Library, version 5.0) and percent fat was calculated based on a mathematical formula published by Williams et al [103].

### 2.1.2 NIRS/DCS

Skeletal muscle typically constitutes 40% of total body mass and during exercise skeletal muscle can use up to 85% of total cardiac output [104]. To maintain overall muscle function and health, microvascular blood flow delivers nutrients (e.g. oxygen) and removes by-products from the muscle [104, 105]. Hemodynamics explain forces that govern blood flow within blood vasculature and are integral in understanding tissue function and its ability to respond and adapt to stressors in both healthy and pathogenic muscle tissue [105]. Muscle tissue function can be evaluated through measurements of blood flow (BF), tissue oxygen consumption rate ( $VO_2$ ), and tissue oxygen saturation ( $StO_2$ ). These measures provide an additional parameter to the process of disease diagnosis and the ability to examine the efficacy of treatment.

Currently, many different modalities exist to measure aspects of hemodynamics. MRI and Positron Emission Tomography (PET) scans are able to continuously monitor microvasculature changes during dynamic protocols [100, 104]. However, these instruments are relatively expensive and highly sensitive to motion during dynamic exercises, which distorts signals and produces motion artifacts. Another drawback is that these approaches require cumbersome equipment that is not portable and may not be available in all clinics. PET also requires the use of radioactive elements, which are harmful to



patients [104]. Another invasive technique is blood draws from major vessels through which blood gas levels can be determined. Measurements using this method yield regional rather than local results including partial pressure of oxygen, partial pressure of carbon dioxide, oxygen saturation, arterial pH, and bicarbonate levels. To further the understanding of tissue function and its adaptations to metabolic stress it is important to be able to measure hemodynamics in a continuous, non-invasive, and inexpensive way.

Two novel and non-invasive optical technologies, NIRS and DCS, can be used to study healthy and pathogenic tissues by quantifying relative changes in the microvascular blood and measuring oxy- and deoxy-hemoglobin concentrations [104]. Tissue hypoxia is commonly associated with various disease states and can occur when arterial oxygen is low, blood flow is slow, or local tissue metabolism is high. Numerous medical conditions including cerebrovascular accidents (CVA), peripheral artery disease (PAD), and even some cancers are associated with anomalies in hemodynamics. PAD is the narrowing of peripheral arteries primarily in the lower limbs and does not encompass the blood supply to the heart or brain. Vascular deficits are the keystone in PAD patients and to some extent PAD patients have a poor response to the metabolic demands of exercise. Following treadmill and pedal exercise tests, an increase in relative blood flow (rBF) was observed in healthy subjects, whereas PAD patients maintained relatively stable rBF [106]. Likewise, the effect of fibromyalgia on the microvasculature was investigated by NIRS/DCS. Fibromyalgia is characterized as the widespread musculoskeletal

pain typically accompanied by reduced exercise tolerance, fatigue, sleep deprivation, memory impairments, and mood disorders [107, 108]. Patients with fibromyalgia had similar rBF with matched healthy controls; however lower oxygen extraction was observed in the disease state [107]. These data highlight the importance of monitoring multiple parameters to provide measures of local muscle metabolism and the advantage of the novel and non-invasive nature of NIRS/DCS.

NIRS oximetry is a reliable instrument used to determine oxy-hemoglobin (HbO<sub>2</sub>), deoxy-hemoglobin (Hb), total hemoglobin concentration (THC), and blood oxygen saturation (StO<sub>2</sub>) [100]. Advantages of NIRS include the ability to assess tissue hemodynamics in a non-invasive continuous manner, monitor at the bedside due to its easy portability, and penetrate tissue up to several centimeters in depth. A well-known spectral window exists in NIRS (650-950nm), in which tissue absorption is relatively low, allowing light to penetrate into deep/thick tissue [105]. As photons pass through the tissue the photons scatter when interacting with cell membranes, organelles, and nuclei or be absorbed when interacting with HbO<sub>2</sub> and Hb. At NIRS wavelength the scatter is greater than the absorption and the photons will reflect back to the surface of the tissue in which a detector can sense the photons. Three different techniques of NIRS exist and differ in illumination type. The three paradigms are 1) continuous-wave, 2) frequency domain, and 3) time-domain. We use frequency domain in which the inputted modulated light interacts with the tissue, such as the muscle, and the change in amplitude and phase shift are detected. Measurements of these

changes at multiple source-detectors allow for the quantification of absolute values of deoxy-, oxy- and total hemoglobin concentrations [i.e. [Hb], [HbO<sub>2</sub>], and [THC].

DCS is used to quantify changes in microvascular rBF in deep tissues. Previously, only red blood cells in superficial tissue (<1mm) were able to be monitored by laser Doppler. This technique utilized the single scattering theory, which quantified the light fluctuations within a specified tissue area. However, in deeper or thicker tissues the photons undergo multiple scattering that are accounted for by the DCS. DCS uses near-infrared light to penetrate deep tissues and measure light speckle fluctuations caused by the motion of red blood cells [104]. Unlike other modalities for measuring BF in which a patient may be exposed to ionizing radiation or contrast agents (eg. PET), DCS is non-invasive. Most importantly it can be utilized for continuous monitoring during dynamic exercise when a gating algorithm is applied.

Separately these two sophisticated tools can be used to monitor oxygenation and BF in tissues. However, in combination, hybrid NIRS/DCS instrument can be used to derive relative oxygen consumption (rVO<sub>2</sub>) employing Fick's principles [100]. Although Fick's principles were initially developed for measuring cardiac output, the principles can be applied to procure other quantifications. Fick's principles include knowing 1) how much of a substance is consumed by the tissue per unit of time, 2) how much of the substance is in the arterial blood supply to the tissue, and 3) how much of the substance is in the venous blood leaving the tissue. Fick's law states that the BF multiplied by the

arteriovenous oxygen difference is equaled to  $rVO_2$ . Using NIRS/DCS and employing Fick's laws,  $rVO_2$ , relative oxygen extraction fraction (rOEF), and relative BF (rBF) can be derived. Recently, the combination of the hybrid NIRS/DCS instruments were used to continuously monitor hemodynamic parameters during a handgrip exercise in forearm muscles [109]. These methodologies were modified and applied to the gastrocnemius, a lower-limb muscle, during a dynamic plantar flexion exercise [100].

In my study, NIRS/DCS was used to quantify muscle oxygen consumption rate ( $VO_2$ ), BF, and tissue (muscle) oxygen saturation ( $StO_2$ ) in the gastrocnemius during a plantar flexion exercise protocol (Appendix 2). A plantar flexion exercise is the ankle movement of moving the foot down and pointing the toes away from the body. A commercial NIRS oximeter (Imagent, ISS inc.) and a custom built DCS flowmeter were used. The Baltimore Therapeutic Equipment (BTE Primus, Hanover, MD) was used for the plantar flexion exercise. Participants were seated at a  $70^\circ$  semi-recumbent position with the right calf exposed for placement of the optical spectroscopy sensors. The right foot was placed into the BTE plantar flexion attachment and secured with Velcro straps. Participants completed a familiarization session followed by a testing session and were given at least 48 hours of rest between these two sessions to avoid the influence of muscle fatigue on tested measures. During familiarization and testing sessions a warm-up was implemented to prime the body for increased oxygen demand occurring with exercise and to reduce the risk of injury. Warm-up consisted of at least 10 foot plantar flexion repetitions at  $30^\circ$  range of motion and

a torque of 25% of bodyweight. Next, at least 5 maximal voluntary isometric contractions (MVC) lasting 4-5 seconds were completed with a 30 second rest period between each MVC [110]. Thirty-five percent of the highest MVC recorded was used to set torque that the participant pushed against during a 75 foot plantar flexion repetition exercise [100]. To ensure consistent effort was provided by the participants during MVC, additional MVC were performed if coefficient of variation (CV) was greater than 10%.

After MVC was determined, the optical sensor was taped to the right gastrocnemius. Right thigh occlusions were performed and used to calibrate the NIRS/DCS device to obtain absolute baseline BF (ml/100ml/min) and absolute baseline VO<sub>2</sub> (ml/100ml/min). Occlusions consisted of three, 10 second venous occlusions at 55 millimeter of mercury (mmHg) which blocked the return of blood flow to the heart from the lower limb. One minute recovery periods between venous occlusions were completed. Data from venous occlusions were used to calibrate rBF (measured by DCS). Next, one, 3 minute arterial occlusion at 235 mmHg was completed and used to calibrate rVO<sub>2</sub> to absolute VO<sub>2</sub>, with 5 minutes of recovery time (measured by NIRS). A 3 minute arterial occlusion allowed for complete occlusion of blood flow into and out of the lower limb. Recovery periods allowed tissue hemodynamics to return to baseline [100, 110].

Muscle BF, StO<sub>2</sub>, and VO<sub>2</sub> rates were continuously measured 3 minutes before the plantar flexion exercise to ensure hemodynamic stability, during the 75 plantar flexion repetitions (2.5-3 minutes), and for 15 minutes post-exercise to monitor blood flow recovery. Participants were asked to complete the concentric

plantar flexion contractions and the plantar flexion attachment automatically completed the eccentric dorsiflexion contractions without participant assistance. Verbal encouragement was used to ensure consistent effort throughout the 75 repetitions. NIRS/DCS instrument collected local muscle tissue parameters (VO<sub>2</sub>, BF, and StO<sub>2</sub>) at the end of every eccentric phase plantar flexion (0.5s measurements period) to minimize muscular motion artifacts due to muscle contraction. These procedures were adapted from Henry et al. [100], in which NIRS/DCS measures were translated from examining hemodynamic measures in the forearm muscle during handgrip exercises to the gastrocnemius muscle during plantar flexion exercise.

## 2.2 Clinical Assessments

Participants' height, weight, blood pressure, and waist circumference were measured. The study coordinator measured participants' height using a wall-mount stadiometer (DETECTO 3P, Webb City, MO or SECA 264). To ensure precise measurements participants were directed to remove their shoes, hair jewelry, hats or hairstyles such as buns or braids from the top of their heads and stand on the stadiometer platform [111]. Participants were further instructed to stand up straight against the backboard of the stadiometer and to the best of their ability keep their head, shoulder blades, buttocks, and heels in contact with the backboard. The head was aligned in the Frankfort horizontal plane, in which the ear canal and the eye were parallel to the floor and perpendicular to the backboard. Next, the participant was instructed to take a deep breath and hold this position as the coordinator lowered the headpiece of stadiometer to rest

firmly on top of the participant's head compressing the hair. Measurements were obtained one time and recorded in centimeters. Weight was determined using a Tanita 800S scale (Tokyo, Japan). Participants were instructed to remove shoes and any objects in pockets. The study coordinator calibrated the scale to zero and instructed the participant to step and stand still on the scale platform. Once the readout on the digital scale became stable the coordinator recorded the value in kilograms. Body mass index (BMI) values were calculated using measured height and weight values as weight (kilograms)/height (meters<sup>2</sup>). BMI categories were defined as: underweight (BMI value <18.5), normal weight (BMI value 18.5-24.9), overweight (BMI value 25-29.9), obese class I (BMI value 30-34.9), and obese class II (BMI value 35-39.9) and extremely or morbidly obese (BMI value ≥40) [111, 112].

The study coordinator obtained blood pressure using a standard (16centimeters (cm) width; 30cm length) or large adult (16cm width; 36cm length) automatic blood pressure cuff. The participant was in a seated position with the arm uncovered and the cuff placed on either the right or left arm approximately 2cm above the bend in the elbow [113]. The center of the inflatable portion of the cuff was positioned over the brachial artery and the cuff was at heart level. Participants were asked to place both feet flat on the floor and sit silently during blood pressure measures. The study coordinator recorded the systolic and diastolic values (mmHg).

Waist circumference (Gulick II Measuring Tape, Wisconsin, USA) was measured by the study coordinator three successive times and the results were

averaged. The inelastic, flexible tape measurer was placed on bare skin surface without compressing the subcutaneous adipose tissue [114]. The smallest circumference above the umbilicus and below the xiphoid process was used for tape measure placement. The participants were asked to stand still, cross their arms and place their hands on the opposite shoulders. The participants were instructed to breathe normally and measurements were recorded at the end of a normal exhalation [111, 114].

Registered nurses, licensed practical nurses and phlebotomists at the Center for Clinical and Translation Sciences at the University of Kentucky completed all blood draws [115]. Prior to blood draw, participant's identification, name and date of birth, were verified and the participant sat in a comfortable position. The tourniquet was placed approximately 3-5 inches above the venipuncture site on either the left or right arm. A winged infusion set, butterfly clip, was used on either the antecubital area or if arm vein attempt was not successful on the dorsal surface of the wrist. If the individual collecting the specimen did not obtain an appropriate specimen after two attempts the assistance of another staff member was requested. A gold top tube (3.5ml) was used to collect serum and 25(OH)D, the sum of 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub>, was analyzed by liquid chromatography/tandem mass spectrometry with a CV of 10%. The University Biospecimens Core of the Center Clinical and Translational Science completed all blood analysis.



### 2.3 Medical History Questionnaire

A non-validated medical history questionnaire was administered (Appendix 3). The questionnaire inquired about prescription drugs and any over-the-counter medications including dietary supplements. In addition, current and past smoking status, allergies, chronic illnesses or viruses, past surgeries, and family history of pulmonary, metabolic disease, stroke or sudden death were documented. The questionnaire also inquired about any other reasons why the participant should not take part in physical activity (eg. neuromuscular or metabolic disorders, breathing issues, asthma, or emphysema). Finally, participants were asked if they were currently seeing their healthcare provider for any reason and if applicable to provide further explanation.

### 2.4 Socioeconomic Status Questionnaire

A series of questions that inquired about participant's socioeconomic status (SES) was administered [116]. The SES questionnaire inquired about educational attainment, household size, annual household income, and current and past employment status. This information was collected in order to examine the extent to which differences in SES may play a role in vitamin D status, physical activity levels, and dietary intake.

### 2.5 Diet Assessment

Participants were asked to continue regular dietary intake throughout the study. Personnel (interviewer) trained in the use of nutrition research software conducted an interview by phone to complete a 24-hour dietary recalls on two to three non-consecutive days. The recalls were obtained on at least one weekend

and one weekday to account for variance in caloric intake based on the day of the week [117]. The Nutrition Data System for Research (NDS-R, Version 2015, University of Minnesota, Minneapolis, MN) was used to obtain and analyze dietary intake [118].

The NDS-R system employs the multiple-pass method to ensure the validity and reliability of the dietary data [117, 119]. The multiple-pass method refers to a standardized five-step procedure designed by the United States Department of Agriculture to minimize the occurrence of misreporting and various forms of bias by confirming food items and portion sizes throughout the interview [117, 119, 120]. The first pass involved obtaining a list of all foods and beverages consumed in the previous 24 hours. During the second pass the list was reviewed with the participant for completeness and correctness. Third, the interviewer collected all details about each reported food and beverage item, including method of preparation and amount consumed. Fourth, the interviewer probed for commonly forgotten foods, such as snacks or beverages consumed throughout the day. Lastly, the detailed information was read back to the participant and reviewed for completeness and correctness [121]. In addition, the participant was asked if the amount of food they consumed in the previous 24 hours was equal to, greater than, or less than the amount typically consumed. Likewise, information on any dietary supplements consumed (type and dose) was recorded.

The NDS-R database includes over 18,000 foods and 8,000 brand name products [121]. Further this system provides the ability to account for differences

in food preparation methods, which allows for a complete and thorough dietary recall. Values for nutrients and nutrient ratios were generated by the database and used to assess the relationship between dietary intake and muscle lipid.

## 2.6 Physical Activity Assessments

Participants wore an ActiGraph GT3X monitoring device (Actigraph, Pensacola, FL), a reliable and valid tool to measure physical activity in older adults [122]. Participants were instructed to wear the device on a belt over their non-dominant hip for seven consecutive days. Participants were instructed to remove the accelerometer before bathing or participating in any other water based activity and before sleeping.

The device is lightweight (27g), small (3.8x3.7x1.8cm) with a rechargeable battery [123]. The device is tri-axial and able to detect and collect data on 3 axis: horizontal right to left (X), vertical (Y), and horizontal front to back (Z). Data is collected using a time sampling interval (epoch) of 10 seconds, which Actigraph software outputs as counts. These counts were linearly related to the intensity of the participant's physical activity levels and were derived from vector magnitude (VM) defined as  $\sqrt{X^2+Y^2+Z^2}$ . We based our definition of sedentary, moderate, and vigorous physical activity in older adults on previously valid and reliable VM cut-off points developed by Santos-Lozano et al [123]. Sedentary or inactivity, such as sitting, ironing, laundry, washing dishes, and cooking, was identified as a VM cut point of <2751, which amount to <3 metabolic equivalents (METs). MET is defined as the amount of oxygen consumed during a specific activity. One MET is defined as the amount of oxygen consumed while sitting quietly in a chair

and is equivalent to 3.5ml/kg/min [124]. Moderate activity (3-6 METs) was defined as 2751 to 9359 (VM) which included activities such as window washing, vacuuming, gardening, yard work, or recreational activities. Activities such as biking or running, with a VM >9359, were considered vigorous (>6METs).

The ActiLife 6 software was used to analyze all data (version 6.11.9). Data from the ActiGraph provided detailed information regarding daily physical activity and length of time (minutes) that each participant spent at various levels of physical intensity. Meeting the Center for Disease Control guidelines on physical activity was defined as engaging in an average of >30minutes/weekday of moderate aerobic activity [125].

A Physical Activity Scale for the Elderly (PASE) questionnaire was administered to obtain a self-reported measurement of occupational, household, and leisure activities during the previous 7 days. Activity items required participants to report the number of days per week the activity was performed followed by the number of hours per day. Study personnel scored the 12-item questionnaire based on instructions published by The New England Research Institutes in which greater reported activity levels were associated with higher scores [126]. Total score were used for statistical analysis and typically ranged from 0 to 400. Validity and reliability of the PASE questionnaire has been previously established in individuals older than 65 years of age (Table 2.2) [126, 127].

Participants also completed the Physical Activity Readiness Questionnaire (Appendix 4) [128], American Heart Association/American College of Sports

Medicine Facility Preparticipation Screening Questionnaire (Appendix 5) [129], and an un-validated physical activity habits survey (Appendix 6) inquiring about the frequency, duration, and type of physical activity.

## 2.7 Physical Functional Assessment

The Four Square Step Test (FSST) was established as a reliable and valid tool used to test dynamic balance and mobility in adults over 65 years of age [130]. The test is quick to administer and requires no special equipment. It is both a sensitive and specific measure that can be used to discern between older adults that have experienced falls compared with non-fallers.

The FSST required participants to move in different directions and step over a one-inch obstacle on the floor (Figure 2.4). An exercise physiologist instructed participants to step over a plastic pipe lying flat on the ground for each of the following directions: 1) forward, 2) sidestep to the right, 3) backwards, and 4) sidestep to the left. This was repeated in the opposite direction and the total time to complete the task was recorded. Participants completed one practice trial to become familiarized with the test followed by three test trials. Only the fastest test trial was used for data analysis. A cut-off score of greater than 15 seconds for completion was used to indicate a greater risk for multiple falls for adults greater than 65 years of age [130].

## 2.8 Graded Exercise Test (GXT)

A multi-stage GXT was used for the assessment of respiratory and circulatory function using Vmax Encore (Carefusion, Yorba Linda, CA). This test was commonly used at University of Kentucky for sedentary or aged individuals

and included a progressive 2-minute workload stages (Table 2.3, Appendix 7). At the final minute of each stage, heart rate, blood pressure, and rate of perceived exertion (RPE) were recorded. During the test, breath-by-breath measurements of oxygen consumption and continuous 12-lead electrocardiogram (EKG) were monitored.

VO<sub>2</sub>max is defined as the maximal rate of oxygen uptake during maximal exercise [131]. When the oxygen uptake plateaus and does not increase by more than 150ml/min with further increases in workload, VO<sub>2</sub>max is reached.

However, many individuals do not reach the oxygen uptake plateau and additional criteria for VO<sub>2</sub>max may be used to indicate if a true VO<sub>2</sub>max has been reached. Additional criteria were the following: failure of heart rate to increase with increases in exercise intensity, venous lactate concentrations  $\geq 8$  mmol/L, respiratory exchange ratio  $\geq 1.1$ , and RPE  $\geq 17$  on the original Borg scale (6-20). VO<sub>2</sub> peak is the greatest rate of oxygen consumption measured during the test regardless if a plateau is reached. VO<sub>2</sub> peak can be higher, lower, or equal to VO<sub>2</sub>max. Many individuals do not reach their true VO<sub>2</sub>max as oxygen uptake does not plateau; however VO<sub>2</sub> peak attained during a maximum effort treadmill test, such as our GXT, is a valid index of cardiorespiratory fitness [131]. Normative data for VO<sub>2</sub>max in older adults are depicted in Table 2.4 [131].

Participants were closely monitored and verbal encouragement was provided throughout the test. Upon completion of the GXT, recovery (cool-down) exercise was performed for at least 5 minutes or until heart rate was  $< 100$  beats per minute. Test termination was based on volitional fatigue or the presence of any

absolute or relative contraindications according to the American College of Sports Medicine Guidelines [114]. A trained exercise physiologist performed all testing and a physician reviewed all medical history prior to testing and post-GXT results for contraindications to study participation.

## 2.9 Aerobic Training

For 7 consecutive days, participants randomized to an aerobic training protocol performed up to 45 minutes of treadmill exercise (Landice Cardio, Randolph, NJ). Training protocol was based on a previous protocol implemented in a similar population [74]. The exercise consisted of treadmill walking at a progressive intensity of about 60-65% of their maximum heart rate (maximum heart rate =  $220 - \text{age}$ ). During each exercise session participants had their blood pressure measured before and after the exercise. Participants wore a heart rate monitor to monitor their individualized target heart rate and maintain intensity. As an added safety measure participants were allowed to significantly reduce their training intensity to a slow walk for 5 minutes at any time they requested. Each training session incorporated a brief standardized warm-up and cool down. The study coordinator supervised every session and compliance with all training sessions was carefully documented (Appendix 8).

## 2.10 Body Composition

Body composition changes occur with aging often without body weight or BMI changes [132]. Generally, fat mass increase and lean mass and bone mineral density (BMD) decrease are observed with aging. Further, the changes in fat mass distribution occur during aging with a greater accumulation of fat in

the abdominal region. Numerous body composition instruments and techniques can be used to measure body composition; however, DXA and CT imaging provide a sophisticated approach to quantify fat and lean mass [97]. Traditionally, DXA scans were clinically used to monitor osteopenia/osteoporosis, but both DXA and CT scans can also be used to estimate fat and lean tissue content in total body or body segments. Further, CT scans can provide a cross-sectional image of visceral fat and fat infiltration in lean tissue.

