Inflammaging: Inflammatory Biomarkers Associated With Nutrition, Metabolism And Muscle Tissue Oxygenation Relative To Sarcopenic Status In Older Adults

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INFLAMMAGING: INFLAMMATORY BIOMARKERS ASSOCIATED WITH NUTRITION, METABOLISM, AND MUSCLE TISSUE OXYGENATION RELATIVE TO SARCOPENIC STATUS IN OLDER ADULTS

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INFLAMMAGING: INFLAMMATORY BIOMARKERS ASSOCIATED WITH NUTRITION, METABOLISM AND MUSCLE TISSUE OXYGENATION RELATIVE TO SARCOPENIC STATUS IN OLDER ADULTS

by

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DISSERTATION

Presented to the Faculty of the Graduate School of The University of Texas at El Paso in Partial Fulfillment of the Requirements for the Degree of

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ABSTRACT

The purpose of this study is to examine if we can differentiate inflammaging between sarcopenic and non-sarcopenic older adults by exploring if there are differences between biomarkers of inflammation and nutritional status in older adults with distinct differences in skeletal muscle mass, strength, and function, metabolic flexibility, and muscle tissue oxygenation. Twenty-one non-sarcopenic (mean±standard deviation, age = 73.1±6.2 yrs, n=5 males, n=5 females) and sarcopenic (age = 81.2±10.5 yrs, n=6 males, n=5 females) were categorized based on muscle mass and strength criteria according to the European Working Group on Sarcopenia in Older People. Body composition, handgrip and leg extension strength, physical performance tests, metabolism, and muscle tissue oxygenation were previously collected. Fasting blood samples were collected for analysis of inflammatory and nutritional biomarkers. Sarcopenic individuals had 45% and 89% higher concentrations of IL-1ra and MIP-1β, respectively, compared to non-sarcopenic. These differences disappeared when normalized to fat mass (FM). Sarcopenic females had 54% greater factor VII concentrations than sarcopenic males. When normalized to FM, I-CAM was 23% higher in non-sarcopenic individuals compared to sarcopenic, and AAT, IgM, VEGF, and VDBP were higher in males compared to females. Ferritin was 242% higher in non-sarcopenic males compared to non-sarcopenic females, and non-sarcopenic older adults had 46% greater IGF-1 concentrations than sarcopenic, with differences eliminated when normalizing by FM. While there were no other differences between groups for nutritional status biomarkers, deficiencies in iron and vitamin D were more prevalent in sarcopenic older adults. These results suggest there are inflammatory and nutritional differences between non-sarcopenic and sarcopenic older adults that is influenced by adipose tissue. Previously, this study population demonstrated distinct differences in metabolic flexibility and energy metabolism and muscle perfusion. Therefore, these distinguishing factors, along with the differences reported in
inflammation and nutritional status may be contributory to development of inflammaging with sarcopenia.
# TABLE OF CONTENTS

**ACKNOWLEDGMENTS** ................................................................................................................................. iii  
**ABSTRACT** ................................................................................................................................................ iv  
**LIST OF FIGURES** ........................................................................................................................................ vii  
**LIST OF TABLES** ........................................................................................................................................... ix  
**CHAPTER I: INTRODUCTION** ..................................................................................................................... 1  
**CHAPTER II: REVIEW OF LITERATURE**  
1) Inflammation with Aging and Sarcopenia................................................................................................. 19  
2) Muscle Blood Flow, Muscle Oxygenation, and Near-infrared Spectroscopy with Aging  
   and Metabolic Dysfunction ......................................................................................................................... 43  
3) Metabolic Flexibility and Muscle Metabolism with Aging......................................................................... 55  
4) Vitamin D Status with Aging and Sarcopenia ............................................................................................... 65  
5) Vitamin D and Inflammation and Immune Function .................................................................................. 101  
6) Iron Status and Anemia with Aging and Sarcopenia ..................................................................................... 109  
7) Interrelationship between Iron Status, Vitamin D Status, and Inflammation ............................................ 122  
**CHAPTER III: METHODS** ......................................................................................................................... 135  
**CHAPTER IV: RESULTS** ........................................................................................................................... 146  
**CHAPTER V: DISCUSSION**  
Summary .......................................................................................................................................................... 149  
Inflammation .................................................................................................................................................. 150  
Nutritional Status ........................................................................................................................................... 156  
Conclusions .................................................................................................................................................... 160  
Limitations ..................................................................................................................................................... 161  
Future Directions ........................................................................................................................................... 162  
**FIGURES** .................................................................................................................................................... 163  
**TABLES** ................................................................................................................................................... 175  
**GLOSSARY** .................................................................................................................................................. 187  
**REFERENCES** ............................................................................................................................................ 190  
**CURRICULUM VITA** .................................................................................................................................. 244
LIST OF FIGURES

Figure 1. Sarcopenia is a multi-factorial condition that is influenced by integrating, contributing factors. Examining multiple factors is causing or exacerbating the risk of developing sarcopenia may provide insight on strategies for prolonging the progression of sarcopenia.......................163

Figure 2. Study design and main outcomes of the original clinical trial (BL39: A pilot study to explore muscle energy metabolism and metabolic flexibility in older men and women, clinicaltrials.gov NCT03701878) and how the variables tested retrospectively may relate to the previous findings.................................................................164

Figure 3. Means ± SD for vitamin D binding protein (VDBP) in non-sarcopenic males, non-sarcopenic females, sarcopenic males, and sarcopenic females..................................................165

Figure 4. Means ± SD for interleukin-1 receptor antagonist (IL-1ra) in non-sarcopenic males, non-sarcopenic females, sarcopenic males, and sarcopenic females.................................166

Figure 5. Means ± SD for macrophage inflammatory protein-1 beta (MIP-1β) in non-sarcopenic males, non-sarcopenic females, sarcopenic males, and sarcopenic females.......................167

Figure 6. Means ± SD for Factor VII in non-sarcopenic males, non-sarcopenic females, sarcopenic males, and sarcopenic females.................................................................168

Figure 7. Means ± SD for intercellular adhesion molecule 1 (ICAM-1) normalized to fat mass (FM) in non-sarcopenic males, non-sarcopenic females, sarcopenic males, and sarcopenic females........................................................................169

Figure 8. Means ± SD for alpha-1-antitrypsin (AAT) normalized to fat mass (FM) in non-sarcopenic males, non-sarcopenic females, sarcopenic males, and sarcopenic females.........170
Figure 9. Means ± SD for immunoglobulin (IgM) normalized to fat mass (FM) in non-sarcopenic males, non-sarcopenic females, sarcopenic males, and sarcopenic females.................................171

Figure 10. Means ± SD for vascular endothelial growth factor (VEGF) normalized to fat mass (FM) in non-sarcopenic males, non-sarcopenic females, sarcopenic males, and sarcopenic females..........................................................................................................................172

Figure 11. Means ± SD for ferritin in non-sarcopenic males, non-sarcopenic females, sarcopenic males, and sarcopenic females..........................................................................................................................173

Figure 12. Means ± SD for insulin growth factor 1 (IGF-1) in non-sarcopenic males, non-sarcopenic females, sarcopenic males, and sarcopenic females.........................................................174
LIST OF TABLES

Table 1. Inflammatory (37) and nutritional (5) biomarkers analyzed in non-sarcopenic and sarcopenic older adults.................................................................175

Table 2. Concentrations of inflammatory biomarkers that were above the lower limit of quantitation in individual participants of biomarkers excluded from statistical analysis due to a majority of participants being below the detectable range. .........................................................177

Table 3. Body composition, muscle function, and muscle strength characteristics of participants that had inflammatory biomarker concentrations that were above the lower limit of quantitation of the biomarkers excluded from statistical analysis due to a majority of participants being below the detectable range........................................................................................................................178

Table 4. Means ± standard deviations (SD) for baseline demographics, anthropometrics, body composition, strength, and repetitions to failure of non-sarcopenic (n=10) and sarcopenic (n=11) older adults. P-values are type I errors of the interactions and main effects..............................179

Table 5. Means ± standard deviations (SD) for inflammatory biomarkers of non-sarcopenic (n=10) and sarcopenic (n=11) older adults. P-values are type I errors between groups.............181

Table 6. Means ± standard deviations (SD) for inflammatory biomarkers normalized to fat mass of non-sarcopenic (n=10) and sarcopenic (n=11) older adults. P-values are type I errors between groups.............................................................................................................................183

Table 7. Means ± standard deviations (SD) for nutritional biomarkers of non-sarcopenic (n=10) and sarcopenic (n=11) older adults. The number of individuals in each group with low values of each biomarker are reported in parentheses. P-values are type I errors between groups..............185
Table 8. Means ± standard deviations (SD) for nutritional biomarkers normalized to fat mass of non-sarcopenic (n=10) and sarcopenic (n=11) older adults. P-values are type I errors between groups.
CHAPTER 1: INTRODUCTION

Sarcopenia is the age-related loss of muscle mass, strength, and function which is theorized to be a multi-factorial condition. Muscle mass and strength significantly decline after age 45 years, and this rate of decline increases further after the age of 65 years. Additionally, muscular strength declines at a greater degree than muscle mass. Approximately 3 – 24% of older adults age 65 years or older are sarcopenic, indicating the importance of identifying interacting, contributory mechanisms, such as metabolic dysfunction, muscle perfusion, muscle disuse or inactivity, inflammation, and nutritional status (Figure 1), leading to this multi-factorial condition. Identifying and developing interventions to reduce the influence of interacting, contributory mechanisms older adults with sarcopenia will have clinical implications to reduce the decline in muscle mass, strength, and function.

It has been well established that a decline in muscle mass, strength and/or function contributes to an increased risk for falls, reduction in ability to perform activities of daily living (ADLs), quality of life, hospitalization, and mortality. More recently, there has been a greater focus on the relationships between the age-related declines in skeletal muscle with chronic inflammation, immune function, blood perfusion and delivery of oxygen to tissues, and metabolism and/or mitochondrial function. These factors are likely closely integrated, thus compounding the effects on muscle mass and strength. In particular, inflammation seems to be a prominent influence on the other factors related to sarcopenia. The aging process is characterized by an increase in oxidative stress and chronic inflammation that infiltrates skeletal muscle cells, thus, contributing to the development of sarcopenia. Sarcopenia may be accelerated by the presence of inflammatory pathway activation due to chronic disease.
conditions. However, it is unknown if inflammation is associated with differences in skeletal health, including metabolic health and muscle perfusion capabilities in an aging population.

Skeletal muscle is important for many metabolic functions including glucose uptake and insulin sensitivity, indicating that loss of muscle mass can result in metabolic shifts. In older adults with impaired metabolic function, insulin-resistance like symptoms, including impaired glucose metabolism and higher rates of free fatty acid (FFA) mobilization occur. Additionally, fat oxidation was reduced in older adults, with muscle mass at least partly contributing to this finding. However, until recently, it was unknown if metabolic function differed between non-sarcopenic and sarcopenic older adults. Recent evidence suggests that sarcopenic older adults experienced signs of metabolic inflexibility, with an inability to shift fuel source utilization based on demand. Furthermore, sarcopenia indicated an increased reliance on carbohydrate (CHO) oxidation and higher fasting glucose concentrations in comparison to non-sarcopenic older adults. Non-sarcopenic older adults also had greater fat utilization fasting, post-prandial, and during aerobic exercise, with an increase in CHO oxidation during anaerobic exercise to meet the increased energy demands. These findings indicate insulin resistance-like characteristics in sarcopenic older adults. Since skeletal muscle accounts for 80-90% of glucose uptake, it is postulated that muscle disuse may lead to symptoms similar to insulin resistance and to the development of metabolic inflexibility.