### 2.10.1 DXA Imaging

The DXA scan is a highly preferable method used in research due to its ability to estimate bone mineral density, fat and lean soft tissue content in an expedient scanning time with minimal cooperation from the participant [131]. The imaging is dependent on the attenuation of the X-ray through various tissues that differ in their densities and chemical composition. DXA body composition assumptions include that 1) a constant attenuation of pure fat and bone-free lean tissue was maintained, 2) measurements were not affected by anteroposterior thickness, 3) the composition of each body region was equally represented in the calculation of total body values, and 4) that one tissue was analyzed at a time.

A DXA scan was performed at the CCTS by a study personnel trained in this procedure. A total body DXA scan was performed using a Lunar Prodigy (GE Lunar Inc., Madison, WI) bone densitometer. Participants were instructed to remove all objects such as jewelry or eyeglasses and wear a hospital gown or light-weight clothing (containing no metal) during the scanning procedure. The scans took approximately 8-10 minutes. Scans were analyzed using the GE



Lunar software (version 10.0). Total body, regional fat and fat-free mass (FFM; kg), mineral-free lean (MFL; kg) mass, and the appendicular skeletal muscle index were assessed.

### 2.10.2 CT Imaging

A subsample of participants completed a CT scan and all scans were administered and results analyzed by trained personnel. Single slice CT images (Siemens) were used to quantify skeletal muscle and fat area of both right and left thighs and abdominal visceral fat. A 100 milliamperere (mA) tube current with a 3 second scanning time was chosen to produce quality images while limiting exposure time of the radiation to the participant. CT image was composed of a 512x512 square matrix resulting in 262,144 pixels ( $512 \times 512 = 262,144$ ). The attenuation coefficient is the measure of how easily the beam can go through the material being imaged [133]. The pioneer of CT scanner, Godfrey Hounsfield, created an attenuation scale to characterize the densities of air, fat, water, and bone. In our study we used a modified version of the Hounsfield scale, in which tissue area was quantified using the corresponding attenuation values: bone  $\geq 200$  Hounsfield Unit (HU); adipose tissue -190 to -30 HU; and skeletal muscle 0-100 HU. With participants in a supine position, mid-thigh measures were completed with a mark between the inguinal crease and the proximal border of the patella. Total thigh fat was further subdivided into two compartments including the fat above the fascia latae (subcutaneous thigh fat) and below the fascia latae (deep thigh fat). In addition, the skeletal muscle was subdivided into areas of low attenuation (0-34 HU) representing fat rich muscle, and high

attenuation values (35-100 HU) representing muscle with normal fat content. The software by the National Institutes of Health Image Processing and Analysis in Java was used to analyze all data [134].

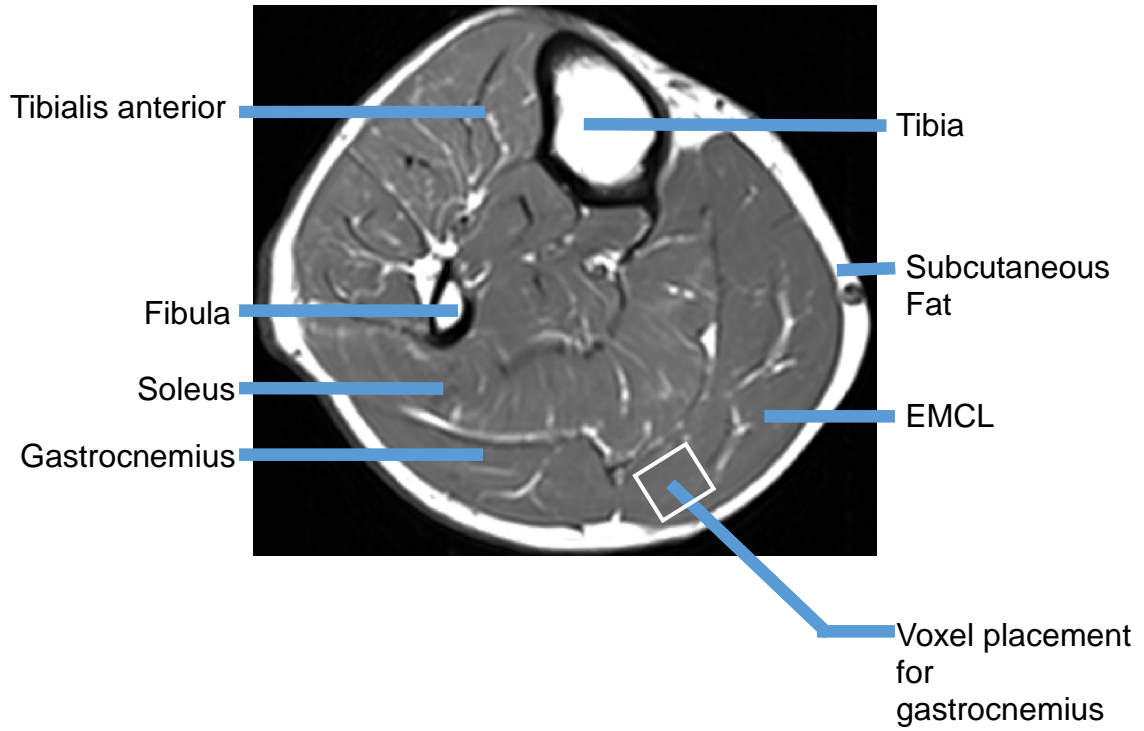
**Table 2.1** Advantages and disadvantages of MRI, DXA, and CT in vivo imaging

<b>Method</b>	<b>Advantages</b>	<b>Disadvantages</b>
<b>MRI</b>	-no ionizing radiation -assessment of muscle quality -quantify muscle compartments and fat depot (IMCL & EMCL)	-expensive -not widely available -scanning and image analysis requires trained personnel -no gold standard in scan and analysis protocols
<b>DXA</b>	-widely available in clinical settings	-low dose exposure to ionizing radiation -no assessment of muscle quality
<b>CT</b>	-widely available in clinical settings -assessment of muscle quality	-low dose exposure to ionizing radiation -lower reproducibility and reliability compared with MRI -no gold standard in scan and analysis protocols

**Table 2.1** Advantages and disadvantages of MRI, DXA, and CT in vivo imaging

The table outlines advantages and disadvantages of various imaging technologies.

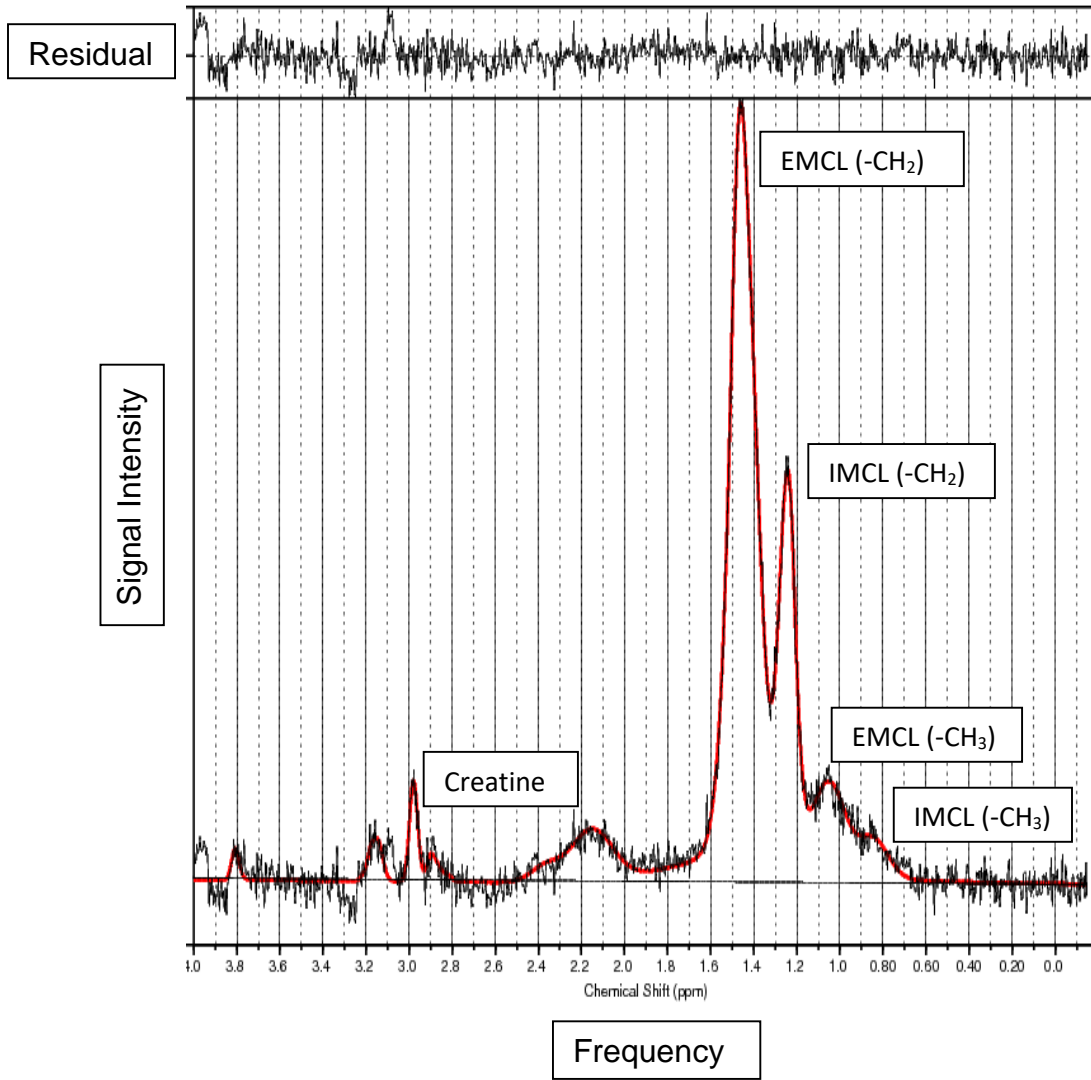
**Figure 2.1** T1-weight MRI axial image of the calf muscle



**Figure 2.1** T1-weight MRI axial of the calf muscle

Right calf T1-weighted transverse slice image. Major muscles and bones were identified and labeled. To further allow for better peak separation between EMCL (-CH<sub>2</sub>) and IMCL (-CH<sub>2</sub>), gastrocnemius voxel was placed to avoid extramyocellular fat depots. Participant was a 66 year old Caucasian male with a BMI of 26.8 kg/m<sup>2</sup>.

**Figure 2.2**  $^1\text{H}$ -MRS spectrum of the gastrocnemius muscle to assess IMCL

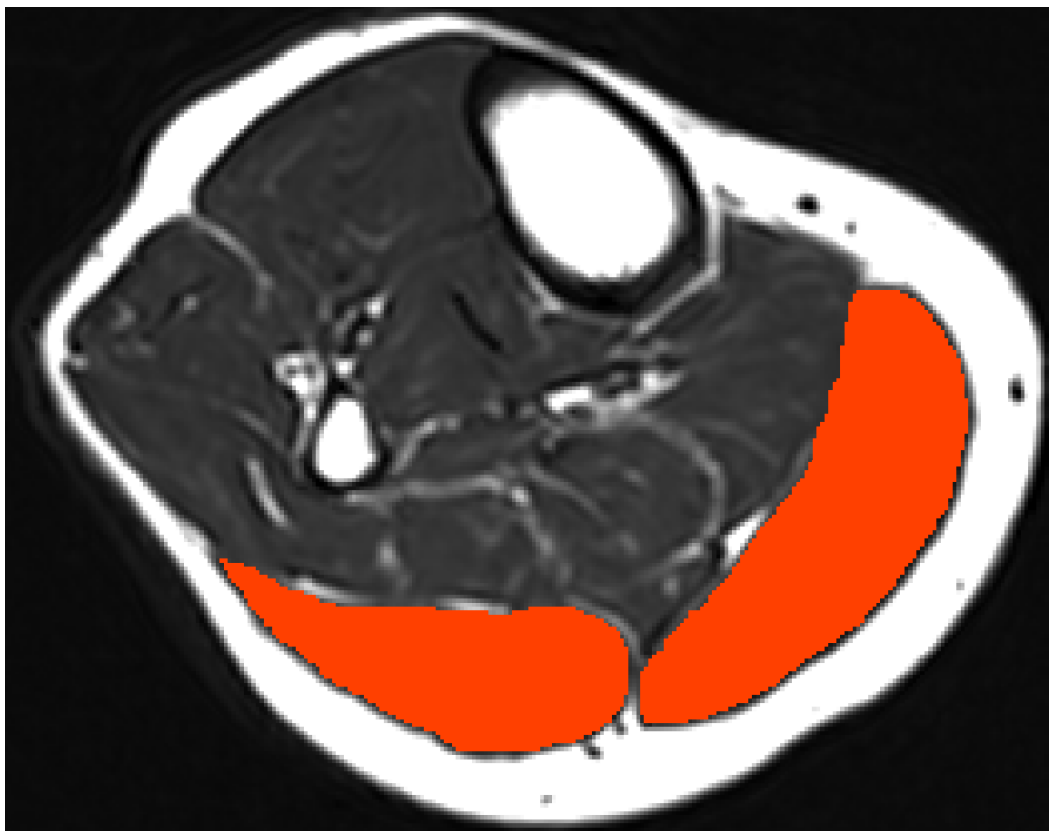


**Figure 2.2**  $^1\text{H}$ -MRS spectrum of the gastrocnemius muscle to assess IMCL

$^1\text{H}$ -MRS spectrum of the human gastrocnemius muscle fitted using LCModel (dark trace is the raw data; red trace is the fitted spectrum, and trace at top of figure is the residual). Participant was a 73 year old Caucasian female with a BMI of  $22.2\text{ kg/m}^2$ . IMCL (-CH<sub>3</sub>), methyl protons at 0.9 ppm; EMCL (-CH<sub>3</sub>), methyl protons at 1.1 ppm; IMCL (-CH<sub>2</sub>), methylene protons at 1.3 ppm; EMCL (-CH<sub>2</sub>), methylene protons at 1.5 ppm; creatine (-CH<sub>3</sub>) resonance at 3.0 ppm.



**Figure 2.3** Highlighted regions of interests in the gastrocnemius to assess EMCL



**Figure 2.3** Highlighted regions of interests in the gastrocnemius to assess EMCL

Gastrocnemius muscles were manually segmented (highlighted in red) from each axial image producing a transverse planar image (only 1 image of 15 are depicted). Participant was 64 year old Caucasian female with a BMI of 19.1kg/m<sup>2</sup>.

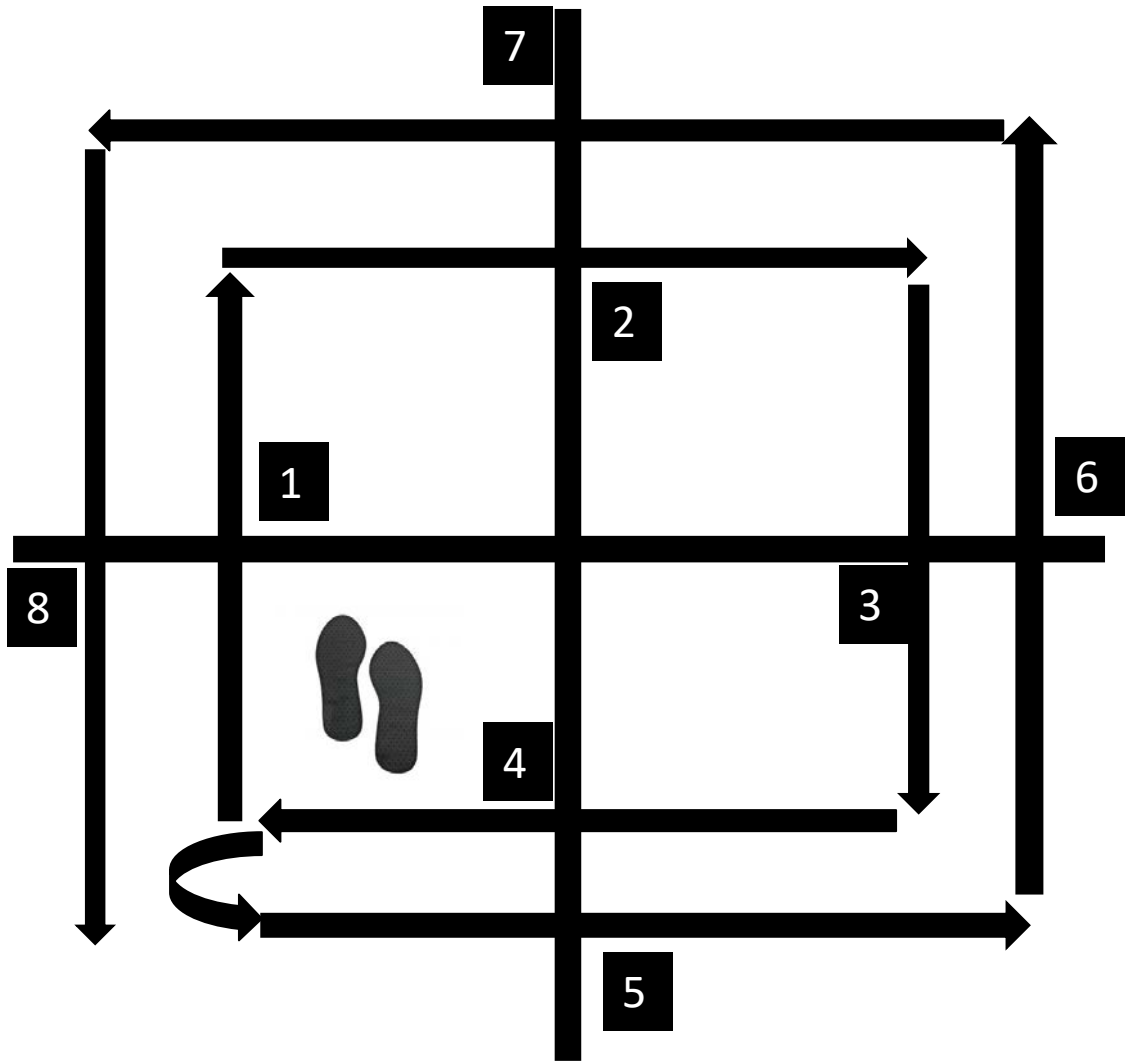
**Table 2.2** The New England Research Institutes' PASE scores by age group and sex

	<b>65-69 YO</b>	<b>70-75 YO</b>	<b>76-100 YO</b>
<b>Males</b>	144.3±58.6	102.4±53.7	101.8±45.7
<b>Females</b>	112.7±64.2	89.1±55.2	62.3±50.7

**Table 2.2** The New England Research Institutes' PASE scores by age group and sex

The New England Research Institutes established the validity and reliability of the PASE questionnaire [126]. Community-dwelling adults 65-100 year of age (n=222) were recruited. Higher PASE scores were significantly correlated with greater performance measures and overall health status. The mean $\pm$ SD for age categories between sexes are presented.

Figure 2.4 Four Square Step Test



**Figure 2.4** Four Square Step Test

The FSST is an inexpensive tool used to measure physical function in older adults. The participant was instructed to stand in square 4 and step over a 1-inch obstacle to square one. The participant continued as fast and safely as possible stepping into each square in the following sequence: 4, 1, 2, 3, 4, 5, 6, 7, and 8.

**Table 2.3 GXT**

<b>Stage</b>	<b>Time (min)</b>	<b>Speed (mph)</b>	<b>Gradient (%)</b>
<b>1</b>	0-2	2.2	0
<b>2</b>	2-4	2.6	0
<b>3</b>	4-6	3.0	2
<b>4</b>	6-8	3.4	4
<b>5</b>	8-10	3.8	6
<b>6</b>	10-12	4.2	8
<b>7</b>	12-14	4.6	10
<b>8</b>	14-16	5.0	12
<b>9</b>	16-18	5.4	14
<b>10</b>	18-20	5.8	16

**Table 2.3 GXT**

Table describe the duration, speed, and grade of each stage.



**Table 2.4** Cardiovascular fitness classifications: VO<sub>2</sub> max (ml/kg/min)

<b>Age (yr)</b>	<b>Poor</b>	<b>Fair</b>	<b>Good</b>	<b>Excellent</b>	<b>Superior</b>
<b>Females</b>					
<b>60-69</b>	≤25	26-28	29-31	32-36	37+
<b>70-79</b>	≤23	24-26	27-29	30-36	37+
<b>Males</b>					
<b>60-69</b>	≤30	31-34	35-38	39-45	46+
<b>70-79</b>	≤27	28-30	31-35	36-41	42+

**Table 2.4** Cardiovascular fitness classifications: VO<sub>2</sub> max (ml/kg/min)

Table describes the cardiorespiratory fitness classification for females and males, 60 to 80 years of age.

## Chapter 3: Specific Aim 1

### 3.1 Summary

A high prevalence of vitamin D deficiency and insufficiency has been documented in older adults. It has been well established that vitamin D is linked to bone health, but more recently, there is a growing interest regarding the relationship between vitamin D and muscle health. With aging, muscle health declines and changes in muscle lipid depots occur. **PURPOSE:** The objective of this retrospective trial was to examine the relationship between 25-hydroxyvitamin D (25(OH)D) concentrations, muscle lipid depots (intramyocellular lipid (IMCL) and extramyocellular lipid (EMCL)), and physical function. We aimed to examine if muscle lipid depots and 25(OH)D concentration were different between individuals of varied BMI and physical activity levels. **METHODS:** Data from adults 60 years of age or older who completed cross-sectional studies between 2012 and 2016 were combined. Blood was drawn to determine 25(OH)D concentrations and at least one form of physical activity assessment was completed (Actigraph accelerometry, Physical Activity Scale for the Elderly (PASE) or physical activity questionnaire). Anthropometrics measures included height, weight, waist circumference, dual-energy X-ray absorptiometry (DXA), and computed tomography (CT) scans. Gastrocnemius IMCL and EMCL were measured with magnetic resonance spectroscopy (MRS) and fat segmentation, respectively. Pearson correlations and independent student t-test were employed. **RESULTS:** Fifty-five participants took part in the 3 studies and a subsample completed waist circumference (n=42) and the Four Square Step

Test (FSST) (n=31). Eighteen of the 31 participants that completed the FSST also completed DXA and CT scans. In the total sample, mean age and body mass index (BMI) were  $70.8 \pm 6$ YO and  $25.9 \pm 4$ kg/m<sup>2</sup>, respectively. A significant negative relationship was observed between 25(OH)D and EMCL ( $p=0.02$ ). IMCL was positively correlated with 25(OH)D concentrations ( $p=0.008$ ). As BMI increased, a general decrease in 25(OH)D concentrations and increase in IMCL and EMCL was observed, but did not reach statistical significance. In females only, greater 25(OH)D concentrations were significantly correlated with faster FSST times ( $p=0.007$ ). In addition, 25(OH)D concentrations and EMCL ( $p \leq 0.01$ ) were significant predictors of FSST when including BMI ( $p=0.558$ ) and age ( $p=0.163$ ) in the model. CONCLUSIONS: Previous studies have observed significant relationship between vitamin D status, BMI, and muscle lipid depots. In accordance with previous literature, we found a relationship between vitamin D status, muscle lipid, and physical function. Discovering that vitamin D status and EMCL predict physical function independent of age and BMI in healthy older adults is novel. Studies suggest a relationship exists between muscle lipid depots, BMI categories, vitamin D status, and exercise; however further data is needed to examine to what extent vitamin D repletion plays a role in muscle health and function in vitamin D deficient older adults.

### 3.2 Introduction

In developed nations, vitamin D deficiency and insufficiency have become a worldwide epidemic and a particular concern in older adults. It is difficult to obtain sufficient vitamin D status through dietary intake alone; therefore, this

epidemic can be primarily attributed to inadequate sun exposure and prolonged time spent indoors without dietary supplementation [27]. Likewise, an increase in adiposity has been related with decreased vitamin D status [41, 42]. In addition, a reduction in the quantity of 7-dehydrocholesterol, the precursor of vitamin D, is observed in aging skin, further contributing to lower vitamin D status in older adults [28]. Studies reported that 20-100% of older adults were vitamin D deficient [27, 50]. The wide variance in vitamin D deficiency is due to non-standardized definitions of several factors including: vitamin D cut-off levels, methodology for vitamin D concentration assessment, and age cut-offs used. The National Institutes of Health set vitamin D deficiency (measured as 25(OH)D) as <20ng/mL, which in healthy individuals is generally considered to be inadequate for both bone health and overall health [26, 27].

Beyond bone health, research suggests that greater 25(OH)D concentrations may be necessary to maintain muscle health [6, 24, 44, 45]. Lower 25(OH)D concentrations were associated with reduced muscle strength and performance-based functional outcomes in the older adults [49, 50, 66]. Vitamin D supplementation trials have yielded mixed results primarily due to varied study designs [49]. However, some low-dose vitamin D supplementation trials, in deficient older adults, have shown improvements in muscle strength and decreases in body sway [135, 136].

The mechanism in which vitamin D supplementation beneficially effects skeletal muscle is still under investigation. Vitamin D can bind to the vitamin D receptor (VDR) located on the muscle cell and act through both genomic and

non-genomic pathways. These pathways contribute to the regulation of calcium and muscle contractions, and an increase in quantity and size of Type II muscle fibers [65, 66, 71]. Further, vitamin D has been associated with the quantity of lipid found in muscle lipid depots, which play a role in muscle metabolism and function [55, 59]. Muscle lipid depots are categorized as IMCL and EMCL. IMCL is located within the muscle cell, typically in close proximity to the mitochondria where the lipid can be readily oxidized during increased metabolic muscle demands [72]. High IMCL content has been associated with greater insulin resistance in obese, sedentary individuals. Paradoxically, in active individuals, an increase in insulin sensitivity has been observed with high IMCL content [77]. EMCL is adipose tissue that has infiltrated the skeletal muscle and is located between muscle fibers [82]. EMCL accumulation is thought to play a negative role in muscle health, as reduction in physical function is observed with greater EMCL content [74, 79, 84, 85].

Vitamin D deficiency has been linked to greater EMCL and reduced muscle strength [50, 55, 66, 86]. In older adults, impaired gait and balance were associated with lower vitamin D status and greater EMCL [55]. Recently, our lab observed no relationship between vitamin D status and EMCL, but a positive linear relationship was observed between vitamin D status and IMCL, independent of reported physical activity and BMI [59]. Although muscle lipid was not associated with the FSST, a measure of functional performance, we observed a relationship between greater 25(OH)D concentrations and faster FSST. These associations may be partly explained at the cellular level with lipid

depot alterations that occur with aging. In C2C12 myotubes, the addition of calcitriol increased the expression and concentration of VDR, lipid packaging proteins, and a protein (OXPAT) that promotes fatty acid utilization [66, 81]. Together these data may suggest that vitamin D is involved in the packaging and mobilization of IMCL.

In addition to 25(OH)D concentrations, modifiable factors including exercise and adiposity contribute to altering the quantity of muscle lipid depots. In obese adolescents, an increase in IMCL, fitness, and power was observed after an 8 week exercise intervention. IMCL increases were associated with increased resting energy expenditure and a lower respiratory quotient suggesting greater lipid oxidation [94]. In older sedentary adults, high IMCL content was observed and associated with decreased force, power, and slower skeletal muscle contractions [80]. Conversely, in older insulin resistant obese men who engaged in 7 consecutive days of aerobic training, an increase in IMCL and decrease in EMCL with concurrent improvements in insulin sensitivity was observed [74]. These studies show that exercise can lead to altered muscle lipid depots and insulin sensitivity.

Adiposity is another modifiable factor that can play a role in muscle lipid depot changes. One study found that central obesity was associated with greater IMCL and EMCL content, independent of age [79]. Velan et al. [84] observed a proportional increase between weight categories (normal weight, overweight, and obese) and both muscle lipid depots (IMCL and EMCL) in adults. These studies suggest that adiposity is related with muscle lipid depot

accumulation; however, the relationship between adiposity, vitamin D status, muscle lipid content, and physical function outcomes have not fully been investigated.

The objective of this retrospective trial was to examine the relationship between vitamin D status, muscle lipid depots (IMCL and EMCL), and physical function in adults 60 years of age or older. Further, we examined the influence of adiposity on these relationships. Finally, we describe muscle lipid depots and 25(OH)D concentrations differences between individuals of varied BMI and physical activity levels.

### 3.3 Methods

#### 3.3.1 Participants

Individuals 60 years of age or older were recruited to participate. These individuals resided in Southeastern United States (latitude 38°N). Exclusion criteria included inability to walk up a flight of stairs without assistance, diabetes, history of kidney disease, neurologic disorders, and myopathy. Additional exclusion criteria included lower extremity injury or surgery and the initiation of a resistance or aerobic training exercise regimen in 3 months prior to study enrollment. Individuals with ferromagnetic implants or foreign bodies that were magnetic resonance imaging (MRI) incompatible were not eligible to take part in the study. The university institutional review board approved this study. Written consent was obtained from all participants prior to study measures. Institutional and governmental regulations concerning human participants in research were followed including study data collection and management. Research Electronic



Data Capture tool (REDCap) [137] was used for study data. REDCap is a secure, web-based application designed to support data capture for research studies hosted at the University of Kentucky.

### 3.3.2 Study Design

Participants completed visits between 2012-2016 (Table 3.1). All participants completed anthropometric measures (height and weight). Blood was drawn to assess vitamin D status (25(OH)D) and a MRI scan of the gastrocnemius was completed to measure gastrocnemius muscle lipid depots (IMCL and EMCL). Each participant completed a medical history questionnaire and at least one form of physical activity assessment (accelerometry, PASE or physical activity questionnaire). A subsample of participants completed waist circumference measures, DXA and CT scans, GXT, and the FSST.

### 3.3.3 Statistical Analysis

Data collected over a 4 year period from 3 different studies was combined and retrospectively analyzed. Power was retrospectively calculated using Statistical Analysis System (version 9.4, Cary, NC) to examine the relationship between 25(OH)D concentrations and IMCL. One participant, with a BMI of 35.2 (obese class II category), was excluded from study analyses due to poor IMCL and EMCL peak separation post MRS analysis. Lack of peak separation resulted in an overestimation of IMCL that was 3 standard deviations (SD) above the mean. Based on a cross-sectional total sample of 55, a Fisher's z-test was employed to retrospectively calculate a power of 75.2%.

Data analyses were completed using Statistical Package for Social Sciences software (version 22, IBM, Armonk, NY). Descriptive statistics were documented and Pearson correlations were employed to examine the relationship between continuous variables. For mean comparisons of two variables, the Levene's test for equality of variance was completed following an independent t-test. For mean comparisons between 2 or more variables a one-way ANOVA was employed. Linear regression was employed to investigate the relationship between our variables of interest. A  $p \leq 0.05$  was set as statistical significance. Data were expressed as mean  $\pm$  SD.

### 3.4 Results

#### 3.4.1 Participant Characteristics

Participants were between 60 and 84 years of age. All participants completed the medical history questionnaire, blood draw, and height/weight measures. Total sample characteristics and sample characteristics by sex group are described in Table 3.2. Twenty-four participants' were normal weight with a BMI range from 18.7 to 24.9 kg/m<sup>2</sup> (mean = 22.5  $\pm$  2 kg/m<sup>2</sup>). Twenty-three participants were overweight with a BMI range from 25.4 to 29.9 kg/m<sup>2</sup> (mean = 27.5  $\pm$  1 kg/m<sup>2</sup>). Eight participants were obese (class I) with a BMI range from 30 to 33.7 kg/m<sup>2</sup> (mean = 31.5  $\pm$  1 kg/m<sup>2</sup>). A subsample of 42 participants completed waist circumference measures (mean = 90.8  $\pm$  13 cm). Waist circumference was significantly different between females (n=18, 82.7  $\pm$  12 cm) and males (n=24, 96.8  $\pm$  11 cm) ( $p < 0.001$ ).

### 3.4.2 Physical Activity and Fitness Characteristics

Thirty-one participants completed the physical activity questionnaire. Participants reported their physical activity lifestyle demands as sedentary (n=1), slightly active (n=5), active (n=19), or very active (n=6). Twenty-four participants completed the GXT, PASE questionnaire, and wore an accelerometer. Due to non-compliance of accelerometer wear, accelerometry data were only reported for 23 participants. Peak VO<sub>2</sub> measures obtained by GXT ranged from 15.5 to 51.4 mL/kg/min (mean = 30±9.3 mL/kg/min) (Table 3.3). Based on previously established cardiovascular fitness categories [131], 5 males and 8 females were classified as either poor or fair and 7 males and 4 females were classified as either good, excellent, or superior. The PASE total score ranged from 57.2 to 431.1 with a mean of 148.7±87.2 (Table 3.4). Participants' time spent in light, moderate, and vigorous activity ranged from 666 to 940.8 min/d, 23.3 to 108.3 min/d, and 0 to 8.5 min/d, respectively (Table 3.5). Nineteen of the 23 participants met the minimum 30 min/d recommendation of moderate activity set forth by the CDC [125]. Further, vector magnitude count (counts/minute/day) was used as a continuous variable to assess accelerometry data and ranged from 323.2 to 884.3 (mean = 569.5±135). VO<sub>2</sub> measures positively correlated with time spent in moderate activity (p=0.047) and negatively correlated with time spent in light activity (p=0.009). PASE questionnaire scores were not correlated with VO<sub>2</sub> or accelerometry measures.

### 3.4.3 Vitamin D Status

Nine individuals were vitamin D deficient ( $25(\text{OH})\text{D} < 20\text{ng/mL}$ ) and 25 were vitamin D insufficient ( $25(\text{OH})\text{D} 20\text{-}30\text{ng/mL}$ ). Twenty individuals were vitamin D sufficient ( $25(\text{OH})\text{D} > 30\text{ng/mL}$ ) and 11 of 20 individuals had a  $25(\text{OH})\text{D} > 40\text{ng/mL}$ .  $25(\text{OH})\text{D}$  concentrations for normal weight participants ranged from 16 to 63ng/mL with a mean of  $35 \pm 14\text{ng/mL}$ . For overweight participants,  $25(\text{OH})\text{D}$  concentrations ranged from 17 to 50ng/mL with a mean of  $29.7 \pm 8\text{ng/mL}$ .  $25(\text{OH})\text{D}$  concentrations for obese participants ranged from 15 to 68ng/mL with a mean of  $27.5 \pm 18\text{ng/mL}$ . Although lower mean  $25(\text{OH})\text{D}$  concentration were observed to be related with greater BMI categories, this relationship was not significant (Figure 3.1). BMI and waist circumference were correlated ( $p < 0.001$ ); however,  $25(\text{OH})\text{D}$  concentrations were negatively correlated with waist circumference only ( $p = 0.03$ ) and not BMI (Figure 3.2). Physical activity measured by PASE questionnaire and accelerometry did not correlate with  $25(\text{OH})\text{D}$  concentrations. Further,  $25(\text{OH})\text{D}$  concentrations were not different between reported physical activity levels (sedentary, slight active, active or very active).

### 3.4.4 Muscle Lipid Depots Characteristics

Mean IMCL was  $0.005 \pm 0.003\text{au}$  with a range of 0.001au to 0.017au. Mean EMCL was  $22.8 \pm 5.6\%$  with a range of 8.9% to 39.8%. BMI was not correlated with IMCL ( $p = 0.11$ ) or EMCL ( $p = 0.06$ ). Although not significant, both muscle lipid depots, IMCL (Figure 3.3) and EMCL (Figure 3.4), increased with increases in

BMI category. Waist circumference was not correlated with either muscle lipid measure.

PASE score, GXT, and accelerometry measures did not correlate with either muscle lipid measure. Participants that reported being active or very active had lower EMCL ( $19.4\pm 4\%$ ) compared with those who reported being sedentary or slightly active ( $23.9\pm 2\%$ ) ( $p=0.02$ ). However, there were no significant differences in IMCL content between individuals of varied activity levels.

#### 3.4.5 Vitamin D Status, Muscle Lipid, and Physical Function

25(OH)D concentrations were positively correlated with IMCL ( $p=0.008$ ) (Figure 3.5). 25(OH)D concentrations were negatively correlated with EMCL ( $p=0.02$ ) (Figure 3.6). EMCL was also significantly higher in vitamin D deficient individuals (25(OH)D $<20$ ng/mL) ( $n=9$ ;  $26.8\pm 6\%$ ) compared with individuals with a 25(OH)D $>40$ ng/mL ( $n=11$ ;  $20.9\pm 3\%$ ) ( $p=0.009$ ).

Thirty-one participants completed the FSST with a mean time of  $7.6\pm 2$ s and range from 4.2 to 12.8s. In the total sample, FSST did not correlate with muscle lipid (Figure 3.7 (A)), 25(OH)D concentrations, BMI, or age. There were no observed quintile differences between the slowest (mean =  $10.1\pm 1$ ) and fastest (mean =  $5.5\pm 1$ s) times to complete the FSST. There were significant differences in FSST between males ( $n=15$ , mean  $6.9\pm 2$ s) and females ( $n=16$ , mean  $8.3\pm 2$ s) ( $p=0.05$ ).

In males no significant relationship was observed between 25(OH)D concentrations, FSST (Figure 3.7 (B)), and muscle lipid. Conversely, females with greater 25(OH)D concentrations had faster FSST times ( $p=0.007$ ) (Figure

3.7 (C)). In females, FSST positively correlated with BMI ( $p=0.012$ ). Although no correlation between FSST and muscle lipid depots were observed, 25(OH)D concentrations ( $p=0.004$ ) and EMCL ( $p=0.013$ ) were significant predictors of FSST when adjusting for BMI ( $p=0.558$ ) and age ( $p=0.163$ ) in the model (Table 3.6).

#### 3.4.6 Muscle Lipid, Vitamin D Status, and Body Composition

IMCL and EMCL did not correlate with DXA or CT measures in the subsample that completed body composition measures. Higher 25(OH)D concentrations correlated negatively with total lean, leg, arms, trunk, android, and gynoid ( $p\leq 0.005$ ). 25(OH)D concentrations were not associated with any fat measures.

#### 3.5 Discussion

The purpose of this retrospective study was to examine the relationship between 25(OH)D concentrations, muscle lipid depots, and physical function. Our primary findings were that 25(OH)D concentrations were negatively associated with EMCL and were not associated with IMCL when outliers, defined as IMCL data points that was 1.5 times greater than the interquartile range, were removed. 25(OH)D concentrations were related with physical function. In females only, 25(OH)D concentrations and EMCL were the best predictors of faster FSST. We assessed muscle lipid depots and 25(OH)D concentrations between different BMI categories and physical activity levels. With higher BMI categories, decreases in 25(OH)D concentrations and increases in muscle lipid depots were observed, but statistical significance was not reached. There were no significant

differences in muscle lipid depots with varied physical activity levels. Together these data suggest that vitamin D status and muscle lipid depots play a role in physical function and support previous study findings [55, 86].

In accordance with previous literature, we observed a negative linear association between EMCL and 25(OH)D concentrations [55, 86]. Although the metabolic role of EMCL remains largely unknown, higher EMCL has been associated with decreased physical function and increased insulin resistance [58, 74]. Gilsanz et al. [86] observed a negative relationship between 25(OH)D concentrations and fat infiltration in 90 postpubertal, healthy females (16-22YO). Women with  $25(\text{OH})\text{D} \leq 29 \text{ ng/mL}$  had 24% greater muscle fat infiltration than women with  $25(\text{OH})\text{D} \geq 30 \text{ ng/mL}$ . We observed that those with lower ( $25(\text{OH})\text{D} \leq 20 \text{ ng/mL}$ ) has 6% greater EMCL compared with those with higher ( $25(\text{OH})\text{D} \geq 40 \text{ ng/mL}$ ) vitamin D status. Although both our studies and Gilsanz et al observed significant differences in EMCL content the magnitude of EMCL varied. This variation of EMCL content between high and low vitamin D status can be attributed to multiple factors including different subject characteristics, sample size, and different techniques used to assess EMCL and physical function. As no gold standard for quantifying EMCL exists, it can be difficult to compare results across studies. We manually highlighted regions of interest and quantified fat content in the gastrocnemius using a previously published formula [103]; whereas others used CT [86] or MRI and subjectively graded images [55]. Tagliafico et al. [55] observed a similar relationship between 25(OH)D concentrations and fat infiltration in 65 year old patients that were admitted to an

internal medicine ward for non-oncological diseases. The authors reported that individuals with lower 25(OH)D concentrations and greater fat infiltration had impaired balance and gait. We did not observe a relationship between EMCL and our physical function measure, FSST. However, in females higher, 25(OH)D concentrations and lower EMCL content were significant predictors of FSST performance times when adjusting for age and BMI. This was not observed in male participants potential due to the wider distribution of 25(OH)D concentrations in females compared with males. The majority (87%) of males were vitamin D insufficient, whereas 25(OH)D concentrations were normally distributed in females. These data indicate that there may be a relationship between vitamin D, EMCL, and physical function as previous studies have noted.

The metabolic role of IMCL has been investigated for over two decades. High IMCL content with physical inactivity has been associated with greater insulin resistance [77]. Paradoxically, active individuals also exhibit high IMCL content, but these relationships have been linked to greater insulin sensitivity. Previously, our lab observed a positive linear relationship between 25(OH)D and IMCL in healthy older adults independent of physical activity and BMI [59]. In this retrospective analysis a significant relationship between 25(OH)D and IMCL was observed; however this significant relationship may not be a true finding as three data points drove this correlation to significance. Further, the discrepancy between our current and past findings may be due to multiple other factors. With a retrospective analysis a sample size of 55 provided us with a power of 75%, thus we may be underpowered to detect significance between IMCL and



25(OH)D concentrations. In addition, the majority of our sample (62%) had a 25(OH)D $\leq$ 30ng/mL, while only 20% of our sample had a 25(OH)D $>$ 40ng/mL. The narrow distribution in the 25(OH)D concentrations may have further contributed to our lack of findings between vitamin D status and IMCL.

Multiple methods were used to assess physical activity and no participant enrolled partook in an aerobic or resistance training regimen prior to start of the study. Compared to physical activity, which encompasses routine daily activities, exercise differs as it is defined as a planned, structured and repetitive task. [93]. Previous trials have observed that short and long term exercise influence muscle lipid depots and positively affected muscle health [74, 94, 95]. In our study, we aimed to examine the relationship between muscle lipid depots and physical activity. We did not observe any relationship between muscle lipid depots and physical activity measures (PASE questionnaire, accelerometry wear, or GXT). However, as measured by the physical activity questionnaire, significantly lower EMCL content was observed in individuals who reported being more active to those individuals who reported being less active. Greater EMCL has been associated with decreased gait and stability in older adults and engaging in an exercise program has been shown to reduce EMCL [55, 74]. Our findings were novel as the data suggests that increased physical activity and not just engagement in an exercise regimen were related with lower EMCL content. Unlike our physically active participants of which 83% met the CDC requirements [125], less than 5% of general adult population engages in 30min/d of physical activity [138]. Greater physical activity can delay or help maintain quality of life

and provide an independent lifestyle with aging. Therefore, physical activity should continue to be emphasized and encouraged in community and clinical settings.

In addition to physical activity, lower adiposity can play an important role in overall health. It is well established that even during periods of stable body weight, lipid deposition changes occur with aging [90]. Central obesity increases and fat infiltrates various tissues including the skeletal muscle. We did not detect significant differences between BMI categories and muscle lipid depots, which may be due to a small obese category sample size. de La Maza et al. [79] observed increased muscle lipid depots (EMCL and IMCL) in both young (27-55YO) and older (66-80YO) men. In the total sample, the authors observed that overweight men had higher IMCL and EMCL compared with normal weight men. Further, a significant positive relationship between muscle lipid depots and both abdominal and trunk fat accumulation was observed. These data suggests that independent of age, central obesity plays a major role in muscle lipid depots accumulation. In our total sample, we did not observe a significant difference in muscle lipid depots between BMI categories or central adiposity measures. However, a general increase in muscle lipid depots was observed with increases in BMI category. To decrease confounding variables we further examined these results between females and males and observed that obese males had greater IMCL content compared with normal weight males. This relationship was not observed in females, which may be due the small sample size of only 3 females classified as obese. Therefore, the discrepancy in significance can be attributed

to the strict study selection. To avoid confounding variables de la Maza et al. [79] recruited only young and older men with comparable body weights and physical activity levels.