With an impairment in the action of insulin to stimulate muscle protein synthesis, development of sarcopenia driven by metabolic dysfunction is favored, resulting in muscle atrophy. Additionally, insulin resistance and metabolic dysfunction are typically observed along with inflammation. For example, presence of hyperglycemia, which is common in those who are metabolically inflexible, as well as amino acid resistant, resulted in an increase in circulating
inflammatory markers such as Interleukin (IL)-6 and Tumor Necrosis Factor (TNF)-α. This relationship between inflammatory markers and insulin resistance suggests that insulin resistance and glucose intolerance may stimulate metabolic dysfunction related to chronic inflammation that may be more observable in those with sarcopenia.

The role of inflammation has been explored in both protein metabolism and in aging, with conflicting evidence, indicating mechanisms underlying chronic low-grade inflammation and the development of sarcopenia should be further explored. For example, Cooney et al. reported that IL-1 infusion decreased protein synthesis rate and an increase in protein breakdown of skeletal muscle. Furthermore, skeletal muscle decline could occur through increased inflammation pathway effects on muscle protein synthesis. However, Buffière et al. indicated that chronically elevated levels of C-Reactive Protein (CRP) did not affect muscle or whole body protein metabolism in older males. These studies indicate the need to examine inflammatory status in relation to skeletal muscle and metabolic function.

Metabolic dysfunction occurs with age and may be related to muscle mass and strength due to the pivotal role muscle has in energy expenditure and glucose uptake. Metabolic impairments due to the decline in muscle mitochondrial function with age may be more likely to occur in older adults who are less active and undergo less muscle contractile stimulus, thus more likely to be sarcopenic. Near-infrared spectroscopy (NIRS) has demonstrated the ability to detect vascular responsiveness in response to a hyperglycemia challenge, indicating the usability of NIRS for examining metabolic differences. Recently, Soares et al. demonstrated detectable differences in vascular responsiveness with NIRS between lean and obese individuals after glucose consumption. Additionally, obese individuals exhibited decreased muscle oxygen utilization after glucose consumption when compared to lean
individuals,\textsuperscript{50} emphasizing the potential NIRS has for observing metabolic flexibility. With evidence supporting the capability of NIRS to observe differences in muscle tissue oxygenation parameters in lean versus obese individuals,\textsuperscript{48–50} differences in metabolic flexibility may also be observable between non-sarcopenic and sarcopenic adults to provide insight on how metabolic flexibility is related to reduced muscle blood flow, thus influencing the nutritional and inflammatory status of older adults.

The age-related increase in vascular and endothelial dysfunction, and resultant decrease in muscle health, may also be underlying mechanisms to the development of metabolic impairment.\textsuperscript{51} Additionally, for optimal muscle function, adequate oxygenation and delivery of nutrients to the muscle is necessary. Aging is associated with a decline in macrovascular and microvascular blood flow, which may have a pronounced effect on muscle health. This reduction in blood flow with aging has been related to endothelial dysfunction\textsuperscript{52} and lead to a reduction in nutrient delivery to the muscle.\textsuperscript{51,53} Age-related decline in muscle blood perfusion may be more pronounced in individuals with lower muscle mass.\textsuperscript{24,54,55} For example, Dinenno and colleagues\textsuperscript{24} reported an association between age-related decline in blood flow and a decline in leg fat-free mass (FFM) and estimated leg muscle oxygenation.\textsuperscript{24} These findings suggest that those with reduced muscle blood flow, thus decreasing the transport of oxygen and other nutrients to the muscle, are more likely to have lower muscle mass. Furthermore, leg muscle oxygenation was found to explain more than 50\% of age-related muscle blood flow, suggesting that older adults, particularly those with less leg FFM, may have reduced oxygen perfusion.\textsuperscript{54} Less muscle blood flow may also result in less oxygenated hemoglobin, again suggesting the lack of oxygen delivery to the muscle to match oxygen demand.\textsuperscript{56} These early studies suggest that sarcopenia may result, at least partially, from decreased muscle blood flow resulting in diminished nutrient
delivery to the muscle, leading to lower anabolic adaptation to the muscle.\textsuperscript{54,56} While exercise has been shown to increase nutrient delivery and an anabolic response to amino acids and carbohydrates,\textsuperscript{53} it is unclear if exercise alone will be effective in those who already experience lower skeletal muscle capillarization and flow capabilities,\textsuperscript{57,58} such as sarcopenic older adults.

Near-infrared spectroscopy has recently been utilized as a non-invasive, cost-efficient method to examine microvascular function, muscle tissue oxygenation, and reflect muscle blood flow.\textsuperscript{59–63} The NIRS device measures relative concentration changes from baseline in oxygenated hemoglobin (Hb) + myoglobin (Mb) (O\textsubscript{2}Hb) and deoxygenated Hb + Mb (HHb). Muscle tissue oxygen saturation (StO\textsubscript{2}) (%) is measured using spatially-resolved spectroscopy to obtain absolute values. From the modified Lambert-Beer law, relative concentrations of O\textsubscript{2}Hb and HHb can be obtained and used to calculate total Hb + Mb (THb), where THb = O\textsubscript{2}Hb + HHb.\textsuperscript{64,65} These variables have been demonstrated to represent oxygen delivery and extraction to provide insight on diffusion and perfusion of skeletal muscle.\textsuperscript{66–68} Age-related declines in muscle oxidative function have been detectable with NIRS.\textsuperscript{69} However, it has been suggested that muscle mass, strength, and function, rather than simply age alone is determinant in vascular responsiveness observed with NIRS.\textsuperscript{70} Recently, differences in muscle tissue oxygenation between non-sarcopenic and sarcopenic older adults were observed at rest and during exercise, suggesting that sarcopenic older adults have reduced muscle perfusion and blood flow and decreased tissue oxygen saturation compared to their non-sarcopenic counterparts (manuscript in preparation). Specifically, those with sarcopenia exhibited a lack of change in THb of the vastus lateralis both post-prandial and during exercise (manuscript in preparation). It was hypothesized that compared to non-sarcopenic older adults, sarcopenic individuals experienced little muscle perfusion response to stimuli (manuscript in preparation), which not only suggests greater
endothelial dysfunction, but also a greater risk for muscle atrophy and a decrease in strength due to lack of nutritive flow to the muscle.\textsuperscript{34,71} Exploring differences in NIRS variables in non-sarcopenic and sarcopenic older adults will provide insight on the potential relationship between skeletal muscle and vascular functioning during the aging process, which may indirectly be influenced by inflammatory status through endothelial dysfunction. An increased pro-inflammatory state may be related to impaired nutrient delivery to skeletal muscle via blood flow, resulting in the accumulation of pro-coagulation factors, cell debris, and endothelial dysfunction resulting in impaired insulin sensitivity.\textsuperscript{72,73}

Nutritional status is another key contributor to sarcopenia. Adequate nutritional status has demonstrated beneficial effects on skeletal muscle,\textsuperscript{74,75} metabolic health,\textsuperscript{76} and inflammatory status.\textsuperscript{77} With malnutrition a probable cause of sarcopenia, this decline in nutritional status may exacerbate chronic inflammation in older adults. Thus, nutrition targeted for skeletal muscle maintenance may be a modifiable method to reduce inflammaging in sarcopenic older adults. Malnourished sarcopenic older adults provided with oral nutritional supplementation high in protein and vitamin D showed improvements in indicators of muscle health (total protein, myoglobin, insulin-like growth factor (IGF-1)), inflammation (IL-16, IL-6-receptor, TNF receptors 1,2), nutritional status (vitamin D, transferrin, vitamin B12), and immune function (Immunoglobulin (Ig)A, IgM).\textsuperscript{78} These findings indicate that adequate nutrition can improve many factors that contribute to sarcopenia.

Vitamin D is one key nutrient that has shown promising effects on skeletal muscle. Vitamin D is commonly deficient in older adults. In fact, prevalence of 25(OH)D deficiency and insufficiency was 4\% and 17.4\%, respectively, in US older adults.\textsuperscript{79} Vitamin D consists of two forms: ergocalciferol (vitamin D$_2$) and cholecalciferol (vitamin D$_3$). Ergocalciferol is found in
plants and fungi, whereas cholecalciferol can be synthesized in the skin by sunlight and is found in some animal source foods such as fortified dairy products, egg yolks, and fatty fish.\textsuperscript{80–83} Once synthesized or absorbed from food, vitamin D\textsubscript{3} is transported to the liver while bound to vitamin D-binding protein (VDBP). In the liver, the vitamin is converted to 25-hydroxycholecalciferol (25(OH)D), the form of vitamin D that is typically used as a measurement of vitamin D status. 25(OH)D is then transported to the kidney, where it is activated to form 1,25-dihydroxycholecalciferol [1,25(OH)\textsubscript{2}D], otherwise known as calcitriol, if needed for regulation of calcium and phosphate.\textsuperscript{84,85}

While known as a vitamin, vitamin D acts a hormone when regulating calcium and phosphorus. Vitamin D receptor (VDR) is found on many tissues in the body including skeletal muscle, brain, prostate, breast, and colon tissues, as well as immune cells, indicating the large influence vitamin D may have on multiple functions of the body and its link with multiple pathological diseases.\textsuperscript{86–90} Although many studies utilize different definitions, 25(OH)D levels < 20 ng·mL\textsuperscript{-1} (50 nmol·L\textsuperscript{-1}) have been defined as deficient and levels 30 - 60 ng·mL\textsuperscript{-1} (75 – 150 nmol ·L\textsuperscript{-1}) are defined as insufficient. Levels of > 75 nmol·L\textsuperscript{-1} are thought to be optimal for health\textsuperscript{91}; however recent evidence has suggested that levels of at least 100 nmol·L\textsuperscript{-1} may be necessary for older adults.\textsuperscript{92} Although the Recommended Daily Allowance (RDA) for vitamin D for individuals between ages 9 – 70 years is 600 IU·d\textsuperscript{-1},\textsuperscript{91} intakes between 1500 – 2000 IU·d\textsuperscript{-1} have been thought necessary to increase blood concentrations to above deficient levels.\textsuperscript{93} Due to insufficient intake,\textsuperscript{79,94} lack of exposure to sunlight,\textsuperscript{95,96} skin pigmentation,\textsuperscript{97,98} and inhibition of absorption,\textsuperscript{99–104} many individuals, particularly older adults, have a low vitamin D status. In particular, older adults are prone to lower levels due to lower bioavailability of a compound needed for synthesis in the skin. This, along with reduced nutrient intake, less time exposed to
sun, and body composition changes leading to higher amounts of adipose tissue,\textsuperscript{105} contribute to the increased risk of low vitamin D status with age.\textsuperscript{79,94,106}

Vitamin D has been hypothesized to have a role in inflammatory response by activation and differentiation of immune and inflammatory cells\textsuperscript{107,108} and decrease risk of infection and inflammation.\textsuperscript{109,110} Immunomodulatory benefits of vitamin D exist as a physical barrier, enhancement of antimicrobial peptides and defensins to improve cellular immunity, and reduction of a cytokine storm. As vitamin D binds to receptors of immune cells, immune response is initiated through regulation of cathelicidins and defensins, therefore reducing cytokine storms linked to infection. Furthermore, adequate levels of vitamin D has been shown to decrease the production of pro-inflammatory cytokines such as IL-12, interferon gamma (IFN-\(\gamma\)), IL-6, TNF-\(\alpha\), IL-17, IL-9 and increase the production of anti-inflammatory cytokines such as IL-4, IL-5, and IL-10.\textsuperscript{111–115}

Associations between vitamin D deficiency and inflammation have been found in older adults,\textsuperscript{116,117} providing support for the modulatory effects sufficient vitamin D levels can have in preventing chronic inflammation. Furthermore, supplementation of vitamin D has revealed beneficial effects on chronic inflammation.\textsuperscript{111–114,118,119} For example, 13 weeks of vitamin D and protein supplementation was effective in preventing increases in inflammatory cytokines compared to a placebo in older adults,\textsuperscript{119} and three months of vitamin D supplementation resulted in decreased levels of IL-6 and TNF-\(\alpha\) in older adults when compared to a placebo.\textsuperscript{111} This relationship, along with the vitamin D’s role in immune function leads to hypotheses that sufficient vitamin D status is essential for prevention and/or mitigation of severe outcomes associated with infections and chronic inflammatory diseases\textsuperscript{114,115,117,120} and may be associated with better outcomes after hospitalization.\textsuperscript{121,122} Recently, the potential role vitamin D may have
in risk reduction for respiratory tract infections, including SARS-CoV-2, has been reviewed, suggesting a mediating function for vitamin D in the cytokine storm that exacerbates infection, especially in vulnerable populations such as older adults (manuscript in press).