Velan et al. [84] observed that with greater BMI categories (normal weight, overweight, and obese) younger men had increased IMCL and EMCL content. Study selection was limited to healthy males not involved in any high-level exercise training. With a small sample size of only eight individuals per group, Velan et al. [84] detected significant differences in muscle lipid depots between BMI categories. The discrepancies between Velan et al. [84] and our findings could be that muscle lipid depots differences between BMI categories were more difficult to detect due to the confounding variables of age and physical activity. With age, changes in adiposity occur even during periods of weight stability [90] and our study included a 24-year range between our youngest and oldest participant. Further, in our study a wide distribution of physical activity was documented. For example, 9 of 22 participants exceeding the CDC recommendations of 30min/d [125] and engaged in more than 1 hour of moderate physical activity. Despite the lack of significance, which in part can be contributed to our heterogeneous sample, our results were in alignment with previous literature as muscle lipid depots increases were observed with increases in BMI categories.

Increases in adiposity have also been associated with decreased vitamin D status. Two hypotheses have been attributed to the aforementioned association, the fat sequestration hypothesis [42] in which the lipophilic nature of

vitamin D promotes significant storage in adipose tissue and the volumetric dilution hypothesis, [41] which suggest that the greater body size and fat mass were associated with lower 25(OH)D concentrations. In our study we observed a lower mean 25(OH)D concentration with greater BMI categories. Compared to normal weight individuals, previous studies have observed a greater vitamin D deficiency prevalence among obese individuals [43] while also showing small but significant increase in 25(OH)D concentrations with weight loss [39]. We observed a general mean decrease in 25(OH)D concentrations with increases in BMI category and a significant negative relationship between waist circumference and 25(OH)D concentrations. Waist circumference can be a more sensitive measure of central adiposity than BMI, as with BMI normal weight, overweight, and obese individuals can have comparable fat mass but fat may be distributed in different parts of the body [139, 140]. We used more sophisticated measures to assess adiposity (DXA and CT scans) and did not observe any relationship with 25(OH)D concentrations and adiposity; however, these measures were only completed in a subsample of participants (n=18) of which 9 were normal weight, 6 were overweight, and 3 were obese. Our data provides further evidence for decreased 25(OH)D concentrations with greater central adiposity.

There are limitations to this specific aim, primarily due to the nature of a retrospective study design. Data was combined from 3 cross-sectional trials and participants were recruited over a 4 year period and completed varied study measures. Despite this, the inclusion and exclusion criteria was similar across

each trial and primary variables of interest (muscle lipid depots and vitamin D status) were measured using the same methodology. We observed that higher 25(OH)D concentrations and lower EMCL content were the best predictors of physical performance. Further, a novel finding was that higher physical activity levels were related with lower EMCL content. Future intervention trials are necessary to determine if vitamin D supplementation in vitamin D deficient adults can result in decrease in EMCL, effect IMCL, and improve overall physical function.

**Table 3.1** Study trials and measures completed

<b>Trials</b>	<b>1 (n=13)</b>	<b>2 (n=18)</b>	<b>3 (n=24)</b>
<b>Active Study Year</b>	2012	2012-2013	2014-2016
<b>Study Measures</b>			
Muscle Lipid Depots	x	x	x
Blood Draw	x	x	x
Height/Weight	x	x	x
Waist Circumference		x	x
Physical Activity Questionnaire	x	x	
PASE Questionnaire			x
Accelerometry			x
GXT			x
DXA		x	
CT		x	
FSST	x	x	

**Table 3.1** Study trials and measures completed

A list of measurements obtained, sample sizes, and the year each cross-sectional trial was completed.

**Table 3.2** Subject characteristics, mean±SD

	<b>Female (n=28)</b>	<b>Male (n=27)</b>	<b>Total (n=55)</b>
<b>Age (years)</b>	71±6	70.6±6	70.8±6
<b>Height (cm)</b>	163±7*	176.4±5*	169.6±9
<b>Weight (kg)</b>	66.3±10*	84±12*	75±14
<b>Body Mass Index (kg/m<sup>2</sup>)</b>	25±4*	27±3*	25.9±4
<b>25(OH)D (ng/mL)</b>	35.3±13*	28±11*	31.7±13
<b>Race (n)</b>			
White	32	22	54
African American	0	1	1

\*Significant differences between females and males (p<0.05)



**Table 3.3** VO<sub>2</sub> peak measures between males and females across age categories

<b>Age (yr)</b>	<b>Females (n=12)</b>	<b>Males (n=12)</b>	<b>Total (n=24)</b>
<b>60-69</b>	25.7±8 (n=7)	34.8±10 (n=9)	30.8±10 (n=16)
<b>70-79</b>	23.8±7* (n=5)	36±4* (n=3)	28.4±9 (n=8)

\*Significant differences between females and males (p<0.05)

**Table. 3.3** Peak VO<sub>2</sub> measures between males and females across age categories

Categories used were based on previous guidelines by Heyward et al.

[131] No differences in peak VO<sub>2</sub> within sex categories and across age groups was observed. Males and females in each age category were statistically different between sexes with females reaching a lower peak VO<sub>2</sub> levels.

**Table 3.4** PASE questionnaire by age category, mean±SD

	<b>60-64 YO (n=8)</b>	<b>65-69 YO (n=8)</b>	<b>70-75 YO (n=6)</b>	<b>76-100 YO (n=2)</b>	<b>Total (n=24)</b>
<b>Males</b>	116.9±58 (n=5)	124.1±32 (n=4)	140.3±41 (n=3)	(n=0)	125.2±43 (n=12)
<b>Females</b>	199.4±201 (n=3)	225.7±85 (n=4)	119.3±42 (n=3)	104.3±44 (n=2)	172.3±113 (n=12)

No statistical differences

**Table 3.4** PASE questionnaire by age category, mean±SD

Age categories were based on the New England Research Institutes' PASE questionnaire. No statistical differences in PASE score between males and females within or across age groups were observed.

**Table 3.5** Minutes per day spent in each activity level, mean±SD

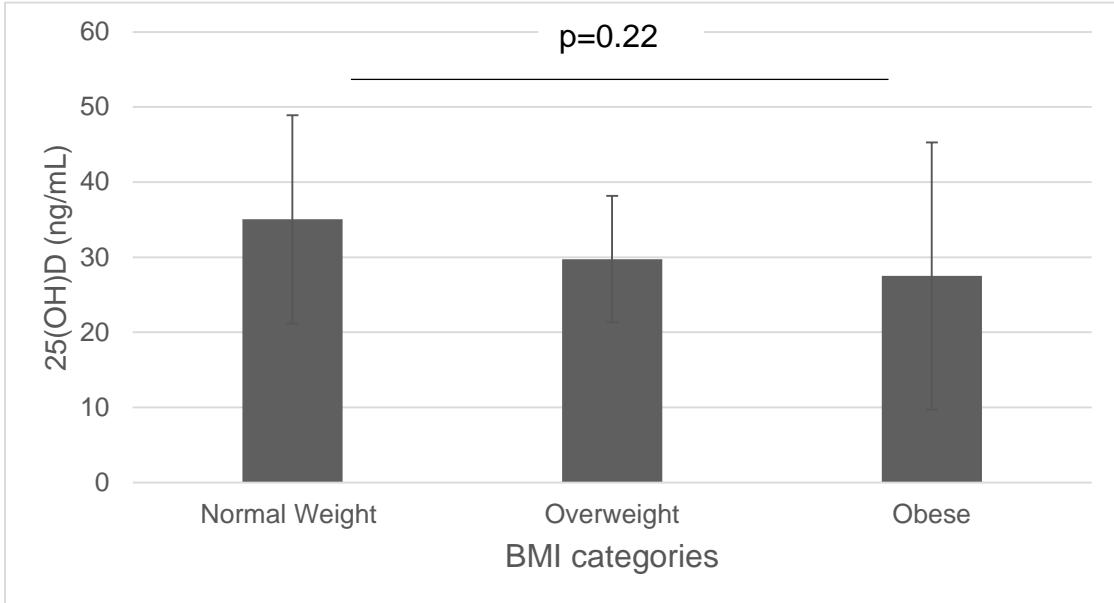
	<b>Female (n=11)</b>	<b>Male (n=12)</b>	<b>Total (n=23)</b>
<b>Light Activity (&lt;3METS)</b>	837.6±62*	785.5±59*	810.4±65
<b>Moderate Activity (3-6 METS)</b>	49.5±22	66.7±25	58.5±25
<b>Vigorous Activity (&gt;6 METS)</b>	0.2±0.2	0.9±2	0.6±2

\*Significant differences between females and males (p<0.05)

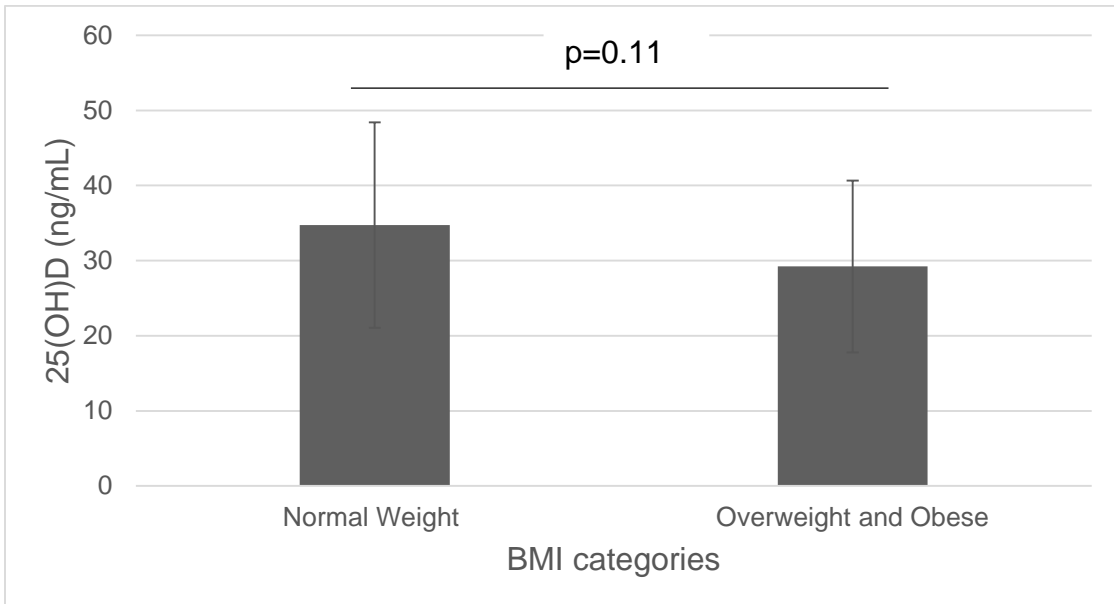
**Table 3.5** Minutes per day spent in each activity level, mean $\pm$ SD

Time (minutes) spent per day in light, moderate, and vigorous activity levels. Females significantly spent more time in sedentary activity category compared with males.

(A)



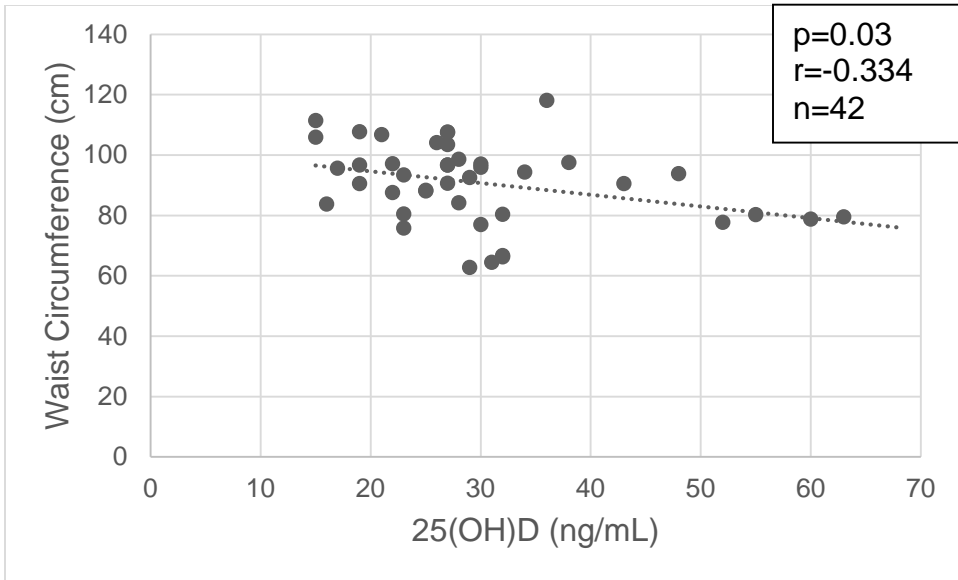
(B)



**Figure 3.1** 25(OH)D concentrations across BMI categories

25(OH)D concentrations were reported across 3 BMI categories (normal weight, overweight, and obese (A)) and across 2 BMI categories (normal weight vs. overweight+obese (B)). Although a decrease in 25(OH)D from normal weight to obese was observed mean 25(OH)D was not significantly different between BMI categories.



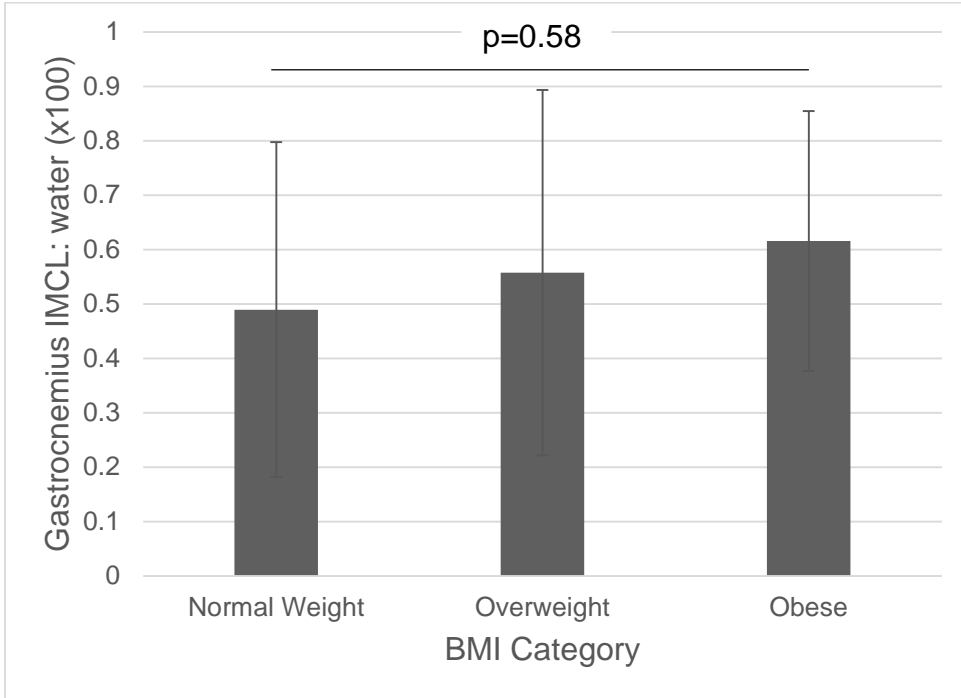


**Figure 3.2** The relationship between 25(OH)D and waist circumference

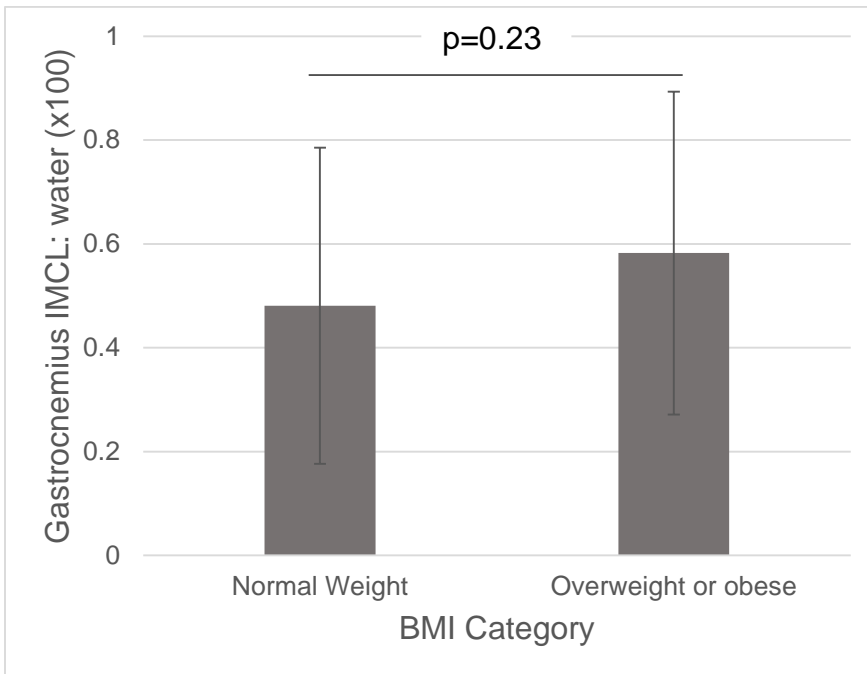
Waist circumference and 25(OH)D measures included males and females.

25(OH)D was negatively correlated with waist circumference.

(A)



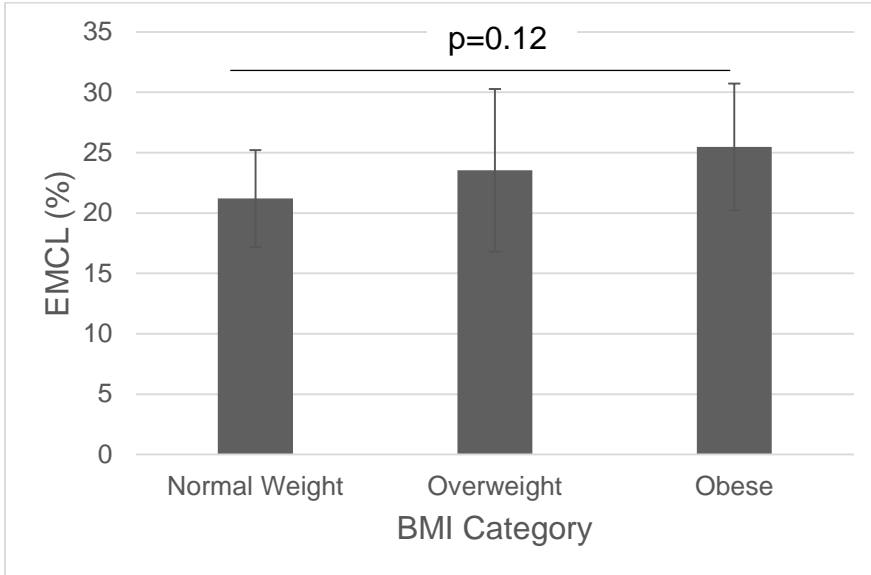
(B)



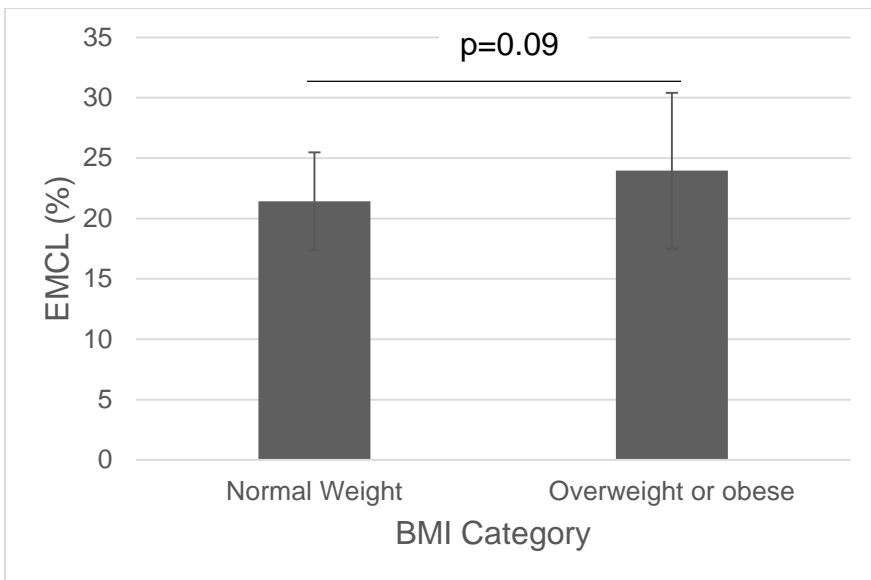
**Figure 3.3** IMCL across BMI categories

IMCL contents were reported across 3 BMI categories (normal weight, overweight, and obese (A)) and across 2 BMI categories (normal weight vs. overweight+obese (B)). Although an increase in IMCL from normal weight to obese was observed, the mean IMCL was not significantly different between BMI categories.

(A)



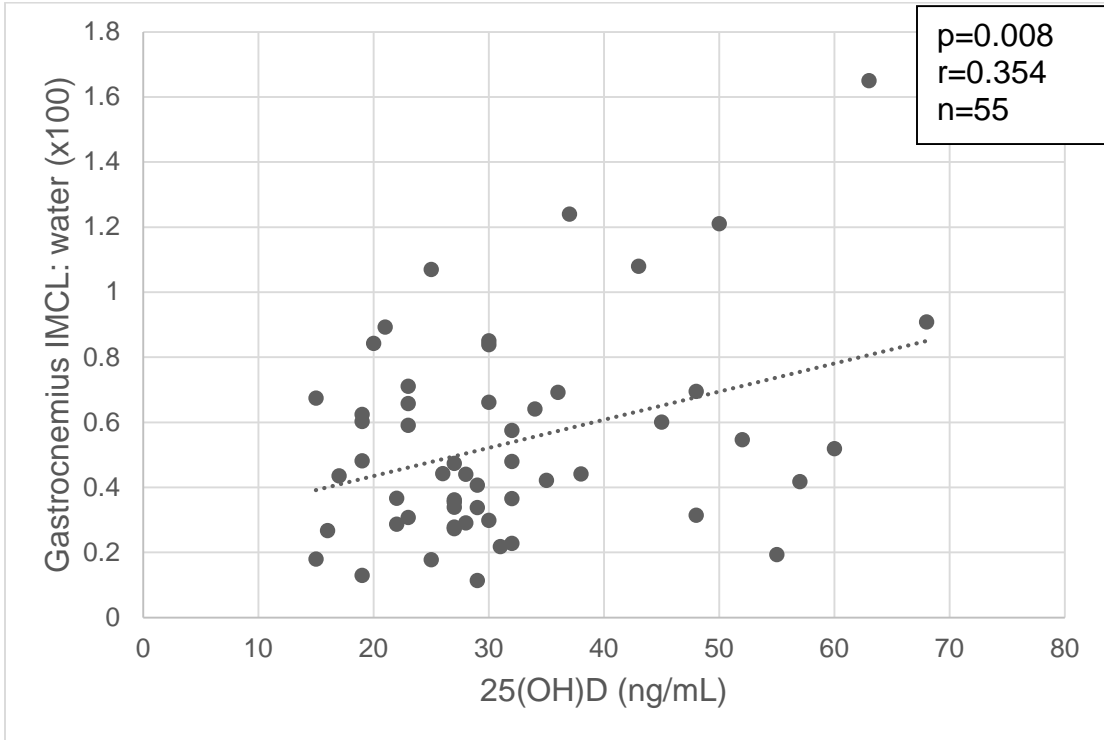
(B)



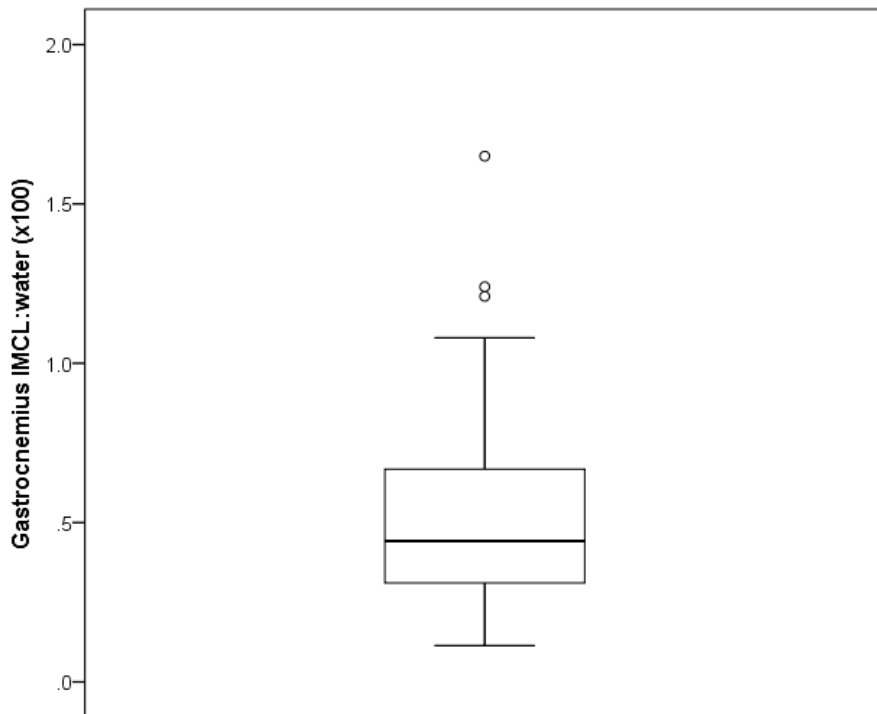
**Figure 3.4** EMCL across BMI categories

EMCL contents were reported across 3 BMI categories (normal weight, overweight, and obese (A)) and across 2 BMI categories (normal weight vs. overweight+obese (B)). Although an increase in EMCL from normal weight to obese was observed, mean EMCL was not significantly different between BMI categories.

(A)



(B)

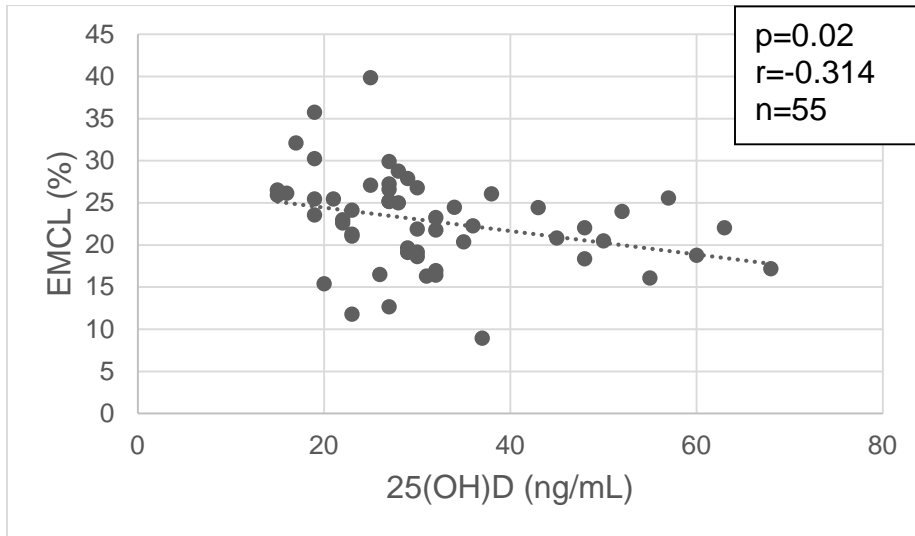


**Figure 3.5** Relationship between 25(OH)D and IMCL

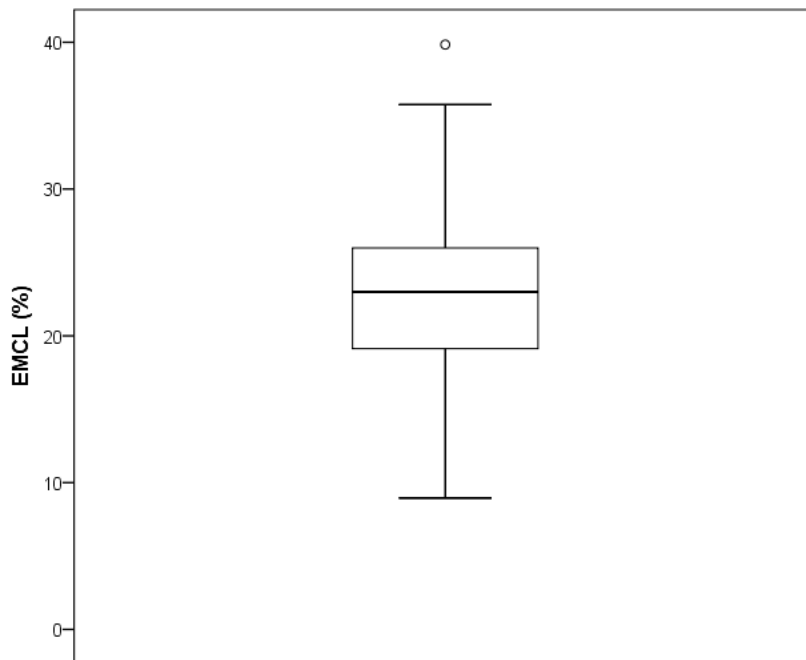
A significant positive linear relationship was observed between 25(OH)D and IMCL (A). IMCL data distribution depicted in a boxplot (B).



(A)



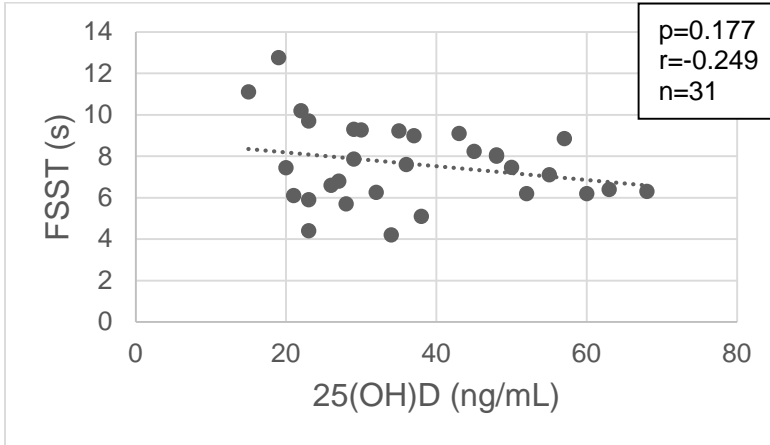
(B)



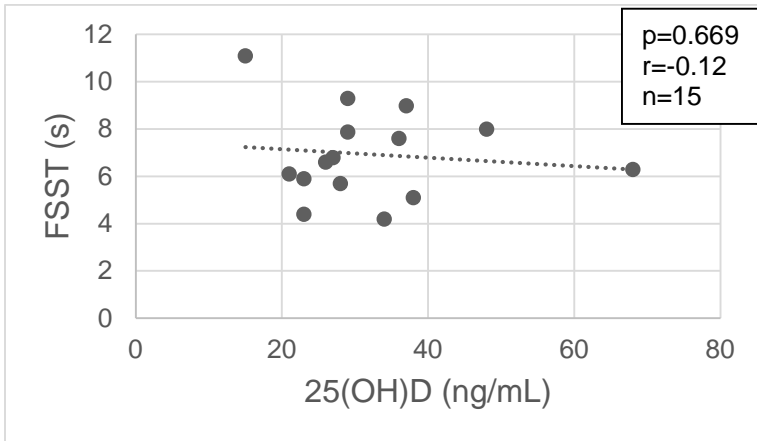
**Figure 3.6** Relationship between 25(OH)D and EMCL

A significant negative linear relationship was observed between 25(OH)D and EMCL (A). The relationship remains significant despite the one data point that was 1.5 times greater than the interquartile range (B).

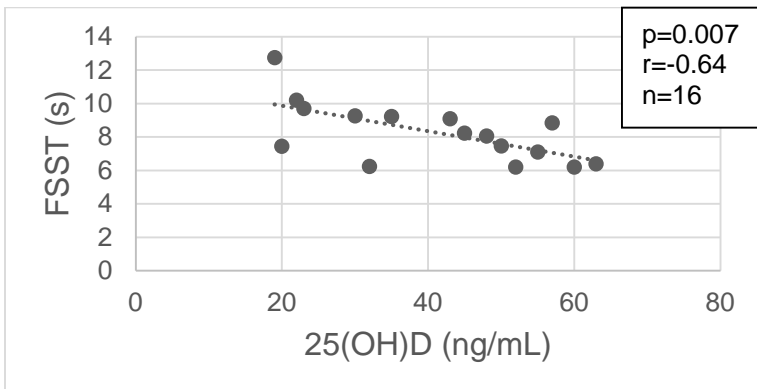
(A)



(B)



(C)



**Figure 3.7** The relationship between 25(OH)D and FSST

The scatter plots indicate a negative relationship between greater 25(OH)D concentrations and improved physical function, as measured by the faster FSST times, in the total sample (A), males (B), and females (C). A greater 25(OH)D concentration was significantly related with faster FSST times in females only.

**Table 3.6** Variables that predict FSST

<b>Predictor Variable</b>	<b>Estimate (Standardized Coefficients (Beta))</b>	<b>p-value</b>
<b>25(OH)D</b>	-0.616	0.004
<b>EMCL</b>	0.585	0.013
<b>BMI</b>	0.116	0.558
<b>Age</b>	-0.271	0.163

**Table 3.6** Variables that predict FSST

BMI and age were not significant predictors of the FSST. Greater vitamin D levels were a significant predictor of lower time to complete the FSST. Greater EMCL content was a significant predictor of greater time to complete the FSST.

## Chapter 4: Specific Aim 2

### 4.1 Summary

Dietary fat intake, physical activity, and body mass index (BMI) may play a role in age-associated changes in muscle lipid depots and local muscle tissue. Higher intake of saturated fatty acids (SFA) was associated with greater intramyocellular lipid (IMCL) content and lower lipid turnover in animal models. Conversely, high intake of polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA) were associated with increased lipid turnover and improved metabolic health. In addition to diet, exercise plays an important role in maintaining muscle health. However, to date human data examining the relationship between modifiable lifestyle factors, diet and physical activity, and muscle lipid depots are not well established. PURPOSE: The purpose of this retrospective trial was to examine how dietary fat intake and physical activity contribute to muscle lipid depots (IMCL and extramyocellular lipid (EMCL)) and local muscle tissue hemodynamics in healthy, aged individuals. METHODS: All participants were enrolled in a double-blinded randomized-controlled trial (four group design: vitamin D repletion or placebo for 12wks with an additional week of aerobic training or no training). Twenty-four hour dietary recalls were completed to assess dietary fat intake. Physical activity questionnaire and graded exercise test (GXT) were completed at baseline (0wks) and accelerometry wear was completed at baseline, midpoint (7wks), and endpoint (13wks). Gastrocnemius IMCL and EMCL were measured with magnetic resonance spectroscopy (MRS) and fat segmentation at baseline and

endpoint near-infrared spectroscopy/diffuse correlation spectroscopy (NIRS/DCS) measures were obtained before, during, and after a foot plantar flexion exercise at 0 and 13wks. RESULTS: Twenty-four participants completed all study measures. Mean age and BMI were  $67.2 \pm 5$ YO and  $25.3 \pm 4$ kg/m<sup>2</sup>, respectively. EMCL was positively associated with SFA intake ( $p=0.026$ ). EMCL was greater in overweight and obese individuals compared with normal weight individuals ( $p=0.006$ ) and BMI was the only significant predictor of EMCL. PUFA:SFA ratio was negatively related with IMCL at endpoint ( $p=0.029$ ). With a stepwise linear regression employed, the aerobic training protocol ( $p=0.014$ ) and the PUFA:SFA ratio ( $p=0.037$ ) were the best predictors of IMCL when adjusting for BMI, age, and time spent in moderate physical activity. Individuals that reported greater physical activity exhibited lower relative blood flow (rBF) and relative oxygen consumption rate (rVO<sub>2</sub>) during and after a plantar flexion exercise. CONCLUSION: In concomitant with previous literature, these data suggest that a higher unsaturated to SFA ratio intake may have a favorable effect on muscle lipid depots and lipid turnover. Although preliminary in nature, increased daily physical activity could relate with local muscle tissue hemodynamics.

#### 4.2 Introduction

Muscle composition alterations are observed with aging including decreases in lean mass and increases in fat accumulation [30, 56, 77]. Inactivity often increases with aging, further contributing to reduced muscle strength and function [125]. In order to mitigate the deleterious muscle changes, it is



imperative to understand the role of modifiable lifestyle factors on skeletal muscle. Modifiable lifestyle factors can effect muscle lipid depots (IMCL and EMCL) and local muscle tissue hemodynamics, which have been implicated in muscle health [30, 100, 141]. In older adults, EMCL accumulation has been associated with increased muscle weakness and decreased mobility [30, 55]. Conversely, high IMCL in combination with high lipid turnover has been associated with insulin sensitivity [77, 141]. With aging, decreases in capillary density were responsible for local tissue hemodynamics changes [142, 143]. Concurrently, lower erythrocyte perfusion is observed because of increased thickness and impairments in the endothelial layer [142, 143]. Modifiable lifestyle factors including exercise and dietary intake may positively alter muscle lipid depots and skeletal muscle hemodynamics.

In both animals and humans, dietary interventions effect muscle lipid depots, IMCL and EMCL. The type of fat (SFA or unsaturated fatty acids) consumed may be an important factor in lipid metabolism. Kakehi et al. [87] observed that a 3d high fat diet (HFD) intervention, primarily composed of SFA, led to an increase in IMCL accumulation and impaired insulin sensitivity. St-Onge et al. [90] observed higher IMCL content with 2 different HF diets (PUFA-rich and SFA-rich) compared with a low fat diet; however, the authors noted that the metabolic significance of a lower or higher IMCL content was unclear. In animal models, a high PUFA diet intervention had a protective effect against the development of insulin resistance [88]. A PUFA-rich diet was also associated with greater IMCL turnover and lower muscle fatty acid uptake. It is thought that,

due to the chemical structures, unsaturated fatty acids were more readily oxidized compared with SFA [88, 89]. Greater lipid oxidation is associated with increased lipid turnover, which may prevent the build-up of deleterious lipid metabolites and promote insulin signal transduction leading to improved insulin sensitivity [88]. Increased EMCL has been consistently associated with insulin resistance and obesity [30, 144]. Overall decrease in caloric intake and weight loss have been shown to decrease EMCL content [30]. After combined 7 consecutive day aerobic training and a low glycemic index dietary intervention, Haus et al. [74] observed decreases in EMCL and increases in IMCL. These muscle lipid depots changes were associated with improved insulin sensitivity and were only attributed to the exercise regimen intervention. As weight loss is accompanied by muscle mass loss, weight loss alone may not be a desirable option for older adults. Therefore, increased physical activity levels and modified dietary fat intake, rich in unsaturated fatty acids, may be one way to mitigate age-associated muscle lipid depots accumulation.

Similar to dietary intake, physical activity is a modifiable lifestyle factor that influences muscle function. Physical activity differs from exercise as it is considered to as activities of daily living; whereas exercise is defined as a structure, planned and repetitive activity [93]. Inactivity increases with age, with 33% of men and 50% of women 75 years of age or older, reporting no engagement in physical activity [125]. Physical activity contributes to aerobic fitness which decreases with aging. Aerobic fitness can be assessed with global body VO<sub>2</sub> measures [131]. Global oxygen consumption is the measure of total

volume of oxygen consumed by the body per minute [145]; however, global oxygen measures fail to monitor and assess the delivery of nutrients to the tissues [104]. NIRS/DCS hybrid techniques allow us to examine skeletal muscle hemodynamic changes during exercise and to further understand how the muscle adapts during times of stress. In older adults the greatest improvement in global VO<sub>2</sub>max was observed in aerobic training programs that were more than 20 weeks in duration with training intensities of 60-70% of VO<sub>2</sub>max [146]. With aging, impairments of local microvascular oxygen delivery and exchange in human skeletal muscle occur [142]. DeLorey et al. [147] observed that adaption of regional muscle oxygen delivery was slower in older adults compared to younger adults at the onset of a moderate-intensity exercise. In younger trained adults a faster re-oxygenation (VO<sub>2</sub> recovery time) of the muscle following an exercise was observed compared with untrained adults [148]. Further, exercise training in heart failure patients has been shown to improve local muscle tissue oxygenation [148]. However, as inactivity increases with aging it is important to assess the relationship between physical activity levels, not exercise, and local muscle tissue hemodynamics during increased energy demands.

Our retrospective analysis was part of a double-blinded randomized-controlled trial, in which participants were enrolled into 1 of 4 groups: a combined treatment of vitamin D repletion and aerobic training, vitamin D repletion alone, aerobic training alone, or control conditions. In this trial we examined the relationship between dietary fat intake and muscle lipid depots (IMCL and EMCL). In addition, our objective was to determine the relationship between

global VO<sub>2</sub>, physical activity levels, and local muscle tissue hemodynamics in individuals 60 years of age or older.

### 4.3 Methods

#### 4.3.1 Participants

Retrospective analyses were based on participants enrolled in the double-blinded randomized, placebo-controlled trial. Both females and males, 60 years of age or older, were recruited through word of mouth and local advertisements. Exclusion criteria included history of myopathy and rhabdomyolysis, uncontrolled hypertension, positive smoking status, inflammatory bowel disease, diabetes, primary hyperparathyroidism, renal disease, or a measured BMI of <18.5 or >34kg/m<sup>2</sup>. In addition, individuals with lower extremity injury or surgery 3 months prior to study enrollment were excluded. Participants with a vitamin D (25(OH)D) status of ≤32ng/mL and asymptomatic findings from the GXT were enrolled and randomized to one of four study arms. The institutional review board approved the study and written consent was obtained prior to study measures.

#### 4.3.2 Study Design

Participants 60 years or older with vitamin D insufficiency (25(OH)D ≤32ng/mL) were randomized to a 13 week double-blinded, four group design intervention. Vitamin D<sub>3</sub> (10,000IU x 5d/wk) or placebo was provided for 12 consecutive weeks. After the initial 12wk period, one additional week (7 consecutive days) with or without aerobic training was completed by both the placebo and vitamin D supplementation groups (Figure 4.1). Screening visits consisted of a blood draw to assess vitamin D status and a GXT to assess for

any contraindication to study enrollment. Baseline measures (week 0) consisted of anthropometry (height, weight, waist circumference) and PASE questionnaire. At baseline and endpoint (week 13) participants completed MRI to assess muscle lipid depots and NIRS/DCS measures during a foot plantar flexion exercise to assess gastrocnemius' hemodynamics. Accelerometry wear and dietary recalls were completed at baseline, midpoint (week 7) and endpoint.

#### 4.3.3 Statistical Analysis

Data were analyzed using IBM's Statistical Package for the Social Sciences (SPSS, version 22.0) and included descriptive statistics, independent t-test, analysis of variance (ANOVA), repeated measures analysis of variance (RMANOVA), Person correlations, and linear regression. Independent t-test was used to compare differences between participant characteristics. A RMANOVA was employed to examine differences in dietary intake, physical activity levels, and muscle lipid depots over time. Pearson correlation was used to assess the relationship between 2 continuous variables. Stepwise linear regression was employed to investigate the predictors of IMCL. Mean and SD were reported as mean $\pm$ SD and statistical significance was defined as  $p\leq 0.05$ .

#### 4.4 Results

##### 4.4.1 Participant Characteristics

This retrospective study included a total of 24 adults between the ages of 60 and 79. Baseline subject characteristics, age, BMI, and waist circumference are described in Table 4.1. Participants' educational attainment ranged from a General Education Development (GED) to a doctoral degree and income range

was between \$11,500 and \$210,000. BMI, weight, and waist circumference did not significantly differ from baseline to endpoint in the total sample or among sex groups.

#### 4.4.2 Muscle Lipid Depots Characteristics

IMCL and EMCL measures over time (baseline to endpoint) were not statistically different (Table 4.2). IMCL was positively correlated with age ( $p=0.009$ ) (Figure 4.2). IMCL did not differ between normal weight individuals and those with a  $BMI > 25 \text{ kg/m}^2$  at baseline. When compared with normal weight individuals, EMCL was greater in individuals with a  $BMI \geq 25 \text{ kg/m}^2$  at baseline ( $p=0.006$ ) (Figure 4.3 (A)). At baseline, EMCL was positively correlated with BMI ( $p=0.001$ ) (Figure 4.3 (B)) and waist circumference ( $p < 0.035$ ) (Figure 4.3 (C)). Baseline BMI was the best predictor of EMCL ( $p=0.002$ ) independent of age or time spent in moderate physical activity (Table 4.3). IMCL and EMCL were not different between the 4 study groups at baseline.