Furthermore, vitamin D has also shown a promising role in muscle health through *in vivo* and *in vitro* studies. In particular, the presence of VDR in skeletal muscle tissue may explain many of these theories between skeletal muscle function and vitamin D status. Expression of VDR on skeletal muscle is necessary for vitamin D uptake into muscle cells\textsuperscript{123}; however, VDR is mainly found on fast-twitch muscle fibers,\textsuperscript{124} which tend to decrease in proportion with age. Interestingly, there is evidence supporting increases in fast twitch muscle fiber size after vitamin D supplementation,\textsuperscript{125} indicating that VDR expression on fast-twitch muscle fibers may be an underlying mechanism for vitamin D deficiency with age. Additionally, it has been found that vitamin D supplementation increases VDR concentration in muscle tissue of older females with low vitamin D status,\textsuperscript{126} indicating that sufficient levels of vitamin D are essential for maintaining adequate uptake into muscle cells.

Vitamin D also has a role in calcium regulation, therefore affecting muscle energy metabolism and muscle contraction.\textsuperscript{127–130} Skeletal muscle cells have also experienced an enhanced oxygen consumption rate after treatment of 1\alpha,25(OH)\textsubscript{2},\textsuperscript{131} indicating the role vitamin D may have in the regulation of muscle metabolism and mitochondrial function. Additionally, vitamin D is thought to have a role in anabolic and catabolic pathways within skeletal muscle.\textsuperscript{132,133} Vitamin D also has a role in increasing IGF-1 concentrations to promote anabolism,\textsuperscript{134} further highlighting the potential for sufficient vitamin D status in skeletal muscle health.

Vitamin D levels have been beneficially associated with skeletal muscle mass and strength, compounding this nutrient’s potential role in the prevention and/or treatment of
sarcopenia. Studies in older adults,74,77,106,119,124,126,130,135–143 athletes,144,145 and youth,146,147 have shown relationships between vitamin D status and parameters of muscle mass, strength, and performance. Specifically, higher vitamin D levels have been associated with better performance in tests of physical performance such as walking speed, chair stand tests, and balance tests, increased handgrip strength,92,139,143,148,151–156 and lower extremity strength.142,154,155,157,158 Vitamin D levels have also been positively related to muscle mass,137,143,149,150,153,158 and shown to be effective in preserving muscle mass over time in adults ages 21 – 97 years old and in older women.159,160 Additionally, studies have reported beneficial effects of supplementation of vitamin D, leading to improvements in muscle mass, muscle strength,161 and muscle function in older adults.162 In fact, there is some evidence that vitamin D supplementation in older adults increased total muscle fiber cross-sectional area and VDR concentration,126 and had positive effects on muscle mitochondrial oxidative function,130 suggesting a potential mechanism of how vitamin D may improve muscle health. These findings indicate vitamin D status to be a preventative and therapeutic option for reducing the effects of sarcopenia.

Iron is another important micronutrient that has shown beneficial effects on performance and immune function.163–165 It has been long established that iron is a nutrient essential for muscle performance due to its role in red blood cell production, oxygen delivery, and electron transport during oxidative phosphorylation.167–169 Iron status has been associated with performance in youth athletes,170–172 adult athletes,163,164 and older adults,173–176 indicating its role in muscle function in a variety of populations. In particular, anemia has been associated with frailty and lower muscular mass and strength in older populations,175–178 indicating that iron status may be important for prevention of sarcopenia in older adults. Declines in dietary iron
intake has also been observed in older adults,\textsuperscript{179–181} further supporting the potential for iron status to have a role in the development of sarcopenia and inflammaging. Additionally, previous studies have indicated the consumption of iron-rich red meat, along with resistance training, has shown beneficial effects on muscle mass, muscle strength, and reduce inflammatory markers in older adults.\textsuperscript{182,183}

Biomarkers utilized to examine iron status have included ferritin, transferrin, transferrin saturation, and Hb concentrations. Ferritin has been reported to reflect body iron stores.\textsuperscript{184} Previously, it has been thought that high iron levels are related to inflammation and chronic disease due to associations between ferritin and inflammatory and metabolic markers.\textsuperscript{185} However, ferritin is an acute phase protein which increases in a state of inflammation.\textsuperscript{186} This may give a false perspective of sufficient or high iron status in the individual, particularly in older adults,\textsuperscript{187} in which iron deficiency may be masked by the high or normal ferritin levels.\textsuperscript{188} Altered iron status is actually prevalent in those in a chronic inflammatory state.\textsuperscript{189–191} Examining multiple biomarkers of iron status that include markers not influenced by inflammation, would provide more insight on the role iron deficiency may have in sarcopenia and inflammaging. For instance, transferrin increases when iron levels in cells are low and can be used to assess iron deficiency and erythropoiesis which allows for the distinction between iron-deficiency anemia and anemia due to inflammation.\textsuperscript{192,193} Transferrin saturation can also add value to examining iron status as a low saturation percentage may reflect an inadequate supply of iron needed for erythropoiesis.\textsuperscript{194} Hemoglobin is an iron-containing protein that largely composes red blood cells.\textsuperscript{195} Concentrations of Hb have been known to reflect anemia, which could be in the form of nutrition-related anemia or anemia of chronic inflammation. This marker is an important health indicator and can provide valuable information regarding the severity of
iron deficiency when measured in conjunction with other markers. Additionally, low Hb levels and/or anemia have been associated with inflammatory markers, indicating that the combination of anemia with iron deficiency and/or inflammation is thought to be potentially causative to declines in muscle function.

Vitamin D is linked to the regulation of iron metabolism, indicating that low vitamin D levels may consequently result in iron deficiency and/or anemia. Due to the emerging relationships observed between vitamin D action on pro-inflammatory cytokines and mechanisms behind iron regulation, there is an increased interest in discovering the physiological functions of vitamin D and iron status on skeletal muscle health and inflammation. This relationship is thought to be associated with hepcidin, an antimicrobial peptide that is essential for regulation of iron metabolism. Iron absorption and excretion are tightly regulated, in which absorption increases with iron deficiency and is suppressed when iron stores are full. This regulatory process is influenced by systemic iron status, the present requirements of erythropoiesis, and the effects of inflammation. An excessive production of hepcidin can result in iron deficiency anemia due to the inability to absorb iron, and in the presence of chronic inflammation, specifically high IL-1β and IL-6 levels, hepcidin production is increased, thus causing iron to sequester and limit iron-supported erythropoiesis. It is thought that vitamin D mediates the expression of hepcidin, through the binding of VDR with a specific gene promotor (HAMP gene) on hepcidin to downregulate hepcidin. Additionally, the role vitamin D has in decreasing inflammatory cytokines (IL-1β and IL-6) that stimulate hepcidin production may indirectly have a contributing part in this relationship. In vitro studies indicated that vitamin D is associated with reduced hepcidin due to the suppression of the HAMP gene by VDR, as well as
decreasing concentrations of IL-1β and IL-6, suggesting the potential of vitamin D regulation of iron balance through hepcidin, particularly in the presence of inflammation.

Relationships between vitamin D deficiency and iron deficiency and anemia, are reported, providing further support for this relationship. Vitamin D deficiency and low iron status in combination, are prevalent in many populations including older adults, children, and athletes, which highlights the necessity to examine how these nutrients may affect inflammation and muscle health with age. Additionally, vitamin D supplementation has been found to decrease hepcidin, and thus may have benefits in altered iron status, particularly in those with chronic inflammation. While typically associated with anemia related to inflammation, it is possible that this mechanism may also be related to iron deficiency with or without anemia due to the reduction in iron necessary to support erythropoiesis. This suggests that those with chronic inflammation may have greater iron requirements to increase circulating iron concentrations and promote red blood cell production, indicating the need for nutritional support with both vitamin D and iron.

Previous studies have indicated a direct relationship between higher levels of inflammatory markers and age, thought to be related to the chronic low-grade inflammation that contributes to age-related diseases. This chronic inflammatory-environment is known as inflammaging, a term coined by Francesci and colleagues, introducing the theory that there is a relationship between age and macrophage activation that is major contributor to the chronic low-grade inflammation present in older adults. Inflammaging is often classified by increased levels of pro-inflammatory cytokines including IL-1β, IL-6 and TNF-α, and a decrease in anti-inflammatory cytokines such as IL-10 and IL-1ra with age and thought to be at least partially responsible for physical decline with age.
For example, the expression of IL-6, along with its receptors (IL-6r and sIL-6r), are limited to a small range of cell types including hepatocytes, neutrophils, leukocytes, monocytes, and skeletal muscle cells.\textsuperscript{221} Additionally, there are accelerating effects seen in neuronal cells exposed to IL-6 and sIL-6r. Neuronal cells are responsive to the combination of both IL-6 and sIL-6r, which may have application to human aging since there are neural components influential to the progression of sarcopenia.\textsuperscript{222–225} Thus, IL-6 and its receptors may be integrative cytokines that link inflammaging to the development of sarcopenia in more than one way. Additionally, TNF-\(\alpha\) is another inflammatory cytokine that is common in muscle wasting conditions including sarcopenia.\textsuperscript{226} This cytokine is produced by multiple cell types including macrophages, lymphocytes, and skeletal muscle cells and acts through two receptors soluble TNF receptor (sTNFR1 and sTNFR2).\textsuperscript{227,228} In addition to its role in pathogenesis, TNF-\(\alpha\) has been reported to increase after just 14 days of inactivity in older adults, indicating the potential role it has with sarcopenia.\textsuperscript{229,230} Therefore, these inflammatory markers, as well as multiple others, may be linked to development of sarcopenia through the inflammaging process.

Inflammaging is largely driven by immunosenescence.\textsuperscript{220} The aging process produces changes in the immune system including a decrease in T cell count, T-cell receptor, and B cells and an increase senescent cells.\textsuperscript{231} These senescent cells produce pro-inflammatory cytokines,\textsuperscript{232,233} further contributing to the inflammaging process and associated age-related diseases. For healthy aging, maintaining immunity and reduced inflammation is necessary, particularly to decrease risk of infection.\textsuperscript{234} The immune response relies greatly on IgA as the first line of defense against pathogens and viruses, and IgM is the first antibody to appear with infection. Lower levels of IgA and IgM are typically associated with reduced immune function and enhanced risk of infection; however, an increase in IgA has been associated with increased
age, which suggests a complex integration with chronic inflammation.\textsuperscript{235,236} While IgM seems to act independently of age, concentrations of IgM and IgA have been reported to be lower in those with malnutrition\textsuperscript{237} and increased in sarcopenic individuals after a nutritional intervention.\textsuperscript{78} These findings suggest that malnutrition in older adults is contributory to immune dysfunction leading to inflammaging and sarcopenia.

Skeletal muscle has also demonstrated a regulatory role in immune system function and inflammation.\textsuperscript{238} Skeletal muscle mass is a main source of anti-inflammatory myokines including IL-7 and IL-15. Active older adults showed higher levels of IL-7 and less evidence of experiencing immunosenescence than non-active older counterparts.\textsuperscript{239} Additionally, IL-15 has shown inhibitory effects on muscle protein degradation and fat deposition,\textsuperscript{240,241} as well as its immunoactivity.\textsuperscript{242,243} Therefore, skeletal muscle may have an important role in the regulation of inflammation and immune function, and thus be a preventative mechanism for inflammaging.

Body composition is influential to inflammaging development through the imbalance of pro- and anti-inflammatory cytokines. Specifically, adipose tissue produces IL-6 and TNF-\(\alpha\), resulting in an increased inflammatory state, whereas skeletal muscle produces anti-inflammatory cytokines.\textsuperscript{238,244} This indicates that the maintenance of skeletal muscle mass and prevention of excess adipose tissue may be essential to prevention inflammaging, and thus, further exacerbating sarcopenia development.

Increased inflammation, potentially from excess adipose tissue, result in the alteration of anabolic pathways, influential on skeletal muscle decline and sarcopenia development.\textsuperscript{73,245} For example, infusion of IL-6 in rodents was reported to induce muscle atrophy by slowing muscle anabolism.\textsuperscript{238,246} Numerous studies have reported associations between inflammatory markers and muscle mass, strength, performance,\textsuperscript{10,15,219,247–255} and frailty status in older adults.\textsuperscript{256,257}
Specifically, there was a greater decrease in leg extension strength over 3.5 years in older women with higher IL-6 levels compared to those with lower levels. In addition, higher levels of IL-6 and TNF-α were found in older adults with lower muscle mass, grip strength and leg extension strength. Recently, higher inflammatory cytokine levels including IL-1β, IL-6, IL-10, IL-12, IL-13, and TNF-α were related to lower muscle volume, leg extension strength, and muscle function in older adults. These findings indicate that sarcopenia may be driven by an increased inflammatory state. Most studies examining inflammation in sarcopenic older adults have included participants with co-morbid conditions typically observed in the aging population. Distinguishing between the presence of inflammaging driven by chronic disease in sarcopenic individuals compared to inflammaging in sarcopenic, but otherwise healthy individuals, will provide insight on the potential role of inflammaging in the development and/or contribution to impaired skeletal muscle health.

The development of inflammaging is related to chronic disease development, and markers of inflammaging have previously been used as predictors of mortality. Elevated levels of inflammatory markers in older adults have been associated with neurocognitive disorders, atherosclerosis, type 2 diabetes, and functional disability, indicating the harmful effects inflammation has on aging skeletal muscle, bone, heart, and brain health. Inflammation and immune dysfunction have been related to increased risk of declining skeletal muscle mass and strength in older adults. However, evidence supports that some long-living older adults do not develop inflammaging, indicating that it remains unclear which lifestyle choices or interventions reduce the risk of inflammaging. Thus, healthy older adults with normal muscle mass, strength, and function may have characteristics that could mitigate risks associated with inflammaging. Therefore, identifying the underlying mechanisms of
inflammaging is essential to develop clinical interventions that will promote improved quality of life in the aging population. While inflammaging is thought to be a naturally occurring process in older adults, increased inflammation may occur in those with sarcopenia due to a decline in skeletal muscle mass and strength, impaired body composition, and diminished nutritional status.

The systemic inflammation that occurs with age is typically associated with the presence of chronic disease; however, there is little known about inflammaging occurring in healthy older adults. It is thought that increased inflammaging is associated with progressive skeletal muscle decline, metabolic impairment, and increased risk of diseases such as diabetes mellitus, Alzheimer’s disease, heart disease, and risk of infection, but it is unknown if the process of inflammaging is different in healthy older adults with low and normal muscle mass and strength. Furthermore, nutrition is a large contributor to both muscle health and inflammation. With the high prevalence of nutritional deficiencies in older adults and the associations they may have with inflammation and parameters of skeletal muscle health, these relationships warrant further exploration. Examining healthy older adults classified as non-sarcopenic and sarcopenic, with distinct differences in metabolic flexibility and muscle tissue oxygenation responses can help detect if the loss of skeletal muscle mass, strength, and function is a primary contributor to increased inflammaging and related nutritional status. If nutritional deficiencies are found in older adults with reduced skeletal muscle strength and size and increased inflammation, this may provide insight on nutritional strategies to mitigate sarcopenia and inflammaging. Therefore, the purpose of this study is to examine if we can differentiate inflammaging between sarcopenic and non-sarcopenic older males and females by exploring if there are differences between biomarkers of inflammation and nutritional status in older adults with distinct differences in skeletal muscle mass, strength, and function, metabolic flexibility, and muscle tissue oxygenation.
Hypotheses

We hypothesize that 1) sarcopenic older adults will have greater basal pro-inflammatory markers, lower anti-inflammatory makers and reduced markers of immune function compared to non-sarcopenic older adults; 2) sarcopenic older adults will have lower nutritional status than non-sarcopenic older adults; 3) females will have greater pro-inflammatory markers and lower nutritional status than males.
CHAPTER II: REVIEW OF LITERATURE

2.1. Inflammation with Aging and Sarcopenia


The purpose of this study\textsuperscript{217} was to examine associations between age-related increased plasma TNF-\textgreek{a} concentrations, atherosclerosis, CRP, leucocytes, and the lipid profile. Older (n=130) and younger (n=44) adults provided fasting blood samples. Occurrence of atherosclerosis was determined and body mass index (BMI) was calculated. TNF-\textgreek{a} was measured in plasma, and standard tests were performed for analysis of lipids, CRP, and leucocytes. The older adults had higher levels of TNF-\textgreek{a}, triglycerides, total cholesterol, low density lipoprotein (LDL) cholesterol, a decreased high density lipoprotein (HDL)/Total Cholesterol ratio, and higher CRP than the younger adults. The older adults were divided into tertiles to determine if high TNF-\textgreek{a} levels were associated with atherosclerosis, and it was determined that high TNF-\textgreek{a} levels were associated with an increased risk of being diagnosed with atherosclerosis, as well as markers associated metabolic dysfunction such as triglycerides and a low HDL to total cholesterol ratio.

2.1.2. Greiwe, Cheng, Rubin, Yarasheski, and Semenkovich (2001)

The purpose of this study\textsuperscript{270} was to test if TNF-\textgreek{a} produced by skeletal muscle contributes to muscle loss with age. A cross-sectional study compared skeletal muscle TNF-\textgreek{a} expression in young (n=12) and older adults (n=12), and a longitudinal design determined the effects of resistance training on skeletal muscle TNF-\textgreek{a} in the frail elderly population (training group, n=8; control group, n=5). Older adults were excluded if a confounding disease was present or if taking medications known to influence muscle function. Body composition was assessed with dual-
energy x-ray absorptiometry (DXA), and for older adults in the resistance training portion, muscle mass was determined using 24 hour urinary creatinine excretion data. Older adults were categorized as frail based on physical performance tests including walking speed, stair climbing speed, lower back range of motion, balance, and upper body strength. Participants also self-reported degrees of difficulty with activities of daily living. A biopsy of muscle tissue from the vastus lateralis was obtained while fasting for determination of TNF-α protein levels. Eight older adults participated in skeletal muscle biopsies before and after an exercise program. Prior to the resistance exercise training program, participants followed a pre-training program three days per week, followed by a progressive resistance training program three days per week for three months. The five older adults in the control group completed the pre-training program and then performed light stretching for the next three months. To determine if TNF-α expression changed with age, the TNF-α content in muscle was compared between young and older participants, with more TNF-α signals, protein, and mRNA in the muscle cells of older adults than young adults. This indicates that increases TNF-α expression is associated with aging even at the pretranslational level. To determine if exercise can affect skeletal muscle TNF-α expression in older adults, maximum strength was tested before and after three months of resistance exercise and compared to a control group. Strength increased in muscles studies for TNF-α expression, but total body, adipose, and lean mass did not change. Muscle TNF-α protein content decreased by 34%, and TNF-α mRNA decreased by 46% with resistance training. Muscle protein synthesis increased by 83%. There was an inverse relationship between muscle protein synthesis rate and muscle TNF-α protein content, while there were no significant changes over time for TNF-α protein and protein synthesis rate. Exercise may delay some of the deleterious effects TNF-α may have on skeletal muscle decline.
2.1.3. Ferrucci, Penninx, Volpato, Harris, Bandeen-Roche, Balfour, Leveille, Fried, and Guralnik (2002)

The purpose of this study\textsuperscript{251} was to see if high IL-6 levels were an independent risk factor for accelerated decline in physical function and disability in older women. Participants were part of an epidemiological study of the causes and course of disability with a Mini-Mental State Examination score of 18 or higher and difficulty performing one or more tasks of functioning. Blood samples from 634 participants were analyzed for IL-6. Leg extensor strength declined 0.14 kg per year in those in the highest IL-6 tertile, but in women in the lower tertiles, strength stayed stable. Higher IL-6 levels may result in a greater risk of developing physical disabilities, along with declines in walking and skeletal muscle strength. These findings indicate the potential relationship an inflammatory state has with muscle in older adults.


The purpose of this study\textsuperscript{256} was to explore the physiological associations with frailty using a newly validated clinical criteria of frailty. Primarily, the purpose was to assess the association between frailty and inflammatory and metabolic biomarkers found to be related to other wasting syndromes. Secondarily, relationships between biomarkers of clotting process in older adults with cardiovascular disease were also examined. Additionally, the frailty criteria was also used to evaluate relationships with lipid and albumin levels. This study used a cohort called the Cardiovascular Health Study that included older adults age 65 and older (n=5,888; female, 2,710; male, 2,025). Criteria to determine frailty included: hand grip strength, walking speed, weight loss, exhaustion, and physical activity. At each site, fasting blood samples were collected. White blood cells, hemoglobin and hematocrit, as well as glucose, insulin, albumin, fibrinogen, factor VII, factor VIII, plasma lipids, and CRP were analyzed. A 75 gram oral glucose tolerance
test (OGTT) was performed on all subjects without diabetes. Coagulation factors were measured. Higher CRP levels were found in the frail group compared to those who were not frail, with or without cardiovascular disease or diabetes. Increased levels of factor VIII and fibrinogen were also associated with frailty. Higher fasting glucose and insulin were also associated with frailty status, as well as levels taken at two hours after the oral glucose tolerance test. The results of this study supported the idea that age-related inflammation is an underlying mechanism of the development of frailty, based on the increased acute-phase markers, glucose intolerance, and increased blood clotting observed in the frail group.