#### 4.4.3 Dietary Intake and Muscle Lipid Depots

Dietary intake including total energy (kcal), fat (g), carbohydrate (g), protein (g) were not different between the 3 study time points (baseline, midpoint, and endpoint) or by study groups. Total and percent energy, fat, and carbohydrate intake are described in Table 4.4. Total and percent dietary fat intake is described in Table 4.5. Males had a greater intake of total SFA ( $p=0.008$ ), PUFA ( $p=0.009$ ), and MUFA ( $p=0.02$ ) compared with females (Table 4.5). However, there were no differences in percent intake of dietary fats between sexes. EMCL at baseline was positively correlated with percent calories

from fat, which can be primarily attributed to SFA, as SFA was the only dietary variable positively associated with EMCL (Figure 4.5). Although SFA was positively correlated with EMCL, BMI remained the best predictor of EMCL (Table 4.6).

PUFA to SFA ratio negatively correlated with endpoint IMCL ( $p=0.029$ ). IMCL content decreased in individuals that consumed a higher PUFA relative to SFA diet (Figure 4.6). Endpoint 25(OH)D concentrations were not associated with endpoint IMCL measures. A stepwise linear regression was employed indicating that aerobic training and PUFA:SFA ratio were the only significant predictors of IMCL (Table 4.6).

#### 4.4.4 Physical Activity and Local Muscle Tissue Hemodynamics

During NIRS/DCS measures one participant was not compliant with procedures because the participants continuously moved their leg throughout testing and completed less than 30 seconds of the 3 minute arterial occlusion protocol. Due to lack of compliance, the participant's data was 1.5 times greater than the interquartile range, and thus the participant was removed from NIRS/DCS analysis. We observed no correlation between hemodynamic measures and subject characteristics, age and BMI. There were no NIRS/DCS measurement differences between males and females. Neither time spent in moderate activity nor total activity were related with local tissue hemodynamics. Further global of aerobic capacity, as measured during GXT, did not correlate with local muscle tissue hemodynamics. PASE questionnaire scores were negatively correlated with  $rVO_2$  during plantar flexion ( $p=0.048$ ), 1 minute after

( $p=0.05$ ), and at full recovery ( $p=0.05$ ) (Figure 4.7 A-C). A lower rBF was also observed with greater PASE scores 1 minute after plantar flexion exercise ( $p=0.059$ ) and at full recovery ( $p=0.083$ ).

#### 4.5 Discussion

The aim of the study was to examine the relationship between dietary fat intake and muscle lipid depots (IMCL and EMCL). In addition, we examined the relationship between aerobic capacity, physical activity levels, and local muscle tissue hemodynamics in healthy older adults enrolled in a randomized controlled trial. Our primary finding was that a greater dietary SFA intake was positively correlated with EMCL and a healthier dietary fat intake ratio (PUFA:SFA) was negatively correlated with IMCL. Past studies examined the effects of short- and long-term dietary interventions on muscle lipid depots. The novelty of this study was that we examined the relationship between muscle lipid depots and daily eating habits in a generally healthy older cohort. Further, in individuals that reported higher activity levels, we observed lower gastrocnemius rBF and rVO<sub>2</sub> during and after a plantar flexion exercise. These data suggest that a higher PUFA:SFA diet is related with lower IMCL and potentially greater physical activity levels may relate with local tissue hemodynamics.

We provide further evidence for age-associated increases in IMCL content. Crane et al. [149] observed that older adults had higher IMCL content and greater lipid droplet size along with a reduced number of muscle mitochondria. Haus et al. [74] observed that older insulin resistant obese men that engaged in a short exercise intervention (7 consecutive day of aerobic



training) had increased IMCL and improved insulin sensitivity; however, mitochondrial changes were not investigated. In addition to increased IMCL content, improved mitochondrial oxidative capacity was seen in older adults engaged in a longer (16wk) exercise regimen [77]. As a result of exercise training, higher IMCL content was associated with insulin sensitivity [77]. Therefore, the importance of IMCL content is closely linked to the mitochondria's ability to metabolize lipid when needed, such as in time of exercise.

Both physical activity and dietary intake are modifiable lifestyle factors that play a vital role in muscle health. Our participants' dietary habits met the Recommended Dietary Allowance (RDA) for percent caloric intake of carbohydrate (45-65%), fat (20-35%), and protein (10-35%) [150]. On average, females met the recommendations for SFA intake ( $\leq 10\%$  from total calories), while males consumed slightly more than the recommended amount (12%). There are no current recommendations for unsaturated fatty acid intake, but it is widely accepted that to reduce the risk of diseases, such as cardiovascular disease or diabetes, reducing SFA to  $<10\%$  of calories per day and replacing SFA with unsaturated fatty acids is necessary [151].

Adipose tissue is the primary site of storage for excess caloric intake [30, 88]. During aging, a redistribution of fat depots occurs even if total body fat is maintained [90]. Characteristically with aging, fat infiltrates various tissues such skeletal muscle as fat storage sites change from the less deleterious subcutaneous fat to visceral fat [30]. We observed a positive correlation between increased SFA intake and EMCL. Although we observed that a higher dietary fat

intake was related with increased EMCL, BMI remained the best predictor of EMCL. In combination with previous data, our findings further support that greater BMI is the major contributor to increased EMCL and that an overall healthy lifestyle is necessary to maintain muscle health. Future studies should focus to examine if weight loss can reduce EMCL.

We also observed that PUFA:SFA ratio intake was negatively correlated with IMCL content. Since our findings were observed at endpoint we further examine if the diet fat ratio or the study intervention was the best predictor of this relationship. We observed that the best predictors of IMCL were both PUFA:SFA ratio and aerobic training. Although our retrospective analysis was not able to assess lipid turnover or mitochondrial oxidation, previous studies have shown that a higher unsaturated fatty acid diet and exercise led to lower dietary fat uptake by the muscle [84, 88, 89]. Jans et al. [89] suggested that these observations may be due to the chylomicrons rich in PUFA and MUFA that were more readily removed from the circulation by hepatic lipase instead of lipoprotein lipase. The authors suggest that post-PUFA meal an improvement in postprandial insulin sensitivity occurs compared with SFA, which would decrease fatty acid uptake by the muscle. Lower muscle lipid uptake accompanied by greater removal of the chylomicrons from the circulation would potentially lead to a reduction in muscle fat accumulation and an increase in muscle lipid turnover. Further, animal studies suggest that the type of fatty acids consumed exert influence on the processing of the lipids during beta-oxidation. Due to their chemical structure, unsaturated fatty acids seem to be preferentially

oxidized over SFA [88]. Unsaturated fatty acids contain one or more double bonds, whereas SFA do not contain double bonds. Double bonds are more reactive and easily susceptible to modifications. These chemical structural differences lead to unsaturated fatty acids' preferential oxidation, which in turn may lead to greater muscle lipid turnover and overall improved muscle health. This was further seen in normal weight individuals who had a greater degree of unsaturation compared with those in a higher BMI category [84]. Synthesis of unsaturated fatty acids through desaturation of SFA was decreased in overweight and obese individuals. The reduced degree of unsaturation in overweight and obese individuals may be involved in increased lipid peroxidation and damage to the myocytes. Combining our retrospective findings with previous studies, these data suggest that maintaining a healthy diet, in particular partially replacing SFA with healthier fats (PUFAs and MUFAs), may play a beneficial role in muscle health.

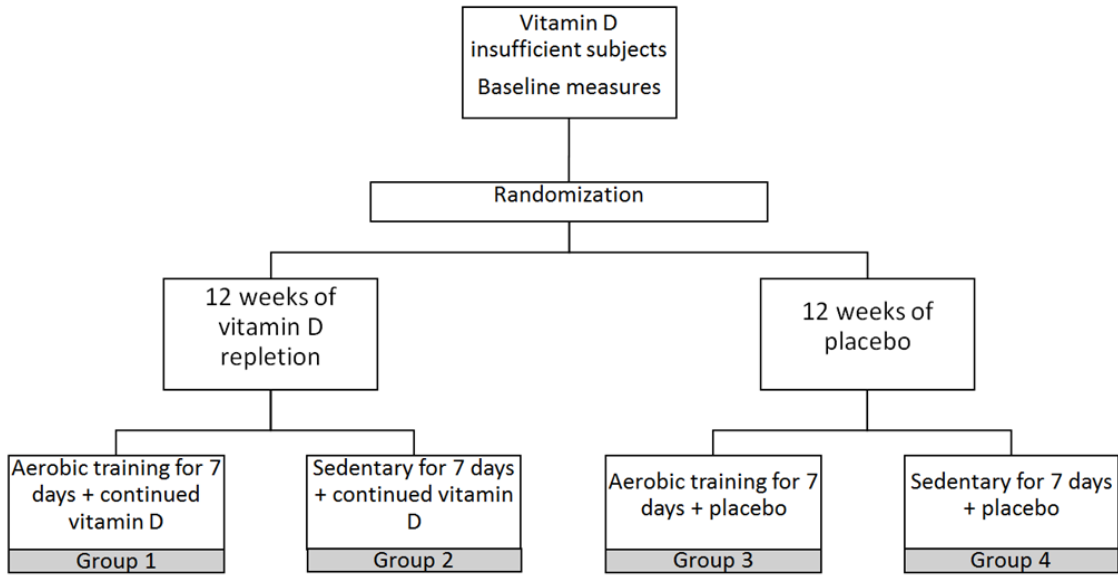
Higher levels of physical activity are important to preserve muscle health and function. We examined the relationship between gastrocnemius hemodynamics during a foot plantar flexion exercise with physical activity levels measured by accelerometry and PASE questionnaire and global oxygen consumption measured during a GXT. Although participants moderate physical activity levels, measured by accelerometry wear, ranged from 20min/d to 108min/d, the majority (82%) of the participants met the CDC's guideline for moderate physical activity [125]. Further, accelerometry vector magnitude counts, a continuous variable was examined, but was not related with local muscle

tissue metabolism. The lack of variance in physical activity could have precluded our ability to detect a relationship between low and high moderate physical activity levels and local muscle tissue hemodynamics.

In addition, we examined the relationship between peak VO<sub>2</sub>, measured by GXT, and local tissue hemodynamics. Peak VO<sub>2</sub> measures provide information about cardiovascular health and in our sample we had a large distribution (15-50mL/kg/min), of which 45.8% of participants had poor to fair cardiovascular fitness. Global oxygen consumption impairments are not always apparent and the need to examine microvascular changes is necessary because it can provide more nuanced assessment of physiological changes associated with aging [152]. Marengi et al. [153] observed that younger adults a tight correlation between global and local VO<sub>2</sub> exists. The authors simultaneously measured local VO<sub>2</sub> of the vastus lateralis and global VO<sub>2</sub> during a graded cycle ergometry test performed until volitional fatigue. The discrepancy between our findings may be attributed to multiple factors. We did not complete global and local VO<sub>2</sub> measures simultaneously. Our global VO<sub>2</sub> measures taken during a graded treadmill exercise test and local gastrocnemius VO<sub>2</sub> measures were completed during a plantar flexion exercise. Participants were also instructed to continue the GXT until volitional fatigue, but the foot plantar flexion protocol was set to 75 repetitions, in which participants may not have reached exhaustion. Therefore, it was not surprising that we did not observe a relationship between peak VO<sub>2</sub> and local VO<sub>2</sub> as measured by NIRS/DCS.

Based on PASE questionnaire findings, we observed that individuals that reported engaging in more physical activity had lower  $rVO_2$  during, immediately after, and at full recovery of the foot plantar flexion exercise. In individuals that were more active, muscles may be more adapt to increases in demand; therefore, during periods of stress the relative increase in BF and  $VO_2$  was lower during and after exercise compared with less active individuals. We observed that physical activity was the best predictor of  $rVO_2$  independent of BMI and age. Although the significance of our findings was that greater physical activity levels in older adults may be helpful in preventing microvascular dysfunction that occurs with aging, these results should be interpreted conservatively.

There are multiple limitations to our findings. First, only PASE questionnaire was correlated with local muscle tissue oxygen consumption and blood flow. The PASE questionnaire is a reliable and valid assessment of physical activity in older adults; however accelerometry is a better measure. As accelerometry data did not correlate with local muscle tissue metabolism the relationship between physical activity and local muscle tissue still remains unclear. Future studies should aim to further the understand of how physical activity, not exercise, may contribute to local muscle oxygen use and impact overall health. Our findings show a relationship between aerobic training, favorable dietary fat intake and lower IMCL. However, the impact of these relationships on overall health warrants further investigations.



**Figure 4.1** Study Design

The randomized double-blinded placebo-controlled trial consisted of 4 arms. Vitamin D insufficient ( $25(\text{OH})\text{D} \leq 32\text{ng/mL}$ ) 60 years of age or older individuals were recruited to participate in the 13 week trial. Participants were randomized to one of four groups 1) vitamin D supplementation + aerobic training, 2) vitamin D supplementation + no aerobic training, 3) placebo + aerobic training, 4) placebo + no aerobic training.

**Table 4.1** Subject Characteristics, Mean±SD

	<b>Female (n= 12)</b>	<b>Male (n= 12)</b>	<b>Total (n=24)</b>
<b>Age (years)</b>	68.8±6	65.6±4	67.2±5
<b>Height (cm)</b>	164.6±5*	177.8±6*	171.2±9
<b>Weight (kg)</b>	66.4±12*	83.4±14*	74.9±15
<b>BMI (kg/m<sup>2</sup>)</b>	24.5±4	26.2±3	25.3±4
<b>Waist Circumference (cm)</b>	82.9±14*	96.4±11*	89.6±14
<b>Ethnicity</b>			
White	12	11	23
African American	0	1	1
<b>SES (n=24)</b>			
High School Graduate or GED	2	2	4
Some College, No Degree	0	1	1
Associates Degree	1	0	1
Bachelor's Degree	4	2	6
Master's Degree	2	4	6
Doctoral Degree	3	3	6
<b>Income (\$; n=21)</b>			
<35,000	2	1	3
35,000 ≤ 50,000	2	0	2
50,000 ≤ 100,000	5	4	9
100,000 ≤ 150,000	1	4	5
>150000	1	1	2

\*Significant differences between females and males (p<0.05)

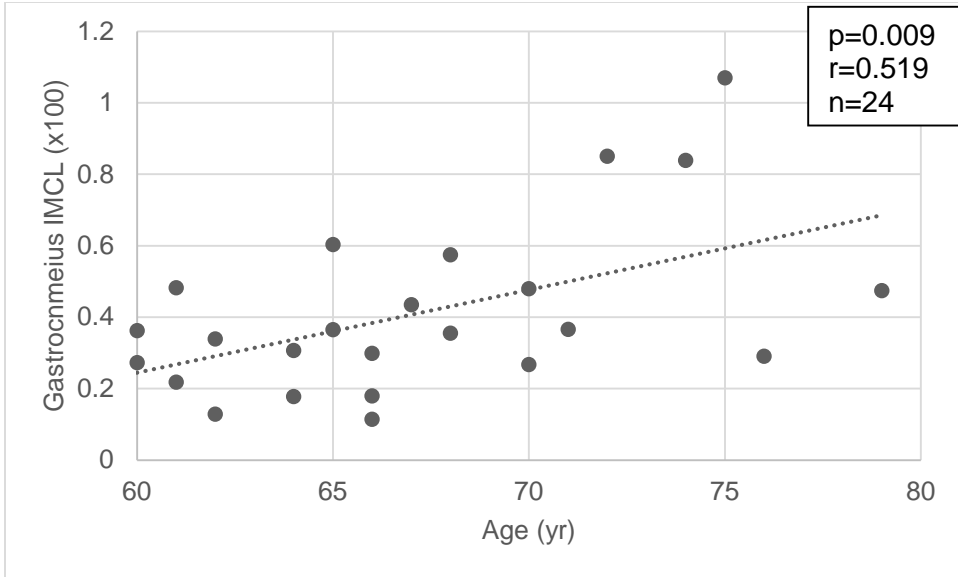


**Table 4.2** EMCL and IMCL Characteristics

<b>Muscle Lipid Depots</b>	<b>Females (n=12)</b>	<b>Males (n=12)</b>	<b>Total (n=24)</b>
<b>IMCL Baseline (au)</b>	0.44±0.28	0.38±0.2	0.41±0.24
<b>IMCL Endpoint (au)</b>	0.37±0.24	0.34±0.22	0.35±0.22
<b>EMCL Baseline (%)</b>	26.2±6	26±4	26.1±5
<b>EMCL Endpoint (%)</b>	25.9±5	25±5	25.4±5

**Table 4.2** EMCL and IMCL Characteristics

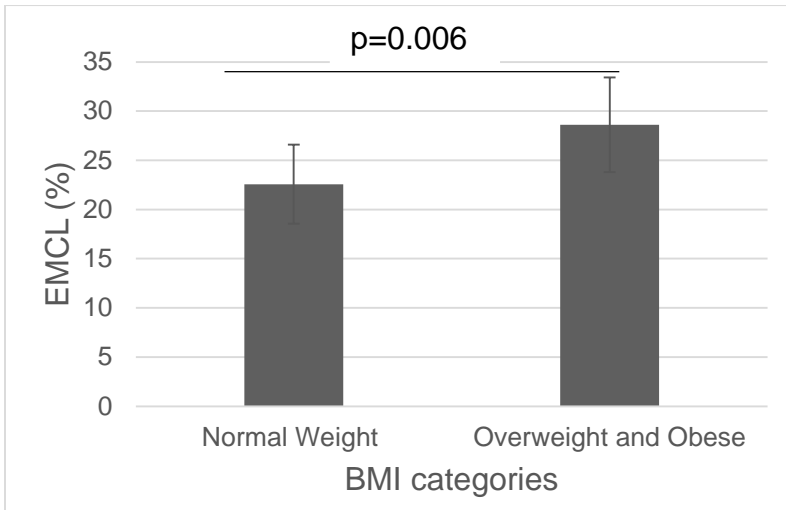
IMCL and EMCL was not different between males and females. Muscle lipid depots at baseline and endpoint were not different in total sample or between sexes.



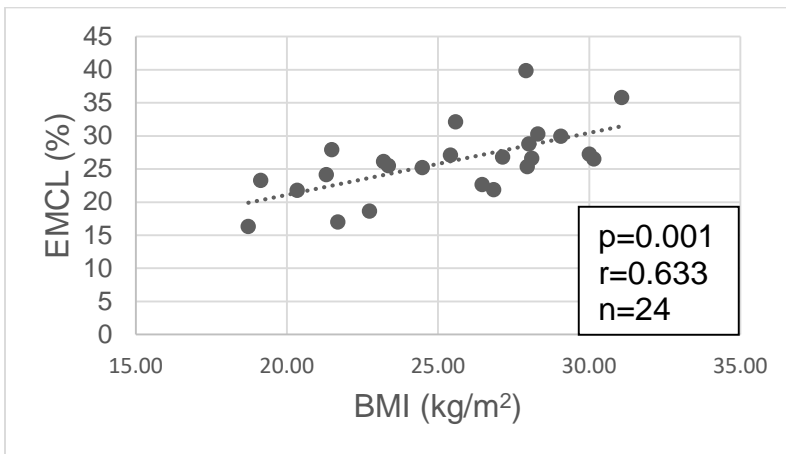
**Figure 4.2** Relationship between IMCL and age

A significant positive linear relationship was observed between IMCL and age. The older the participants were the higher their gastrocnemius IMCL content.

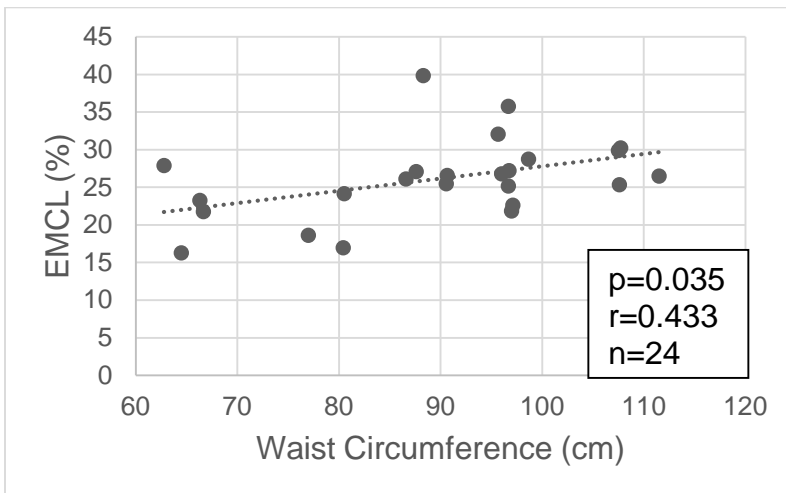
(A)



(B)



(C)



**Figure 4.3** The relationship between EMCL, BMI, and waist circumference  
EMCL content was statistically lower in normal weight vs.  
overweight+obese individuals at baseline and at end study (A). EMCL was  
positively correlated with BMI (B) and waist circumference (C).

**Table 4.3** Variables that predict EMCL

<b>Predictor Variable</b>	<b>Estimate (Standardized Coefficients (Beta))</b>	<b>p-value</b>
<b>Moderate Physical Activity</b>	0.055	0.774
<b>Age</b>	-0.048	0.962
<b>BMI</b>	0.652	0.002

**Table 4.3** Variables that predict EMCL

Age and time spend in moderate activity were not significant predictors of EMCL content. Higher BMI levels were a significant predictor of greater EMCL content.