2.1.5. Visser, Pahor, Taaffe, Goodpaster, Simonsick, Newman, Nevitt, and Harris (2002)

The purpose of this study\textsuperscript{255} was to examine if higher levels of IL-6 and TNF-\textalpha were associated with lower muscle mass and muscle strength in well-functioning older adults aged 70-79 years. A large cohort (Health ABC) of 2,746 participants (1,344 males and 1,402 females) participated. Fasted blood samples were collected to analyze IL-6 and TNF-\textalpha. Muscle mass was determined with appendicular skeletal muscle mass by DXA and mid-thigh cross-sectional area (CSA) by computed tomography. Muscle strength was determined with isometric grip strength and isokinetic knee extensor strength at 60°·s\textsuperscript{-1}. An increase in IL-6 was associated with a decrease in grip strength in both males and females. An increase in TNF-\textalpha was associated with a decrease in grip strength in females. In black men, muscle mass and strength were associated with the pro-inflammatory cytokines. In black women, those with higher TNF-\textalpha had lower muscle mass. The prevalence of high cytokine status (combining levels of IL-6 and TNF-\textalpha) was 31.2\% and 28.5\% in white and black men, respectively, and 24.1\% and 22.4\% in white and black women, respectively. In general, older adults, except white men, with high levels of IL-6 and TNF-\textalpha had less appendicular muscle mass, smaller muscle area, lower grip strength, and lower
knee extensor strength compared to those with low levels. These findings suggest that higher pro-inflammatory cytokine levels may contribute to the development of sarcopenia by contributing to the loss of muscle mass and strength.


The purpose of the study was to examine the relationship of plasma concentrations of IGF-1 and IL-6 with muscle function in a population-based sample of older adults. Participants (mean age: male = 65 years; female = 66 years) from the InCHIANTI study (n=526) were screened and assessed for height, weight, and waist circumference. Fasted blood samples were analyzed for glucose, insulin, IGF-1, IL-6, and IL-6 receptors. Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) was calculated. Handgrip strength was measured with a handheld dynamometer. Explosive muscle power was determined for the lower extremity via an extension movement by pushing against a flat pedal. Age was found to be negatively related to handgrip strength and lower extremity power, while IL-6 levels were positively related to age and BMI and negatively related to lower extremity power and handgrip strength. Additionally, IGF-1 lower as age increased, and high levels of IGF were related to greater strength and power. Higher levels of IL-6 and IL-6 receptors were associated with lower IGF-1 concentrations. Levels of IL-6, IL-6 receptor, and IGF-1, as well as them combined, were predictors of muscle strength and power in this older adult population. Furthermore, when stratified by IL-6 levels, IGF-1 was an independent predictor of muscle strength and power in only subjects with the lowest IL-6 levels, indicating that the effects IGF-1 may have on muscle strength and power depends on inflammatory levels in the body.

The purpose of this study\textsuperscript{219} was to examine the effects of IGF-1 and IL-6 in disabled women over age 65 years. Women (n=1002) who had difficulty with functioning but a high mental state were able to participate. Questionnaires and examinations were performed every six months for three years. Baseline blood samples were collected and analyzed for IGF-1 and IL-6. Physical function was assessed by self-report and diseases were determined at baseline. Mortality was assessed by a five year follow up. Those with low IGF-1 levels were more likely to have a walking limitation. Women with high IL-6 levels were more likely to have a walking limitation, mobility disability, and severe activities of daily living disability. Women with both risk factors had a higher likelihood of being disabled with a walking limitation, mobility disability, and activities of daily living disability. The research presented in this study provides some insight on how changes with the endocrine and inflammatory systems may affect health as individuals age.


The purpose of this study\textsuperscript{271} was to examine possible relationships between plasma levels of TNF-α and IL-6 and body composition in healthy older adults, older adults with type 2 diabetes, and young controls using multivariate regression analysis. Young controls (n=20), healthy older adults (n=20), and older adults with type 2 diabetes (n=16) were screened to identify metabolic conditions that may affect body composition and immune system function. A clinical chemistry profile, complete blood count (CBC), cell differential count, and CRP were assessed, and all were within normal ranges. Fasted blood samples were analyzed for TNF-α, IL-6, and insulin. Body composition was assessed via DXA for measurements of appendicular skeletal muscle mass and relative truncal fat. Whole body potassium counting was performed to indirectly measure skeletal muscle mass. Both older adult groups had higher levels of IL-6 and
TNF-α compared to the younger adults, with difference between the older adult groups. Older adults also had higher absolute and relative truncal fat. Truncal fat mass was associated with both TNF-α and IL-6, even after adjusting for age and sex. However, the association disappeared when adjusting for diabetes due to collinearity issues with truncal fat. In males, TNF-α was associated with appendicular skeletal muscle mass, even after adjusting for age, height, weight, and diabetes. There were no associations in females, or with IL-6. Body cell mass from potassium counting was associated with both IL-6 and TNF-α in males, but were no longer significant when adjusting for age, height, weight, and diabetes. The results of this study indicate that truncal fat is related to high levels of TNF-α and IL-6, while TNF-α is also related to lower muscle mass, suggesting that higher truncal fat may contribute to increased inflammatory levels that may be partly responsible for the loss of muscle with age (development of sarcopenia).


The purpose of this study was to examine if higher levels of activity were associated with lower levels of IL-6 and CRP and if these association remain after adjusting for other confounding factors related to high levels of inflammatory markers. Subjects aged 70 – 79 years participated in this cross-sectional design. Subjects had high physical and mental capabilities and self-reported physical activity. Additionally, baseline plasma levels were analyzed for IL-6 and CRP. This study indicated that high levels of activity were associated with lower levels of IL-6 and CRP. These findings suggest that loss of muscle mass and strength with sarcopenia, which is related to physical inactivity and muscle disuse, may be related to higher levels of inflammation.

The purpose of this study was to evaluate the potential association between inflammation and physical performance by exploring the relationship between levels of inflammatory markers (CRP, IL-6, IL-10, TNF-α, and IL-1β) and cytokine-soluble receptors (sIL-6r and IL-1ra) with physical performance (lower extremity and handgrip strength) in an elderly population. This study examined 1020 participants that were part of a larger prospective study. Physical performance was assessed with walking speed, chair stand, and balance and handgrip strength was tested. Fasted blood samples were taken and analyzed for inflammatory markers and cytokine-soluble receptors. Higher levels of CRP, IL-6, and IL-1ra were inversely and independently associated with poorer physical performance and muscle strength. These findings extend support for utilizing inflammatory markers for a screening test for sarcopenia and/or disability and may be a potential target to mitigate the detrimental effects of aging.

2.1.11. Ferrucci, Corsi, Lauretani, Bandinelli, Bartali, Taub, Guralnik, and Longo (2005)

This study aimed to see if higher levels of inflammatory markers with age result, in part, to progressively increased burdens of cardiovascular risk factors and morbidity. A total of 1,327 participants within the InCHIANTI study participated and provided fasting blood samples to analyze for serum IL-6, soluble IL-6 receptors (sIL-6r), IL-1β, IL-1ra, TNF-α, IL-18, TGF-β, CRP, fibrinogen, total cholesterol, HDL cholesterol, triglycerides, and LDL cholesterol. Demographic factors such as alcohol intake and physical activity was assessed, BMI was calculated, occurrence of disease and disability was reported, particularly subclinical cardiovascular disease. In both men and women, IL-6, IL-18, IL-1ra, CRP, and fibrinogen increased with age. Serum sIL-6r also increased slightly with age in females but not males. After adjusting for cardiovascular risk and morbidity, the effect of age on IL-6 was reduced but age remained a significant predictor of higher IL-18 and CRP in females. These findings provide
support that cardiovascular risk factors are contributory to inflammatory status and aging but are not the only influence, indicating that examining inflammatory status in healthy older adults with differences in muscle mass and strength may provide new insights on the inflammaging.


The purpose of this study was to examine whether higher levels of inflammatory markers (IL-6, CRP, and α1-antichymotrypsin (ACT)) are associated with the loss of muscle strength and muscle mass over three years in the Longitudinal Aging Study Amsterdam sample. Muscle mass was assessed with DXA to determine appendicular skeletal muscle mass. Handgrip strength was measured using a dynamometer. Relative change in muscle mass and strength was calculated as the difference between baseline and follow-up values, divided by baseline strength x 100. Loss of more than 40% strength was defined as loss of muscle strength over follow up. Sarcopenia was defined as a loss of appendicular muscle mass greater than 3% and was determined in 25.5% of the study population. Blood samples were collected and analyzed for IL-6, CRP, and ACT. Older adults who had higher IL-6, but not CRP or ACT, had a greater decline in grip strength than older adults with low IL-6 levels. Additionally, high IL-6 and CRP levels were associated with a 3-fold and 2-fold increased risk of strength loss, respectively. No associations between inflammatory markers and sarcopenia (> 3% of muscle mass) were found. This study implies that higher levels of IL-6 and CRP are related to the loss of muscle strength with aging, but there were no relationships observed between inflammation and muscle mass.


The purpose of this study was to examine a cohort from the CHS to look at associations between metabolic syndrome and its determinants with frailty. Out of the cohort,
3,141 participants who were not considered frail or having chronic diseases at the time, were eligible. Follow-ups were performed after five and nine years to examine development of frailty, insulin resistance (HOMA-IR), and metabolic syndrome. Participants who became frail were more likely to have higher White blood cell (WBC) counts, HOMA-IR scores, and fasting concentrations of insulin, CRP, factors VII and VIII, and IL-6 at baseline than those who did not develop frailty. Those who became frail were also 50% more likely to have metabolic syndrome at follow-up. Measurements of HOMA-IR and CRP were consistently related to an increased risk of developing frailty. The authors suggest that these associations may be related to a defect in muscle metabolism, indicating the need to compare inflammatory status in older adults with distinct differences in muscle mass and strength.


The purpose of this study was to examine if circulating levels of CRP, IL-6, and TNF-α were associated with physical function in older persons, independent of disease status and if these associations were independent of body composition. This study utilized participants within four clinical studies that assessed common measures of physical function, inflammation, and body composition in older adults ≥ 55 years old, with chronic obstructive pulmonary disease, congestive heart failure, high cardiovascular risk, or self-reported disability. For physical function, the Short Physical Performance Battery (SPPB) was assessed. Grip strength was measured in both hands and the maximal value was utilized. Fasted blood samples were obtained to analyze inflammatory biomarkers such as IL-6, TNF-α, and CRP. For measurements of body composition, body mass and height were measured to calculate BMI, and DXA was utilized to assess total body fat, fat mass, and lean mass. After adjusting for age, gender, race, and study, higher CRP levels were related to lower SPPB scores, longer four meter walk time, and longer
chair rise times, but were not associated with grip strength. Higher IL-6 levels were related to longer four meter walk and chair stand times and lower grip strength and SPPB scores. Concentrations of TNF-α was not related to any function assessments. When adjusting for body composition, relationships between chair rise time and both CRP and IL-6 were attenuated after controlling for fat mass, however, when adjusting for lean mass, the associations were stronger between CRP and IL-6 and grip strength.