**Table 4.4** Dietary intake between sexes, mean±SD

	<b>Female (n=12)</b>	<b>Male (n=12)</b>	<b>Total (n=24)</b>
<b>Energy (kcal)</b>	1510±353*	2055±736*	1782.7±630
<b>Fat (g)</b>	56.7±19*	82.6±31*	69.7±28
<b>% Calories from Fat</b>	32±4	34.9±5	33.5±5
<b>Carbohydrate (g)</b>	185.7±42	245.2±94	215.5±78
<b>% Calories from Carbohydrate</b>	48.2±6	46.9±7	47.6±7
<b>Protein (g)</b>	65.1±16	77.3±27	71.2±23
<b>% Calories from Protein</b>	17.7±4	15.3±3	16.5±4

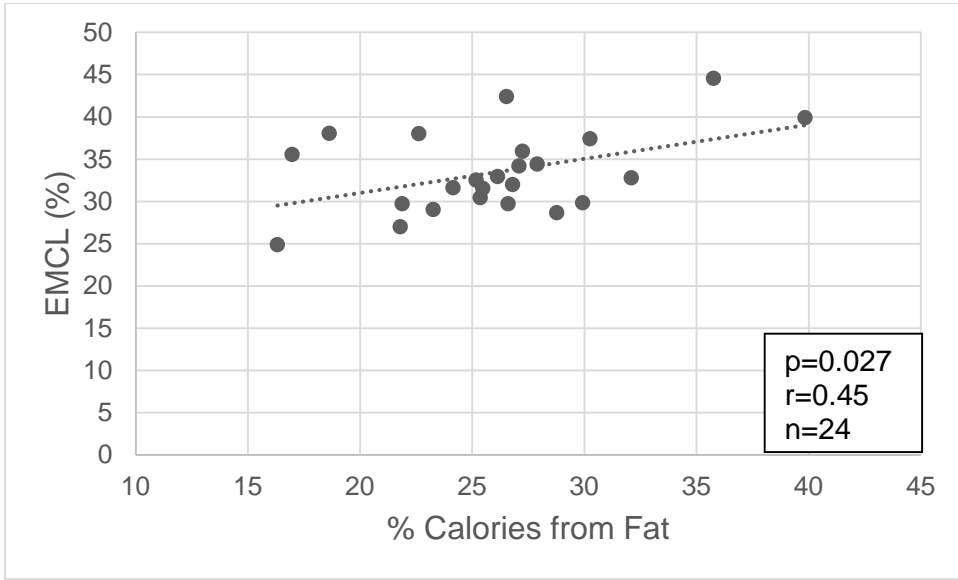
\*Significant differences between females and males (p<0.05)

**Table 4.5** Dietary fat intake between sexes, mean±SD

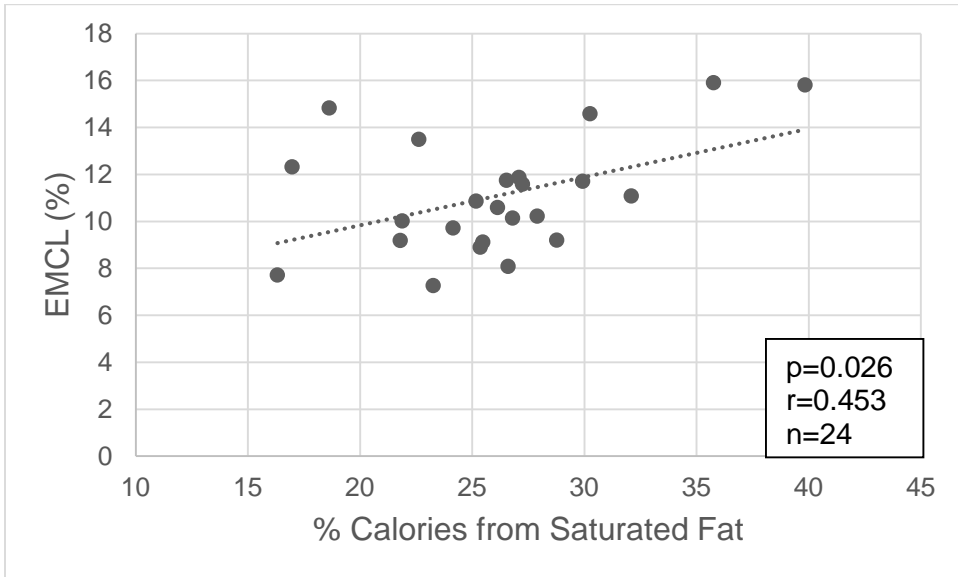
	<b>Female (n=12)</b>	<b>Male (n=12)</b>	<b>Total (n=24)</b>
<b>SFA (g)</b>	18.4±7*	27.7±11*	23±10
<b>% Calories from SFA</b>	10.3±2	11.8±2	11.1±2
<b>PUFA (g)</b>	12.9±4*	19.6±9*	16.3 8
<b>% Calories PUFA</b>	7.3±1	8±2	7.7±2
<b>MUFA (g)</b>	20.6±7*	28.5±10*	24.6±9
<b>% Calories MUFA</b>	11.6±2	12.2±2	11.9±2
<b>PUFA to SFA ratio</b>	0.84±0.3	0.78±0.2	0.81±0.2

\* Significant differences between females and males (p<0.05)

(A)



(B)



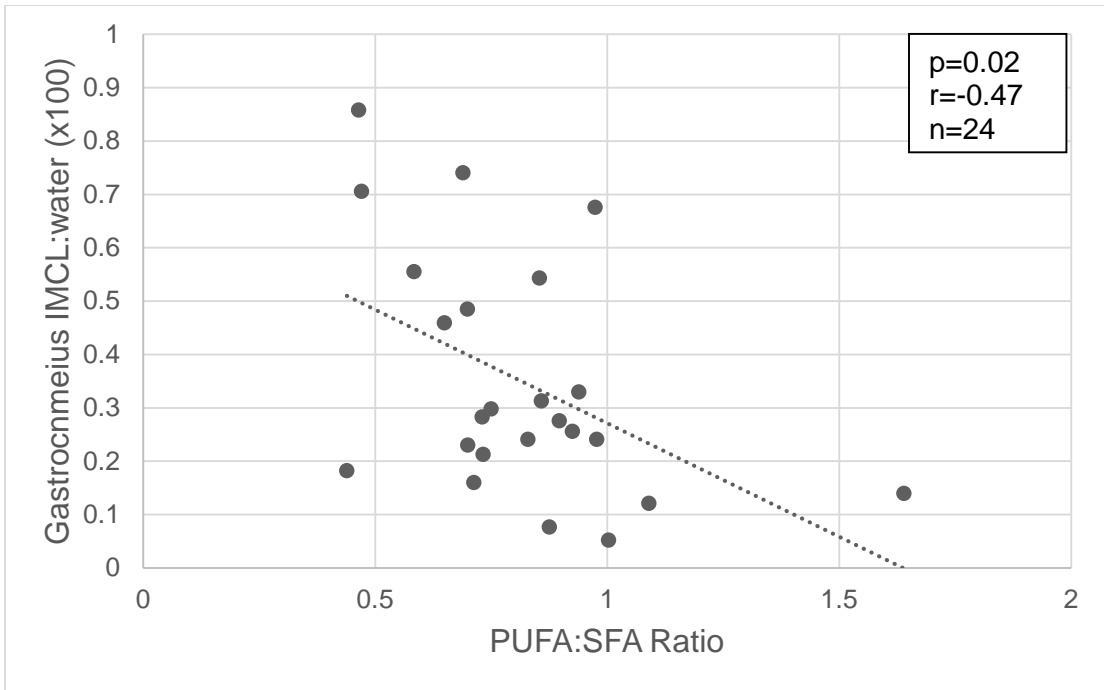
**Figure 4.4** Relationship between percent calories from fat and EMCL

A positive linear relationship was observed between intake of percent calories from total fat (A) and saturated fat (B) and EMCL. Individuals with a higher consumption saturated and total fat had greater EMCL content.

**Table 4.6** Variables that predictors of EMCL when adjusting for SFA intake

<b>Predictor Variable</b>	<b>Estimate (Standardized Coefficients (Beta))</b>	<b>p-value</b>
<b>Moderate Physical Activity</b>	0.033	0.863
<b>Age</b>	-0.043	0.820
<b>BMI</b>	0.562	0.009
<b>% calories from SFA</b>	0.234	0.235

**Table 4.6** Variables that predictors of EMCL when adjusting for SFA intake  
Age, SFA, and time spend in moderate physical activity were not significant predictors of EMCL content. Higher BMI levels were a significant predictor of greater EMCL content.



**Figure 4.5** Relationship between PUFA to SFA Ratio and IMCL

A negative linear relationship was observed between an average intake of PUFA to SFA and endpoint IMCL measures. Individuals with a higher consumption of PUFA relative to SFA intake had lower IMCL content.



**Table 4.7** Variables that predict IMCL

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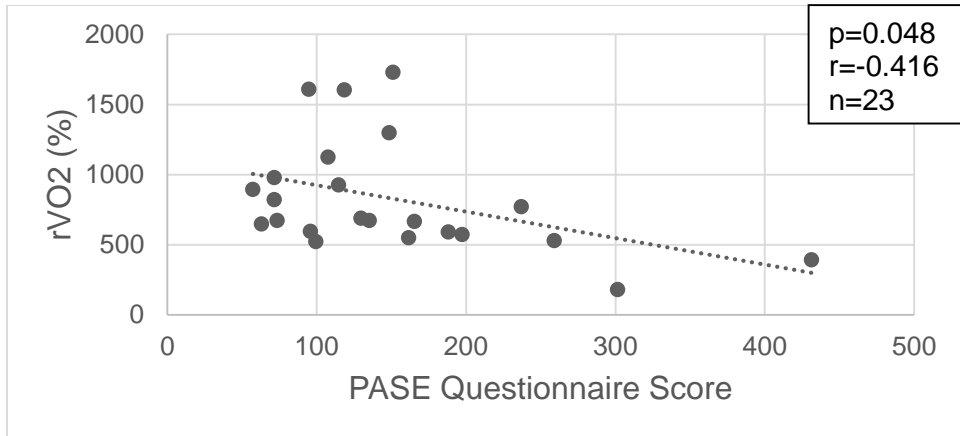
<b>Predictor Variable</b>	<b>Estimate (Standardized Coefficients (Beta))</b>	<b>p-value</b>
<b>Aerobic Training</b>	-0.383	0.046
<b>PUFA:SFA ratio</b>	-0.484	0.014

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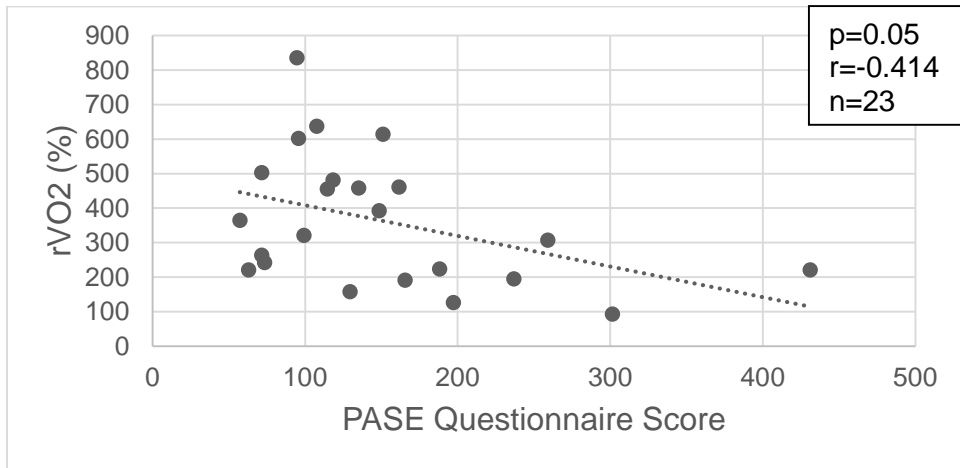
**Table 4.7** Variables that predict IMCL

The stepwise linear regression indicated that aerobic training and greater intake of PUFA:SFA significantly predict IMCL content.

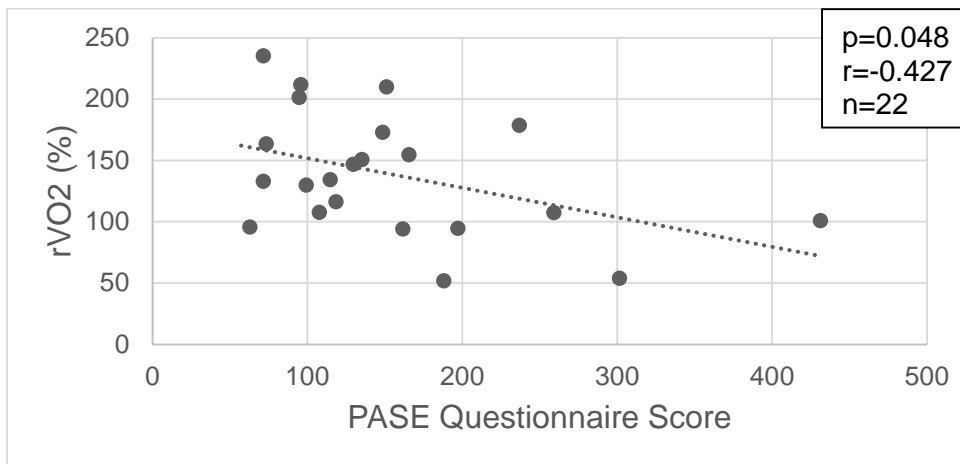
(A)



(B)



(C)



**Figure 4.6** Relationship between PASE questionnaire and local muscle hemodynamics

A significant negative linear relationship was observed between PASE questionnaire scores and rVO<sub>2</sub> during (A), 1 minute after (B), and at full recovery (C) of the plantar flexion exercise.

## Chapter 5: General Discussion

### 5.1 Discussion

During the aging process muscle strength and function decline, but modifiable lifestyle factors can play a key role in alleviating muscle dysfunction. Additionally, age-associated muscle lipid depot changes can negatively affect muscle health and function. Higher EMCL content has been associated with reduced muscle strength and function in older adults [30, 55]. Likewise, higher IMCL content in combination with low IMCL turnover has been linked to greater insulin resistance [141]. Dietary intake, vitamin D status, and exercise have been associated with muscle lipid depots. Previous work has observed an inverse relationship between 25(OH)D concentrations and EMCL [55, 86] and our lab has observed a positive linear relationship between 25(OH)D concentrations and IMCL in healthy older adults [59]. For our first objective, we examined the relationship between muscle lipid depots, vitamin D status, and physical function. No significant relationship between 25(OH)D concentrations and IMCL content was observed; however, in accordance with previous studies [55, 86] a significant negative relationship between EMCL content and 25(OH)D concentrations was noted. Further in females, lower EMCL content and higher 25(OH)D concentrations were significant predictors of faster FSST times independent of age and BMI. These observational data suggest that maintaining higher vitamin D status may play a role in lowering EMCL and positively impacting physical function.

Exercise can positively influence overall muscle health by lowering EMCL content and increasing IMCL turnover [30, 141]. Previous studies have observed muscle lipid depot changes in younger and older adults enrolled in an exercise intervention [44, 74, 77, 95]. However, we aimed to examine the relationship between daily physical activity levels and muscle lipid depots. We used multiple methods to measure physical activity levels and no relationship was observed between physical activity and muscle lipid depots. These results may suggest that increased daily physical activity alone may not be enough to contribute to healthful alteration in muscle lipid depots. Higher-intensity, structured exercise regimens may be necessary to alter muscle lipid depots. However, the relationship between physical activity and muscle lipid depots warrants further investigation, as our sample was relatively small and lacked variance in physical activity levels.

Similar to vitamin D and physical activity, BMI is another modifiable lifestyle factor that can influence muscle lipid depots. Previous studies observed a relationship between higher BMI categories and greater IMCL and EMCL content [79, 84]. Also, greater BMI has been associated with lower vitamin D status [39, 43]. We further assessed these relationships in a cohort of adults 60 years of age or older. Although our findings were not significant, we observed increases in muscle lipid depots with increases in BMI category. We observed lower vitamin D status in higher BMI categories and a significant negative correlation between 25(OH)D concentrations and central adiposity measured by waist circumference. We observed a positive relationship between EMCL and

BMI in the cohort enrolled in the randomized double-blinded placebo-controlled trial. BMI was also a significant predictor of EMCL when adjusting for physical activity levels and age. Together these data suggest that without exercise, higher BMI and central adiposity, relate to muscle lipid depots accumulation and may potentially leave higher BMI individuals more susceptible to lower vitamin D status.

In addition to the aforementioned modifiable lifestyle factors, dietary intake can influence muscle lipid depots. We retrospectively analyzed the relationship between dietary intake and muscle lipid depots in participants enrolled in a double-blinded randomized controlled-trial. Ectopic fat accumulation was observed in skeletal muscle of individuals with an overweight or obese BMI classification [30]. In addition, changes in fat distribution, independent of body weight and waist circumference, occur with aging with increased fat deposition seen in the skeletal muscle [154]. Increases in fat storage, decline in mitochondria content and function, and poor capacity for oxidative metabolism leaves older adults susceptible to increased EMCL content and stagnant IMCL content [72, 74, 77].

Adding to previous findings [30], we observed a positive relationship between increased EMCL and dietary fat intake. Also for the first time to our knowledge, a relationship between increased PUFA:SFA dietary intake and decreased IMCL was observed. Further, the aerobic training program and increased dietary intake of PUFA:SFA were the best predictors of IMCL. Both human and animal dietary intervention trials have observed a relationship

between greater unsaturated fatty acids and higher IMCL turnover [84, 88, 89]. Combining our data with previous findings, these results may suggest that individuals that consume a higher unsaturated fatty acid diet in combination with aerobic exercise, may have lower lipid accumulation potentially due to greater lipid turnover.

We did not assess lipid turnover, but we used NIRS/DCS measures to monitor microvascular hemodynamic changes during a foot plantar flexion exercise this provided us with an index to monitor local muscle tissue hemodynamics. Individuals that reported engaging in more physical activity had lower rBF and rVO<sub>2</sub> during and after the foot plantar flexion exercise. These findings may be due to active individuals being more acclimated to increases in muscle demand and therefore having a relatively lower hemodynamic response compared with less active individuals. This would suggest that greater physical activity levels and not necessarily exercise may help to prevent microvascular dysfunction that occurs naturally with aging.

## 5.2 Limitations

A major limitation of this dissertation was the nature of a retrospective study analysis. A retrospective study uses data that has previously been documented and may not be related to the research aims [155]. The first specific aim, used combined datasets from 3 different trials. Participants were recruited over a 4 year period and completed varied study measures. Incomplete data was inevitable, as waist circumference, DXA, CT, and FSST measures were not completed for each individual trial. Also, physical activity levels were difficult to



compare across the total sample as diverse assessment methods were implemented to measure physical activity levels. Physical activity levels were measured either by using a physical activity questionnaire, accelerometry wear, or PASE questionnaire. Although the physical activity questionnaire inquired about frequency, duration, and intensity of exercise the nature of an open-ended question may have led to varied subjective responses. For example, one question inquired if participants exercised and if so to provide additional detail. This type of open-ended question allowed for individuals' to subjectively determine whether their activities constituted as exercise or physical activities of daily living.

A major limitation in both aims was that the data was underpowered for some variables due to the small sample size. Between both aims and generally among the literature racial diversity is lacking. Our study participants were primarily White and since genetic factors may play a role in the interpretation of vitamin D status there is a need to investigate these relationships in all races. Further, currently no gold standard exists for the analysis and reporting of IMCL and EMCL, therefore muscle lipid depots can be difficult to review across studies.

### 5.3 Significance and Implications

The findings from both aims support previous literature and provide new information about the relationship between modifiable lifestyle factors and muscle lipid depots in older adults. First, we were able to provide further evidence for the associations between 25(OH)D concentrations, EMCL, and physical function. Although not significant, similar to previous findings we observed a relationship

between increased muscle lipid depots and BMI categories. Decreased 25(OH)D concentrations in higher BMI categories provide further evidence to the established relationship. In both animal and human models, studies have focused on dietary fat interventions with varied fat composition and study duration. The novelty of our findings was that we examined the relationship between muscle lipid and daily dietary habits. Even without any supplemental dietary intervention, we observed that higher IMCL was related with increased daily intake of PUFA:SFA. In combination, a higher daily unsaturated fatty acid diet and exercise were significant predictors of IMCL content, which was consistent with previous work that examined dietary and exercise interventions on IMCL turnover.

With observational retrospective data, we were able to provide information about the relationships between our variables. Future intervention trials are needed to examine the cause and effect between modifiable lifestyle factors and muscle lipid depots in older adults. Further, both animal and human studies need to focus on identifying mechanisms related to how modifiable lifestyles factors may cause favorable lipid changes (EMCL decreases and increased IMCL turnover).

#### 5.4 Future Directions

The findings from our study demonstrate that modifiable lifestyles factors relate with age-associated alterations in muscle lipid depots. However, as our results were observational in nature, future studies should consider a more robust study design aimed to examine the effect of lifestyles factors on

deleterious muscle lipid accumulation. Further, studies should be appropriately powered and include both females and males of varied age and diverse races.

The discrepancy between our previous work [59] and our current findings regarding IMCL and 25(OH)D concentrations warrant further investigation. Future studies should examine the effect of vitamin D supplementation in vitamin D deficient older adults on muscle lipid depot changes and physical function. Due to the dynamic nature of IMCL, it is important to measure muscle lipid turnover and mitochondrial function as well as IMCL quantity. Further, multiple physical function measures should be completed in order to provide a comprehensive approach to assess muscle strength and performance. It is also important to discern between daily physical activity and structured exercise and the role physical activity may play on age-associated muscle lipid depots. Less active older populations may be more receptive and compliant to meeting physical activity goals compared with initiating and maintaining a rigorous exercise regimen. Therefore, the effect of physical activity on muscle lipid depots needs to be further investigated.

Dietary intake should also be considered when assessing muscle lipid depots. Unsaturated fatty acids seem to have a favorable effect on muscle lipid depots and turnover; however human dietary fat interventions are necessary to examine the effect of a high unsaturated fatty acid diet on muscle lipid depot changes and overall health. Finally, with aging microvascular changes were observed and future studies should incorporate novel techniques, such as NIRS/DCS, to assess the effect of modifiable lifestyle factors on local muscle

tissue changes. Taken together, our findings show that modifiable lifestyles factors (BMI, vitamin status, physical activity, and dietary fat intake) relate with muscle lipid metabolism and may contribute to overall health and quality of life in older adults.