2.1.15. Hsu, Kritchevsky, Liu, Kanaya, Newman, Perry, Visser, Pahor, Harris, and Nicklas (2009)

The purpose of this study was to use principle component analysis to identify a single or multiple inflammatory components, and evaluate the associations between these components and measures of physical function. Participants within the Health ABC study were eligible to participate if they were 70 – 79 years old, had no difficulty with mobility or disability, did not have cancer in the past three years, and were not participating in a lifestyle intervention. Fasted blood samples were obtained and analyzed for CRP, IL-6, sIL-2R, sIL-6r, plasminogen activator inhibitor-1, sTNFR1, sTNFR2, and TNF-α. Leg extensor strength was measured with an isokinetic dynamometer at 60°·s⁻¹ and isometric handgrip strength was measured in both hands and maximum values were summed. Walking performance was assessed as time to complete 400 meters. Physical function was assessed by the Health ABC physical performance battery consisting of five repeated chair stands, usual six meter walk time, six meter narrow walk between lines 20 cm apart, and a balance test of three tiers. Age, sex, race, smoking and alcohol history, medications, clinical pulmonary disease, coronary disease, diabetes, physical activity level, total lean mass, and total fat mass were assessed as covariates. The inflammatory markers fit into two components: TNF-α related markers and CRP related markers. Lower age was
related to lower TNF-α related component, but was not associated with CRP related component. Lower physical performance and leg extensor strength was associated with higher TNF-α related component level. Both TNF-α and CRP components were positively related to 400 meters walking time and inversely associated with grip strength.


The purpose of this study was to examine if high levels of inflammatory markers and soluble receptors were associated with five year changes in muscle mass and strength in well-functioning older adults. Participants from the Health, Aging, and Body Composition Study (n=2,177) ages 70 – 79 years at baseline who had complete data of inflammatory markers at baseline and complete data for examining a five year change in muscle mass and/or strength, were included in this analysis. Muscle mass was analyzed by computed tomography scans of the mid-thigh CSA. Muscle strength was assessed with handgrip strength and isokinetic strength of the leg extensors at 60°·s⁻¹. Inflammatory markers including IL-6, TNF-α, CRP, sIL-2r, sIL-6r, sTNFR1, and sTNFR2 were analyzed. Potential confounders such as age, sex, race, study site, chronic diseases, physical activity, steroid use, estrogen use, anti-inflammatory drug use, statin use, and weight change were assessed as covariates. Higher levels of IL-6, CRP, TNF-α, sIL-6r, and sTNFR1 were related to changes in muscle thigh area. Overall, levels of TNF-α were consistently associated with the decline in muscle mass and strength, even after adjusting for weight change. Additionally, sIL-6r was positively associated with the change in muscle mass while negatively associated with muscle strength changes, which further studies may need to investigate. These findings indicate that inflammatory status may be influential towards the
degree muscle mass and strength decline with age, and that changes in weight may be a contributing part of this relationship.

2.1.17. Stenholm, Maggio, Lauretani, Bandinelli, Ceda, Di Iorio, Giallauria, Guralnik, and Ferrucci (2010)

The purpose of this study was to examine if high levels of catabolic and low levels of anabolic biomarkers predict muscle strength decline in older adults. Participants (n=716) from the InCHIANTI study that had biomarker data and handgrip strength at baseline, three year follow up, and six year follow up, performed physical performance tests such as maximal handgrip strength, and walking speed. Fasted blood samples were collected and analyzed for catabolic biomarkers (CRP, IL-6, IL-1ra, and sTNFR1) and anabolic biomarkers (DHEA-S, IGF-1, and testosterone). Participants were then divided into sex-specific tertiles based on levels of inflammatory markers. Potential confounding factors such as diseases, BMI, waist circumference, physical activity level, estimated daily energy intake, and smoking history were assessed. Higher levels of IL-6 and IL-1ra were associated with a greater decline in grip strength. Additionally, participants who had high levels of one or more catabolic biomarkers had a greater decline in grip strength compared to those without high levels. Conversely, decreasing DHEA-S levels were associated with greater decline in strength. High levels of TNF-αR1 and lower levels of DHEA-S were also related to a decline in walking speed. This study indicates that accumulation of multiple inflammatory markers is associated to a decline in handgrip strength, indicating that measuring a multitude and not just single biomarkers provides a more accurate assessment of inflammatory state and muscle decline in older adults.

The purpose of this study was to examine the similarities and differences of Fried and Rockwood frailty measures and how they apply to frailty within a large cohort of individuals aged 85 years and older. A health assessment and data on pre-existing conditions were examined. A fasted blood sample was collected to analyze for inflammatory markers (IL-6, TNF-α, CRP, albumin, total WBC, neutrophils, monocytes, eosinophils, and basophils), immunosenescence markers (lymphocyte count, ratios of CD4/CD8 T cells, memory/naïve CD4 and CD8 T cells and B cells), cytomegalovirus IgG, and cellular aging markers (telomere length, markers of oxidative stress, and markers of lipid peroxidation). Frailty status was scored based on the Fried method and the Rockwood frailty index based on 40 potential deficits. Additionally, chronic diseases were recorded, cognitive status was assessed, and BMI, sociodemographic data, and disability score was recorded. Logistic regression indicated that the Fried frailty status was significantly related to seven biomarkers: IL-6, TNF-α, CRP, neutrophil count, albumin, lymphocyte count, and memory/naïve CD8 T cell ratio. Lower IL-6 and TNF-α were associated with lower risk of frailty, while higher levels of CRP and neutrophils were associated with a greater risk of frailty. Spearman’s rank correlation indicated significant relationships between RFI and eight biomarkers: positive correlations existed for IL-6, TNF-α, CRP, neutrophil count, and WBC and negative correlations existed for albumin, lymphocyte count, and memory/naïve B cell ratio. Low levels of IL-6 and TNF-α were associated with being less frail, while individuals with high CRP and neutrophils and low albumin and B cell ratio were frailer. This study shows relationships between frailty status and inflammatory markers in a very old population, but did not have enough evidence to support a role of immunosenescent with frailty in this population.

The purpose of this study was to examine the relationship between physical performance (SPPB) and grip strength, inflammatory markers, and muscle mass in old, community-dwelling adults. Participants (n=567) aged 80 years and older performed assessments of body composition estimated with bioelectrical impedance analysis (BIA), handgrip strength, SPPB, and fasted blood samples to analyze CRP, IL-6, and TNF-α. Comorbidities were assessed and were summed together for a score between 0 and 13. Activities of daily living and cognitive function were assessed. Low grip strength was associated with low physical performance, but SPPB was not related to muscle mass. However, this could have been due to estimating with BIA. More precise methods may have produced different results. In this cross-sectional study, a relationship between inflammation and age-related muscle mass and strength was not found; however, studies examining two distinct populations of older adults based on differences in muscle mass and strength may see disparities in inflammatory status.

2.1.20. Addison, Drummond, Lastayo, Dibble, Wende, McClain, and Marcus (2014)

The purpose of this study was to compare intramuscular adipose tissue (IMAT) and muscular inflammation in non-obese, age- and BMI-matched older frail (n=8) and non-frail (n=16) adults. Participants were classified as frail if they had a score of < 25 on the modified physical performance test and reported little to no planned physical activity over the last year. Exclusion criteria included diseases associated with increased IMAT and decreased functional mobility. A subgroup was asked to participate in a muscle biopsy if free from heart disease or any other disease to increase systemic inflammatory levels. Physical activity levels were assessed with the Physical Activity Scale for the Elderly. Mobility was assessed using distance covered in the six minute walk test and self-selected gait speed over 50 feet. Muscle force was determined using the average of three maximal voluntary isometric (MVC) contractions of the
leg extensors. Magnetic resonance imaging determined the CSA of lean mass and IMAT. Muscle biopsies of the vastus lateralis were performed after an overnight fast in a subgroup (7 frail and 11 non-frail). Pro-inflammatory cytokines of the muscle (IL-6 and TNF-α protein and mRNA) expression was analyzed from the muscle tissue. The frail group had more IMAT and less lean tissue than the non-frail. IL-6 mRNA and protein expression was higher in the frail group than the non-frail group, but there were no differences between groups for TNF-α mRNA and protein expression. Correlations indicated a moderate, positive association between thigh IMAT and muscle IL-6 mRNA and protein, but no correlation with TNF-α. IL-6 measurements were strongly related to all mobility measurements, with a negative association with gait speed, 6 minute walk test, and muscle force. These findings indicate a potential link between inflammation and muscle and physical function that may be associated with the amount of IMAT in older adults.


The purpose of this study was to examine inflammatory markers to detect specific patterns that would characterize older adults with differing levels of physical performance. Multivariate models examined relationships between systemic inflammation and physical function. Participants were included if they were 70 years of age or older and did not meet any of the exclusion criteria of confounding lifestyle and disease-related attributes. Physical performance was assessed by gait speed over four meters at the participant’s normal walking speed. Participants were categorized as a slow (n=11) or normal walker (n=27) with 0.8 m·s⁻¹ as the criterion cutoff. Fasted blood samples were obtained and analyzed for 14 inflammatory markers, growth factors, and vascular adhesion molecules. The relationship between gait speed
categories and patterns of biomarkers was explored by constructing and validating a predictive classification model. The slow walkers were older than the normal walkers, so a preliminary analysis was performed to rule out an existing relationship between inflammatory markers and age. In this study sample, the inflammatory markers were not significantly influenced by age. By using the PLS-DA model, slow walkers were characterized by higher circulating levels of IL-8, myeloperoxidase, and TNF-α, and lower levels of P-selectin, IFN-γ, and GM-CSF. These findings indicate that regardless of age, older adults with slow gait speed had higher levels of inflammatory markers. This profile may represent associations with the innate immune system senescence with slower gait speed, and thus may be more prevalent in older adults with sarcopenia.

2.1.2. Morrisette-Thomas, Cohen, Fülöp, Riesco, Legault, Li, Milot, Dusseault-Bèlanger, and Ferrucci (2014)

The purpose of this study was to use principal components analysis (PCA) to identify groups of key inflammatory markers in older adults to better understand changes in the inflammatory system with aging. Participants (n=1,010) ages 20 – 102 years from the InCHIANTI study were included in this cross-sectional analysis at baseline if they had full biomarker data at the baseline visit. Cytokines, receptors, and chemokines (IL-1β, IL-1RA, IL-6, IL-8, IL-10, IL-12, IL-18, IL-15, SGP130, sIL-6R, IFN-γ, TGF- β1, TNF-α, TRAIL, sTNFRI, sTNFR2, monocyte chemoattractant protein (MCP), MIP, and CRP were included in the 19 inflammatory markers studied. Assessment of comorbidities were performed. The main axis of variation with PCA indicated that inflammaging suggests changes in both pro- and anti-inflammatory markers with age and that overall activation of the inflammatory system rather than balance between pro and anti-inflammatory markers may be more relevant in the
population. These findings encourage the need to examine multiple markers simultaneously to look at overall inflammatory system activation patterns.

2.1.23. Sanders, Ding, Arnold, Kaplan, Cappola, Kizer, Boudreau, Cushman, and Newman (2014)

The purpose of this study\textsuperscript{276} was to examine changes in biomarkers of aging, physical function, and cognitive function over nine years in older adults. Older adults (n=901) age 65 years and older with measurements obtained at baseline and at nine years later participated in this study. Fasting blood samples were analyzed for DHEAS, adiponectin, IL-6, IGF-1, IGFBP-1, and IGFBP-3. Physical function was assessed with gait speed and grip strength. Cognitive function was assessed with tests for mental state function and psychomotor speed and working memory. Age, sex, race, marital status, smoking, weight change, and number of chronic conditions were collected as covariates. Over the nine years, levels of adiponectin, IL-6, and IGFBP-1 increased and DHEAS, IGF-1, IGFBP-3 and cholesterol decreased. Additionally, grip strength, gait speed, and psychomotor speed and working memory decreased about 21\% while mental state decreased 4\%. An increase in IL-6 and decrease in DHEAS consistently and strongly correlated with parameters of declining function. This indicates that physical function is related to increased inflammation, indicating the need to examine if differences in muscle mass and strength in older adults results in differences in inflammatory markers.