## Appendices

### A.1 MRI Screening Form for Participants

<b>MAGNETIC RESONANCE (MR) PROCEDURE SCREENING FORM FOR PATIENTS</b>					
Date ____/____/____		Patient Number _____			
Name _____		Age _____	Height _____	Weight _____	
Last name      First name      Middle Initial					
Date of Birth ____/____/____		Male <input type="checkbox"/>	Female <input type="checkbox"/>	Body Part to be Examined _____	
month      day      year					
Address _____			Telephone (home) (____) ____-____		
City _____			Telephone (work) (____) ____-____		
State _____ Zip Code _____					
Reason for MRI and/or Symptoms _____					
Referring Physician _____			Telephone (____) ____-____		
<hr/>					
1. Have you had prior surgery or an operation (e.g., arthroscopy, endoscopy, etc.) of any kind?				<input type="checkbox"/> No	<input type="checkbox"/> Yes
If yes, please indicate the date and type of surgery:					
Date ____/____/____		Type of surgery _____			
Date ____/____/____		Type of surgery _____			
2. Have you had a prior diagnostic imaging study or examination (MRI, CT, Ultrasound, X-ray, etc.)?				<input type="checkbox"/> No	<input type="checkbox"/> Yes
If yes, please list:      Body part      Date      Facility					
MRI	_____	____/____/____	_____	_____	_____
CT/CAT Scan	_____	____/____/____	_____	_____	_____
X-Ray	_____	____/____/____	_____	_____	_____
Ultrasound	_____	____/____/____	_____	_____	_____
Nuclear Medicine	_____	____/____/____	_____	_____	_____
Other	_____	____/____/____	_____	_____	_____
3. Have you experienced any problem related to a previous MRI examination or MR procedure?				<input type="checkbox"/> No	<input type="checkbox"/> Yes
If yes, please describe: _____					
4. Have you had an injury to the eye involving a metallic object or fragment (e.g., metallic slivers, shavings, foreign body, etc.)?				<input type="checkbox"/> No	<input type="checkbox"/> Yes
If yes, please describe: _____					
5. Have you ever been injured by a metallic object or foreign body (e.g., BB, bullet, shrapnel, etc.)?				<input type="checkbox"/> No	<input type="checkbox"/> Yes
If yes, please describe: _____					
6. Are you currently taking or have you recently taken any medication or drug?				<input type="checkbox"/> No	<input type="checkbox"/> Yes
If yes, please list: _____					
7. Are you allergic to any medication?				<input type="checkbox"/> No	<input type="checkbox"/> Yes
If yes, please list: _____					
8. Do you have a history of asthma, allergic reaction, respiratory disease, or reaction to a contrast medium or dye used for an MRI, CT, or X-ray examination?				<input type="checkbox"/> No	<input type="checkbox"/> Yes
9. Do you have anemia or any disease(s) that affects your blood, a history of renal (kidney) disease, renal (kidney) failure, renal (kidney) transplant, high blood pressure (hypertension), liver (hepatic) disease, a history of diabetes, or seizures?				<input type="checkbox"/> No	<input type="checkbox"/> Yes
If yes, please describe: _____					
<b>For female patients:</b>					
10. Date of last menstrual period: ____/____/____		Post menopausal?		<input type="checkbox"/> No	<input type="checkbox"/> Yes
11. Are you pregnant or experiencing a late menstrual period?				<input type="checkbox"/> No	<input type="checkbox"/> Yes
12. Are you taking oral contraceptives or receiving hormonal treatment?				<input type="checkbox"/> No	<input type="checkbox"/> Yes
13. Are you taking any type of fertility medication or having fertility treatments?				<input type="checkbox"/> No	<input type="checkbox"/> Yes
If yes, please describe: _____					
14. Are you currently breastfeeding?				<input type="checkbox"/> No	<input type="checkbox"/> Yes

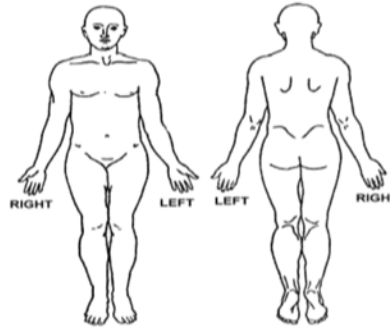


**WARNING:** Certain implants, devices, or objects may be hazardous to you and/or may interfere with the MR procedure (i.e., MRI, MR angiography, functional MRI, MR spectroscopy). Do not enter the MR system room or MR environment if you have any question or concern regarding an implant, device, or object. Consult the MRI Technologist or Radiologist **BEFORE** entering the MR system room. The MR system magnet is **ALWAYS** on.

**Please indicate if you have any of the following:**

- Yes  No Aneurysm clip(s)
- Yes  No Cardiac pacemaker
- Yes  No Implanted cardioverter defibrillator (ICD)
- Yes  No Electronic implant or device
- Yes  No Magnetically-activated implant or device
- Yes  No Neurostimulation system
- Yes  No Spinal cord stimulator
- Yes  No Internal electrodes or wires
- Yes  No Bone growth/bone fusion stimulator
- Yes  No Cochlear, otologic, or other ear implant
- Yes  No Insulin or other infusion pump
- Yes  No Implanted drug infusion device
- Yes  No Any type of prosthesis (eye, penile, etc.)
- Yes  No Heart valve prosthesis
- Yes  No Eyelid spring or wire
- Yes  No Artificial or prosthetic limb
- Yes  No Metallic stent, filter, or coil
- Yes  No Shunt (spinal or intraventricular)
- Yes  No Vascular access port and/or catheter
- Yes  No Radiation seeds or implants
- Yes  No Swan-Ganz or thermodilution catheter
- Yes  No Medication patch (Nicotine, Nitroglycerine)
- Yes  No Any metallic fragment or foreign body
- Yes  No Wire mesh implant
- Yes  No Tissue expander (e.g., breast)
- Yes  No Surgical staples, clips, or metallic sutures
- Yes  No Joint replacement (hip, knee, etc.)
- Yes  No Bone/joint pin, screw, nail, wire, plate, etc.
- Yes  No IUD, diaphragm, or pessary
- Yes  No Dentures or partial plates
- Yes  No Tattoo or permanent makeup
- Yes  No Body piercing jewelry
- Yes  No Hearing aid
- Yes  No *(Remove before entering MR system room)*
- Yes  No Other implant
- Yes  No Breathing problem or motion disorder
- Yes  No Claustrophobia

Please mark on the figure(s) below the location of any implant or metal inside of or on your body.



**IMPORTANT INSTRUCTIONS**

Before entering the MR environment or MR system room, you must remove all metallic objects including hearing aids, dentures, partial plates, keys, beeper, cell phone, eyeglasses, hair pins, barrettes, jewelry, body piercing jewelry, watch, safety pins, paperclips, money clip, credit cards, bank cards, magnetic strip cards, coins, pens, pocket knife, nail clipper, tools, clothing with metal fasteners, & clothing with metallic threads.

Please consult the MRI Technologist or Radiologist if you have any question or concern **BEFORE** you enter the MR system room.

**NOTE: You may be advised or required to wear earplugs or other hearing protection during the MR procedure to prevent possible problems or hazards related to acoustic noise.**

I attest that the above information is correct to the best of my knowledge. I read and understand the contents of this form and had the opportunity to ask questions regarding the information on this form and regarding the MR procedure that I am about to undergo.

Signature of Person Completing Form: \_\_\_\_\_ Date \_\_\_\_/\_\_\_\_/\_\_\_\_  
Signature

Form Completed By:  Patient  Relative  Nurse \_\_\_\_\_  
Print name Relationship to patient

Form Information Reviewed By: \_\_\_\_\_  
Print name Signature

MRI Technologist  Nurse  Radiologist  Other \_\_\_\_\_

## A.2 NIRS/DCS formulas

### NIRS for oxygenation measurement:

Total hemoglobin concentrations [tHb]:  $[tHb] = [HbO_2] + [Hb]$

Tissue blood oxygen saturation ( $S_tO_2$ ):  $S_tO_2 = 100\% \times ([HbO_2]/[tHb])$

### DCS for blood flow measurement:

Relative blood flow (rBF):  $rBF = (BFI)/(BFI_{baseline})$

### Hybrid NIRS/DCS measurement:

(1)  $VO_2 = BF \times ([O_2]_a - [O_2]_v)$

(2)  $OEF = ([O_2]_a - [O_2]_v)/[O_2]_a$

(3) combining equation (1) and (2):  $VO_2 = OEF \times BF \times ([O_2]_a)$

(4) relative change (rVO<sub>2</sub>, rOEF, rBF, (r[O<sub>2</sub>]<sub>a</sub>)) can be determined by dividing the baseline value:  $rVO_2 = rOEF \times rBF \times (r[O_2]_a)$

(5) assuming [O<sub>2</sub>]<sub>a</sub> is constant:  $rVO_2 = rOEF \times rBF$

(6)  $OEF = (S_aO_2 - S_tO_2)/(\gamma \times S_aO_2)$

(7) assuming  $\gamma$  is constant and  $S_aO_2$  is constant at 100%:

$rOEF = (1 - S_tO_2) / (1 - S_tO_{2 \text{ baseline}})$

(8)  $rVO_2 = rBF \times (1 - S_tO_2) / (1 - S_tO_{2 \text{ baseline}})$

Abbreviation	
BFI	Blood flow index
[O <sub>2</sub> ] <sub>a</sub>	Arterial oxygen concentration
[O <sub>2</sub> ] <sub>v</sub>	Venous oxygen concentration
OEF	Oxygen extraction fraction
VO <sub>2</sub>	Oxygen consumption rate
$\gamma$	% of blood volume contained in the venous compartment of the vascular system

### A.3 Medical History Questionnaire

- YES NO 1. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure, heart, or any other condition?

Medicines/dosage:

Taken For:

---

---

- YES NO 2. Do you know of any other reason (such as any neuromuscular or metabolic disorders, eg diabetes, breathing issues, asthma or emphysema) why you should not do physical activity?

List:

---

---

---

- YES NO 3. Are you currently seeing your doctor or health care provider for any reason? If yes, please explain any findings (heart murmur, abnormal pulmonary findings etc):

List:

---

---

---

- YES NO 4. Do you currently smoke (or have a history of smoking) or use tobacco products? When did you last quit?

---



---

YES NO

5. Is there any family history of pulmonary, metabolic disease, stroke, or sudden death?

If yes, please list:

---

---

---

YES NO

6. Any other known allergies (medications, food, latex, etc)

If yes, please list:

---

---

---

YES NO

7. Do you have any chronic illnesses or known viruses (hepatitis, HIV) etc?

If yes, please explain:

---

---

---

YES NO

8. Have you had any surgeries of any kind?

If yes, please list type, reason and year:

---

---

---

YES NO

9. Are you taking any over the counter medications, dietary supplements (vitamins or minerals (multivitamins)?  
Please list both type/dosage and reason for taking

---

---

---

#### A.4 Physical Activity Readiness questionnaire (PAR-Q)

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly; circle YES or NO.

YES NO 1. Has your doctor ever said that you have a heart condition/high blood pressure and that you should only do physical activity recommended by a doctor?

YES NO 2. Do you feel pain (tingling, heaviness, burning, tightness, squeezing) in your chest when you do physical activity?

List any heart condition here:

---

YES NO 3. In the past month, have you had chest pain when you were not doing physical activity?

YES NO 4. Do you lose your balance because of dizziness or do you ever lose consciousness?

YES NO 5. Do you have an orthopedic (bone or joint) problems that could be made worse by a change in your physical activity?

## A.5 AHA/ACSM Health/Fitness Screening Questionnaire

### AHA/ACSM Health/Fitness Facility Preparticipation Screening Questionnaire

Assess your health status by marking all *true* statements

---

#### History

You have had:

- a heart attack
- heart surgery
- cardiac catheterization
- coronary angioplasty (PTCA)
- pacemaker/implantable cardiac
- defibrillator/rhythm disturbance
- heart valve disease
- heart failure
- heart transplantation
- congenital heart disease

If you marked any of these statements in this section, consult your physician or other appropriate health care provider before engaging in exercise. You may need to use a facility with a **medically qualified staff**.

#### Symptoms

- You experience chest discomfort with exertion.
- You experience unreasonable breathlessness.
- You experience dizziness, fainting, or blackouts.
- You take heart medications.

#### Other health issues

- You have diabetes.
  - You have asthma or other lung disease.
  - You have burning or cramping sensation in your lower legs when walking short distances.
  - You have musculoskeletal problems that limit your physical activity.
  - You have concerns about the safety of exercise.
  - You take prescription medication(s).
  - You are pregnant.
- 

#### Cardiovascular risk factors

- You are a man older than 45 years.
  - You are a woman older than 55 years, have had a hysterectomy, or are postmenopausal.
  - You smoke, or quit smoking within the previous 6 months.
  - Your blood pressure is >140/90 mm Hg.
  - You do not know your blood pressure.
  - You take blood pressure medication.
  - Your blood cholesterol level is > 200 mg/dL.
  - You do not know your cholesterol level.
  - You have a close blood relative who had a heart attack or heart surgery before age 55 (father or brother) or age 65 (mother or sister).
  - You are physically inactive (i.e., you get <30 minutes of physical activity on at least 3 days per week).
  - You are > 20 pounds overweight.
- 

If you marked two or more of the statements in this section you should consult your physician or other appropriate health care provider before engaging in exercise. You might benefit from using a facility with a professionally qualified exercise staff to guide your exercise program.

- None of the above

You should be able to exercise safely without consulting your physician or other appropriate health care provider in a self-guided program or almost any facility that meets your exercise program needs.

## A.6 Physical Activity Questionnaire

In terms of physical demands, how would you rate your lifestyle?

- Sedentary
- Slightly Active
- Active
- Very Active

In terms of physical or muscular demands, how would you rate your workplace?

- Sedentary
- Slightly Active
- Active
- Very Active
- Not Applicable

Are you currently exercising?

- YES
- NO

What type of exercise do you participate in regularly?

- Resistance
- Cardio
- Resistance and Cardio

How many times per week do you participate in these types of exercise?

---

If resistance training, please briefly describe your typical workout (full body, one muscle group per day, intensity, duration etc):

---

Please list any other activities below:

---

## A.7 GXT Data Collection Form

Subject id: \_\_\_\_\_ Date: \_\_\_\_\_

Resting Data:

HR	BP	Angina?

Graded Exercise Testing

Stage	Workload		Min	HR	BP	RPE			Angina?
	Speed	Incline				Chest	Legs	Overall	
1	2.2	0%	0-2						
2	2.6	0%	2-4						
3	3.0	2	4-6						
4	3.4	4	6-8						
5	3.8	6	8-10						
6	4.2	8	10-12						
7	4.6	10	12-14						
8	5.0	12	14-16						
9	5.4	14	16-18						
10	5.8	16	18-20						

Recovery

Workload Speed/Incline		Min	HR	BP	Angina?
0	0%	1			
0	0%	3			
0	0%	5			

**Notes:**

**Form completed by:** \_\_\_\_\_

**Testing completed by:** \_\_\_\_\_

**Notes:** \_\_\_\_\_

## A.8 Aerobic Training Data Collection Form

Subject Id:

Date/Time:

Session:

Weight:

Target heart rate:

Study personnel:

BP before AT:

BP after AT:

HR before AT:

HR after AT:

Min	Workload Speed/Incline		Average HR	Comments



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## Vita

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## Vita

Department of Pharmacology and Nutritional Sciences  
University of Kentucky

### Education

2006-2010 Michigan State University, East Lansing, Michigan  
Bachelor of Science, Nutritional Sciences  
2010-2011 University of Kentucky, Lexington Kentucky  
Master of Science in Nutritional Sciences

### RESEARCH

2012-2016 Graduate Research Assistant, University of Kentucky  
Title: Vitamin D Contribution to Muscle Metabolic Function in Aged Adults  
Mentor: D. Travis Thomas, Clinical Sciences, College of Health Sciences

### GRANTS AND AWARDS

2006 Academic Competitiveness, Michigan State University  
2006-2008 Michigan MEAP Scholar, Michigan State University  
2009-2010 Federal National SMART Grant, Michigan State University  
2010 Bradford-Adams Endowed Scholarship for Chemistry, MSU  
2013 1st place: Poster Presentation: Epidemiology Research of Vitamins and Minerals in the Vitamin and Mineral Research Interest Section of the American Society of Nutrition at the Experimental Biology Conference, Boston, MA  
2011-2014 Thornton Scholarship, University of Kentucky  
2014 Tracy Farmer Institute for Sustainability and the Environment Promoting Healthy Lifestyle in Targeted Minority Setting, University of Kentucky  
2014 Nutrition Intervention to Reduce Symptoms in Patients with Advanced Heart Failure, Lennie (PI), University of Kentucky  
2014 Vitamin D Contribution to Muscle Metabolic Function in Aged Adults, Thomas (PI), University of Kentucky  
2015 Human Health Sciences Certificate of Appreciation in Mentorship, University of Kentucky  
2015 College of Health Sciences' Robinson Graduate Award for Research Creativity, University of Kentucky

2014-2016 T32 Nutrition and Oxidative Stress Training Fellowship,  
University of Kentucky

## PUBLICATIONS

- 2013 Redzic M, Lewis RM, Thomas DT. Relationship between 25-hydroxyvitamin D, muscle strength, and incidence of injury in healthy adults: a systematic review. *Nutrition Research*. 2013;33(4):251-8.
- 2013 Lewis RM, Redzic M, Thomas DT. The effects of season-long vitamin D supplementation on collegiate swimmers and divers. *International Journal of Sport Nutrition and Exercise Metabolism*. 2013;23(5):431-40
- 2014 Thomas D, Redzic M. Vitamin D and Athletes, Part I: A Growing Interest. *Athletic Training and Sports Health Care*. 2014; 6(1):5-6.
- 2014 Thomas DT, Redzic M. Vitamin D and Athletes, Part II: Vitamin D Relationship to Injury & Clinical Recommendations. *Athletic Training and Sports Health Care* 2014; 6:(2):53-54
- 2014 Redzic M, DK Powell, DT Thomas. Vitamin D Status is Related to Intramyocellular Lipid in Older Adults. *Endocrine* 2014; 47(3):854-61
- 2015 Cassity EP, Redzic M, Teager CR, Thomas DT. The effect of body composition and BMI on 25(OH)D response in vitamin D-supplemented athletes. *European Journal of Sport Science*. 2015; 1-7.

## ABSTRACTS

- 2012 Lewis RM, Redzic M, Shores PB, Thomas DT. 25(OH)D Status is not Related to Bone Mineral Density or Inflammatory Cytokines in Collegiate Athletes. *Medicine & Science in Sport & Exercise*, 2012; 44(5)S pp. S721.
- 2013 Redzic M, Powell, DK, Thomas, DT. Vitamin D status is related to intramyocellular lipid in older adults. *The Journal of the Federation of American Societies for Experimental Biology* 2013; 27:635.7.
- 2013 Lewis RM, Redzic M, Thomas DT. The Effects of Season-long Vitamin D Supplementation on Collegiate Swimmers and Divers. Center for Clinical and Translational Sciences, University of Kentucky, 2013.
- 2014 Achenjang NS, Pampati S, Redzic M, Lutz CT, Powell DK, Thomas DT. Vitamin D Status, Physical Function, and Muscle Lipid in Aged Adults. National Conference on Undergraduate Research, University of Kentucky, 2014.
- 2014 Redzic M, DK Powell, CT Lutz, DT Thomas. Differences in extramyocellular lipid and physical function in older adults with sufficient and insufficient vitamin D status. *Journal of the Federation of American Societies for Experimental Biology* 2014; 28:260.2.

- 2014 Redzic M, Cassity E, Hawes C, Thomas DT, Bruckner G. A Pilot Study Promoting Healthy Lifestyles in a Targeted Minority Setting. Tracy Farmer Institute for Sustainability and the Environment 4th Annual Research Showcase, University of Kentucky, 2014.
- 2015 Teager C, Redzic M, Cassity E, Thomas DT. Differences in Bone Turnover and 25(OH)D Change in Indoor Athletes. The Journal of the Federation of American Societies for Experimental Biology 2015; 29:733.2.
- 2015 Redzic M, Thomas DT. The Contribution of Dietary Intake Patterns and Physical Activity Levels to Muscle Lipid in Aged Individuals. Center for Clinical and Translational Sciences Conference, University of Kentucky, 2015.
- 2015 Redzic M, Thomas DT. The Contribution of Dietary Intake Patterns and Physical Activity Levels to Muscle Lipid in Aged Individuals. Barnstable Brown Obesity and Diabetes Research Day, University of Kentucky, 2015.
- 2016 Thomas DT, Redzic M, Zhao M, White M, and Yu G. Changes in Muscle Lipid and rVO<sub>2</sub> following Vitamin D Repletion and Aerobic Training in Aged Individuals. American College of Sports Medicine Conference, 2016.
- 2016 Redzic M, Mitchell LV, Thomas DT. Unsaturated to Saturated Fatty Acid Intake Ratio is Inversely Associated with Intramyocellular Lipid in Healthy, Aged Individuals. Center for Clinical and Translational Sciences Conference, University of Kentucky, 2016.
- 2016 Redzic M, Mitchell LV, Thomas DT. Unsaturated to Saturated Fatty Acid Intake Ratio is Inversely Associated with Intramyocellular Lipid in Healthy, Aged Individuals. Barnstable Brown Obesity and Diabetes Research Day, University of Kentucky, 2016.
- 2016 Redzic M, Mitchell LV, Thomas DT. The Relationship between Fat Intake, Physical Activity, and Muscle Lipid in Aged Individuals. American College of Sports Medicine Conference, 2016.
- 2016 McLean H, Redzic M, Thomas DT. Higher Unsaturated Fatty Acid Intake and Aerobic Training are Related with Lower Intramyocellular Lipid in Older Adults. Posters-at-the-Capitol in Frankfort, KY 2016

#### SEMINAR PRESENTATIONS

- 2013 Vitamin D Contribution to Muscle Metabolic Function in the Elderly. Muscle Forum for the Center of Muscle Biology, University of Kentucky
- 2013 Vitamin D Contribution to Muscle Metabolic Function in the Elderly. Graduate Center For Nutritional Sciences Seminar, University of Kentucky

- 2014 Differences in extramyocellular lipid and physical function in older adults with sufficient and insufficient vitamin D status. Experimental Biology Conference, San Diego, CA
- 2015 The relationship between vitamin D and muscle health. Department of Pharmacology and Nutritional Sciences Seminar Series, University of Kentucky
- 2015 Influence of vitamin D repletion, diet composition, and physical activity on muscle lipid distribution in healthy aged individuals. Muscle Forum for the Center of Muscle Biology, University of Kentucky
- 2016 Influence of vitamin D repletion, diet composition, and physical activity on muscle lipid distribution in healthy aged individuals. Department of Pharmacology and Nutritional Sciences Seminar Series, University of Kentucky

#### MENTORING/TEACHING ACTIVITY

- 2011-2016 Taught and mentored 9 undergraduate and 16 master's students, University of Kentucky
- 2016 Nutrition Counseling and Interviewing Techniques, Wellness and Sports Nutrition (NS 605), University of Kentucky
- 2016 Nutritional Consultation lecture and lab, Nutrition and Physical Fitness (KHP 240), University of Kentucky
- 2015 Seminar in Interprofessional Healthcare (HHS356), University of Kentucky
- 2015 Nutrition Counseling and Interviewing Techniques, Wellness and Sports Nutrition (NS 605), University of Kentucky