2.1.24. Varadhan, Yao, Matteini, Beamer, Xue, Yang, Manwani, Reiner, Jenny, Parekh, Fallin, Newman, Bandeen-Roche, Tracy, Ferrucci, and Walston (2014)

The purpose of this study\textsuperscript{260} was to evaluate if a measurement that is influenced by NFkB activation would be independently predictive of mortality and other adverse outcomes after
adjusting for age, sex, BMI, education, smoking, and cardiovascular disease status. Additionally, an objective was to identify a subset of 15 NFkB-related inflammatory markers that were most predictive of mortality for 10 years. Exploratory analyses were used to evaluate if the inflammatory phenotype for predicting mortality differed according to sex and cardiovascular disease status and whether impact of inflammation on mortality changed with age. Participants from two large prospective studies were utilized and stored blood was analyzed for inflammatory markers. Out of the 15 NFkB-related markers (CRP, IL1β, IL1ra, IL6, sIL6r, IL-8, IL-10, IL-12, IL-15, IL-18, TNF-α, STNFR1, sTNFR2, MCP-1, and IFN-γ) measured in one of the studies, five measurements were determined as predictive of five year mortality (CRP, IL-1ra, IL-6, IL-18, and sTNFR1). These markers were then measured in the other study population to predict 10 year mortality risk based on individual markers and then three aggregate measures of inflammation. The aggregates were then validated in the first study. It was found that an index that combine sTNFR1 and IL-6 was the best cytokine predictor of 10 year mortality in both populations.


The purpose of this study was to examine the relationship between the incidence of sarcopenia and inflammatory factors (IL-6 and TNF-α). This was a cross-sectional study of 441 participants over 60 years old. Participants were divided into two groups: 1) Sarcopenia (Appendicular skeletal muscle index (ASMI) < 7.0 kg·m² and handgrip strength < 26 kg in males and ASMI < 5.7 kg·m² and handgrip strength < 18 kg), and 2) Non-sarcopenia (ASMI ≥ 7.0 kg·m² and ≥ 5.7 kg·m² in males and females, respectively). Fasted blood samples were taken for hemoglobin, lymphocyte, and blood chemistry including cholesterol, triacylglycerol, blood glucose, albumin, and creatinine, and inflammatory markers included IL-6 and TNF-α. Serum
levels of IL-6 and TNF-α were higher in those with sarcopenia compared to the control group. Plasma albumin, BMI, and visceral fat were inflammatory factor predictors of TNF-α and IL-6, indicating that nutritional status and body composition may be drivers of inflammaging.


The purpose of this study was to characterize relationships between systemic inflammation, body composition, and physical function in old age with assessments of inflammatory markers, tissue composition of the thigh, and tests of muscle strength and function of the lower extremities. This study examined sedentary young individuals aged 18 – 35 years old and old community-living individuals greater than 70 years old. Eligibility criteria was in place to minimize confounding factors. Magnetic Resonance Imaging was used to analyze tissue composition of the dominant thigh. Muscle tissue, subcutaneous adipose tissue, and intermuscular adipose tissue were quantified volumetrically. Muscle strength was assessed with maximal isokinetic leg extension strength testing to achieve peak torque. In the older individuals, physical function was also assessed with the SPPB. A panel of 14 inflammatory markers, growth factors, and vascular adhesion molecules related to systemic or vascular inflammation were measured. Differences in cytokines, thigh composition, and physical performance were significant between groups. Greater subcutaneous adipose tissue and intramuscular adipose tissue were related to higher levels of P-selectin, myeloperoxidase, sICAM-1, and sVCAM-1. Lower muscle volume and leg extensor strength were correlated with higher levels of inflammatory cytokines including IL-1β, IL-6, IL-10, IL-12, IL-13, TNF-α, and GM-CSF. Older adults with an SPPB score ≤ 9 were considered to have lower muscle volume and knee extensor strength, and have simultaneously higher levels of IL-1β, IL-6, IL-10, IL-12, IL-13, TNF-α, and
GM-CSF. These findings indicate that having lower muscle mass, strength, and function may be prone to increased inflammaging.


The purpose of this study\textsuperscript{259} was to evaluate the relationship between sarcopenia and biomarkers that may be involved in its pathogenesis and used for early detection. This cross-sectional study included 36 sarcopenic and 36 non-sarcopenic individuals from a geriatric outpatient clinic. A comprehensive geriatric assessment evaluated physical, functional, mental status, nutritional status, medical history, comorbidities, and medications. Fasting blood samples were evaluated for blood glucose, liver and renal function tests, lipid profiles, erythrocyte sedimentation rate, and CBC. Additionally, levels of adiponectin, pentraxin 3, and thioredoxin were analyzed. Sarcopenia was associated with inflammatory markers CRP, erythrocyte sedimentation rate, and adiponectin. There was a significant difference in serum inflammatory markers between groups (elevated CRP and erythrocyte sedimentation rate and reduced adiponectin in the sarcopenic group). However, it is unknown if other confounding comorbidities contributed to the increased inflammation. Studies controlling for chronic diseases may be able to highlight the influence of muscle on increased inflammation in older adults.


The purpose of this study\textsuperscript{151} was to identify inflammatory components from seven inflammatory markers using PCA and to examine the association between identified components and the change in grip strength over five years in older adults. A total of 813 participants provided a measurement of grip strength at baseline and were followed up three times over five years, with 294 providing grip strength at year five. Baseline inflammatory marker data was
available for 724 participants. Inflammatory markers included IL-6, TNF-α, hsCRP, homocysteine, and albumin. Grip strength was measured twice in each hand and the mean of all four measurements was used. Confounding lifestyle variables were assessed. Significant relationships were found between the CRP and grip strength and albumin and grip strength at baseline but not the decline over five years. Examining if older adults with differences in hand grip strength have differences in inflammatory and nutritional status will provide more information about this relationship.

2.1.29. Marcos-Pérez, Sánchez-Flores, Maseda, Lorenzo-López, Millán-Calenti, Gostner, Fuchs, Pásaro, Laffon, and Valdiglesias (2018)

The purpose of this study was to examine the connection between frailty and inflammaging and immunosenescence biomarkers to see if frailty is associated with changes in immune activation markers, pro-inflammatory markers, and lymphocyte sub-populations. Older adults (n=259) aged 65 years or older were recruited if they did not have any chronic infection, autoimmune disease, or cancer. Blood samples were collected and analyzed for IL-6, CRP, sTNFR2, TNF-α, and lymphocyte subsets including CD3+, CD4+, CD8+, CD10+, and CD16+56+. Frailty was established based on unintentional weight loss, self-reported exhaustion, hand grip strength, walking speed, and physical activity. A comorbidity index was used to general comorbidity and number of comorbid diseases. Frail participants were older. For immune function, only the percent change in CD19+ decreased significantly in frail participants. However, there were increases in inflammatory markers with frailty. Compared to non-frail participants, there was a 70% increase in IL-6 and a twofold increase in TNF-α in frail participants. The findings from this study indicate that inflammaging is related to the frailty
status of older adults and this population may experience greater chronic inflammation than occurs in the normal aging process.

2.1.30. Li, Yu, Shyh-Chang, Li, Jiang, Yu, Xu, Liu, Guo, Xie, Li, Ying, Li, and Li (2019)

The purposes of this study were to examine if high levels of pro-inflammatory cytokines are associated with the onset of sarcopenia and the severity of sarcopenia, and if the cytokines change based on lifestyle interventions including diet and exercise in sarcopenic older adults. Older adults participating in the Peking prospective longitudinal sarcopenia study in China were recruited for this study. Participants from the PPLSS were eligible if were able to perform activities of daily living, had normal cognitive function or mild cognitive dysfunction, were age 65 years or older, and were determined to be sarcopenic based on relative skeletal muscle mass index (RSMI) and grip strength or gait speed based on criteria from the Asian Working Group for Sarcopenia cut-offs. Out of the 68 sarcopenic older adults enrolled, 56 completed fasting blood samples were taken and analyzed CRP, IL-6, IL-18, TNF-like weak inducer of apoptosis (TWEAK), TNF-α, leptin, IGF-1, insulin, adiponectin, and fibroblast growth factor-21 (FGF21). Fifty-six participants who were not diagnosed as sarcopenic also completed the blood assessments. After the baseline assessments were performed, the sarcopenic older adults participated in a 12-week nutrition and resistance training program. The nutrition intervention included energy intake of 30 kcals·kg⁻¹·day⁻¹, protein of 1.5 g·kg⁻¹·day⁻¹, and a protein supplement of 30 g whey protein. The resistance training consisted of 20 minutes of strength training three times per week. A total of 32 participants completed the intervention and pre- and post-assessments. The sarcopenic group was older and more likely to be inactive, female, and diabetic, had lower BMI, muscle mass, and grip strength. Sarcopenic individuals also had higher IL-6, IL-18, TNF-α, TWEAK, and leptin and lower adiponectin, IGF-1, and
insulin levels than the non-sarcopenic individuals. These findings indicate the pro-inflammatory cytokines are generally higher in sarcopenic individuals compared to non-sarcopenic individuals, and anabolic hormones are generally lower with sarcopenia. Additionally, higher levels of TWEAK were particularly associated with a higher risk of sarcopenia and more severe muscle loss. After examination of the 12 week intervention, levels of TWEAK, TNF-α, and IL-18 were close to normal levels, suggesting that regaining proper nutritional status and muscle mass has a role in reducing inflammation. These findings provide evidence that the loss of muscle mass and strength with sarcopenia may be related to higher inflammatory levels. Future studies should examine if this distinction is also related to nutritional markers and in a population free of diabetes and other conditions associated with high inflammatory levels.


The purpose of this study was to identify inflammatory characteristics of physical frailty and sarcopenia through a comprehensive panel of cytokines and a multivariate statistical approach. Participants age 70 years and older were determined as physical frail and sarcopenic (n=100) or non-frail and sarcopenic controls (n=100) by assessment of physical frailty determined by the SPPB, appendicular muscle mass via DXA, and absence of mobility disability determined by ability to complete a 400 meter walking test. Fasting blood samples were obtained for analysis of 27 inflammatory markers, growth factors, and chemokines. Those with sarcopenia had higher levels of CRP and lower concentrations of MPO, IL-8, MCP-1, and PDGF-BB. These findings indicate that there may be benefit to examining multiple inflammatory markers simultaneously in sarcopenia and non-sarcopenic older adults to determine a particular
inflammatory profile that may provide insight on the role inflammation has in relation to muscle mass and strength.

2.2. Muscle Blood Flow, Muscle Oxygenation, and Near-infrared Spectroscopy with Aging and Metabolic Dysfunction

2.2.1 Dinenno, Jones, Seals, and Tanaka (1999)

The purposes of this study were to examine 1) if limb blood flow at rest is lower in healthy older adults compared to younger adults and if this is a function of lower systemic arterial blood flow; 2) if age-related reduction in limb blood flow is due to a reduction in vascular conductance related to vasoconstriction nerve activity; and 3) if lower whole-limb blood flow with age is related to a lower limb oxygen demand and tissue mass. Non-obese younger (n=16) and older (n=15) males arrived fasted to perform body composition assessments from DXA or underwater weighing. Blood velocity and vessel diameter were measured on the right femoral artery to calculate blood flow. Blood pressure was measured and used to calculate vascular resistance and vascular conductance. Echocardiography was performed to determine stroke volume and cardiac output. Muscle sympathetic nerve activity was determined by microneurographic technique. In a subset of nine young and 11 older individuals, leg oxygen consumption was estimated from whole-body resting oxygen consumption. Leg tissue mass was performed with DXA scans to determine leg fat-free mass, total leg mass, and total leg volume. Femoral artery blood flow was 26% lower in older than younger men due to a 25% lower blood velocity. Additionally, vascular conductance was 32% lower and vascular resistance was 45% higher. Nerve activity was higher in older men and when controlling for nerve activity, the age-related difference in blood flow, vascular conductance, and vascular resistance decreased. Whole-body and leg oxygen consumption was lower in older men and blood flow was directly related to estimated leg oxygen consumption, thus controlling for leg oxygen consumption.
reduced age-related differences in blood flow. Differences in leg oxygen consumption explain more than 50% of the age-related differences in leg blood flow, suggesting that older adults may have lower oxygen demand to the tissues, which may cause a reduced limb perfusion.

2.2.2. Dinenco, Seals, DeSouza and Tanaka (2001)

The purposes of this study were to examine if 1) the age-related reductions in basal limb blood flow and vascular conductance are attenuated in men who exercise regularly, 2) basal limb blood flow and vascular conductance decline linearly across age, and 3) the age-associated decline in basal limb blood flow is related to declines in limb fat-free mass (FFM) and oxygen demand. A total of 89 men that were young (20 – 35 years) or older (55 – 75 years) completed the first study, and 142 healthy men age 18 – 79 years completed the second study. Femoral artery blood flow and vascular conductance was determined by measurement of blood velocity and vessel diameter with a duplex ultrasound machine and blood pressure. Stroke volume was calculated and used to determine cardiac output. Oxygen consumption of the leg was estimated from whole-body resting oxygen consumption, and resting metabolism was determined with indirect calorimetry. Body composition and leg FFM was determined with DXA. Maximal oxygen consumption was measured using a modified Balke incremental treadmill test. Whole-body FFM, basal femoral artery blood flow, vascular conductance, leg FFM and estimated leg oxygen consumption were lower in the older men compared to the young. Vascular resistance was higher in the older group. Additionally, blood flow, vascular conductance, and vascular resistance were all related to age, while leg FFM and oxygen consumption showed a decrease with age. Age-related decline in blood flow were associated with the decline in leg FFM and oxygen consumption. These age-associated declines were not different in the men based on aerobic physical activity. These findings indicate the role in skeletal muscle mass and
oxygenation capabilities and demand to maintaining blood flow with age. Aerobic fitness did not play a role, but the effect of muscle strength was not assessed. This may indicate that older adults who maintain a higher level of muscle mass and strength may be able to prevent some of the age-related decline in muscle blood flow.

2.2.3. Dinenco, Tanaka, Stauffer, and Seals (2001)

The purpose of this study was to examine if lower basal whole-limb blood flow and vascular conductance with human aging is somewhat mediated by increases in sympathetic alpha-adrenergic vasoconstrictor tone using intra-femoral infusion of phentolamine. Young (n=7) adults aged 22 – 33 years and older (n=8) adults aged 57 – 70 years were assessed for body composition and leg volume by DXA. Femoral artery blood flow and vascular conductance was determined with ultrasound measurements of blood velocity and vessel diameter. Phentolamine was infused fasted to measure basal baseline measurements of blood flow. Additionally, propranolol was infused to eliminate confounding factors of age-related β-mediated vasodilation. A cold pressor test was used to examine local α-adrenergic receptor blockage in the leg. Older adults had lower baseline blood flow and vascular conductance, and higher vascular resistance. Propranolol had no effects but phentolamine showed increases in blood flow and vascular conductance and decreases in resistance from baseline in older adults that were greater differences than the younger adults experienced. This indicates that the age-related decrease in blood flow and vascular conductance is mediated mainly by sympathetic α-adrenergic vasoconstrictor tone. This indicates that conditions that contribute to heightened sympathetic nervous system activity such as metabolic disturbances (hyperinsulemia) may contribute to decreased blood flow with age.
2.2.4. Donato, Uberoi, Wray, Nishiyama, Lawrenson, and Richardson (2006)

The purposes of this study\textsuperscript{55} were to see if older adults have reduced blood flow prior to exercise, have comparable blood flow to younger adults during submaximal forearm exercise, and have a reduced blood flow during submaximal leg exercise. Young males 20 – 29 years (n=8) and older males 65 – 80 years old (n=6) performed unilateral leg extensions and handgrip exercise until failure to establish maximal work rate at each modality. For both arm and leg, submaximal workloads were chosen: forearm exercise consisted of rest, 3, 6, and 9 kg, and leg exercise consisted of rest, 3, 6, and 9 W). Relative workloads at 20, 40, and 60\% maximal work rate. Blood velocity and vessel diameter were measured with ultrasound to determine blood flow. Oxygen consumption was measured during leg extension exercise and leg VO\textsubscript{2} at a given work rate was estimated. Forearm and thigh circumferences and length were measured to determine tissue volume. Older adults had lower thigh tissue volume and quadriceps muscle mass, maximal work rate in both handgrip and leg extension exercise, resting leg blood flow, and leg blood flow at absolute workloads. However, the age-related difference in blood flow disappeared when normalized to quadriceps muscle mass both at rest and during exercise. Arm blood flow was not different between age groups. These findings indicate that muscle mass is the determining factor for the decrease in blood flow with age to the lower limbs, indicating the importance of muscle mass maintenance with age.

2.2.5. Kirby, Crecelius, Voyles, and Dinengo (2012).

The purpose of this study\textsuperscript{56} was to examine if local control of skeletal muscle blood flow is impaired in aging humans during systemic hypoxia and muscle contractions and if plasma adenosine tri-phosphate (ATP) of blood draining the skeletal muscle is lower in these conditions in older adults. Young (n=37) and older (n=25) healthy adults participated in protocols to see the
effects of forearm plasma ATP infusion, systemic hypoxia, graded intensity forearm exercise, ATP hydrolysis from forearm exercise, and erythrocyte ATP release during Hb deoxygenation with age. Skeletal muscle blood flow with erythrocyte deoxygenation (such as hypoxia and exercise) was decreased in older adults due to impaired local vasodilation. Due to the relationship between skeletal muscle blood flow and the oxygenation state of hemoglobin, these findings indicate that those with less muscle mass, and consequently less muscle blood flow, may have less oxygenated hemoglobin. In certain states of hypoxia and exercise, oxygen delivery may not match oxygen demand in older adults which could affect muscle perfusion.

2.2.6. Timmerman, Dhanani, Glynn, Fry, Drummond, Jennings, Rasmussen, and Volpi. (2012).

The purpose of this study was to determine if an acute and moderate increase in physical activity with aerobic exercise improves the response of muscle protein anabolism to intake of essential amino acids and sucrose in older adults. In this cross-over design study, six healthy but sedentary older participants performed two experimental visits separated by 4 – 6 weeks. These visits were identical except that aerobic exercise at 60 – 70% of heart rate reserve for 45 minutes was performed the night before one of the visits. Fasted arterial blood was analyzed for insulin, glucose, and indocyanine green concentrations. After four hours, the supplement was ingested in small amounts every 10 minutes for three hours. Leg blood flow was measured throughout this period with Doppler ultrasound. Blood samples from the femoral artery and vein were taken during the basal state and after ingestion and analyzed for amino acids, glucose, insulin, and free phenylalanine and glucose enrichments. Muscle biopsies were taken from the vastus lateralis at four timepoints (two during the basal period and two after ingestion). While there were no differences in blood flow or microvascular perfusion fasted, change in blood flow from fasted state was higher in the exercise condition. Microvascular
perfusion also increased with the exercise condition during ingestion. Overall, this study indicated that exercise resulted in an enhanced anabolic response to amino acids and carbohydrates in older adults, suggesting that exercise may be useful tool in treating and preventing sarcopenia by increase leg blood flow, leading to greater nutrient delivery to the muscle.


The purpose of this study was to compare skeletal muscle oxidative function between individuals less than 35 and greater than 65 years of age using NIRS approach of rapid arterial cuff occlusions to measure post-exercise muscle oxygen consumption recovery kinetics to see if aging would prolong recovery kinetics (mitochondrial function) compared to younger individuals. Post-exercise muscle oxygen consumption was assessed in 39 individuals either age 18 – 30 years or 60 – 85 years. A NIRS optode on the flexor digitorum profundus was used to measure O$_2$Hb and HHb during arterial occlusion and isometric handgrip exercise at 50% MVC until oxygen saturation dropped by about 50%. After exercise, multiple rapid cuff inflations were performed to construct a muscle $\dot{V}$O$_2$ recovery curve. Tissue saturation slope of change was used to calculate muscle $\dot{V}$O$_2$ and its recovery rate from exercise. Due to poor NIRS signals, 32 individuals were analyzed. Post-exercise muscle oxygen consumption recovery kinetics were prolonged in older adults compared to younger. These findings indicate an age-related decline in muscle oxidative function that can be detected in a non-invasive method using NIRS.

The purpose of this study\textsuperscript{48} was to examine whether a vascular occlusion test technique with NIRS was able to detect differences in vascular responsiveness following a hyperglycemia challenge (OGTT). Male (n=8) and female (n=6) participants arrived fasted and blood pressure, blood glucose and vascular concentration was evaluated. A 75 gram glucose drink was consumed and vascular responsiveness with an occlusion test and blood glucose concentrations were evaluated at 30, 60, 90, and 120 minutes after glucose ingestion. Vascular responsiveness of the tibialis anterior was measured for five minutes at baseline, five minutes occlusion, and eight minutes following cuff release at each timepoint. Reperfusion rate and area under the curve (AUC) was calculated. The reperfusion slope increased from 0.8 \(\%\cdot s^{-1}\) to 1.1 \(\%\cdot s^{-1}\) at 90 minutes post glucose challenge and was negatively correlated with AUC. This indicates that after blood flow occlusion, reperfusion of tissue oxygen saturation was steeper, and the AUC decreased at 90 minutes and was detectable with NIRS. NIRS may be able to detect responses in older adults following consumption of rapidly digesting CHO to see if there are differing responses between sarcopenic and non-sarcopenic older adults.


The purpose of this study\textsuperscript{50} was to examine differences in oxidative metabolism non-invasively in obese and normal weight individuals using NIRS and a vascular occlusion technique before and after a hyperglycemic challenge. Normal weight males (n=10) and females (n=6) and obese males (n=9) and females (n=4) arrived fasted to complete blood pressure, blood glucose, and monitoring of HHb with NIRS before consumption of a 75 gram glucose beverage. Blood glucose concentrations and HHb of the tibialis anterior were also assessed at 30, 60, 90, and 120 minutes after ingestion. NIRS was also measured during occlusion of blood flow with a
pneumatic cuff at each of the five timepoints. The baseline, AUC during occlusion, and area above the curve (AAC) during reperfusion for HHb were calculated. In obese individuals, the HHb AUC decreased after the glucose challenge while the normal weight individuals experienced an increase. This indicates that during occlusion of blood flow, hyperglycemia results in an increase in muscle oxygen utilization after 90 minutes in normal weight individuals. Those with obesity showed a decrease in muscle oxygen utilization at 30 and 60 minutes after glucose consumption. It is possible that this decrease in oxygen utilization of the muscle may be indicative of metabolic inflexibility of the muscle and can be non-invasively measured with NIRS.


The purpose of this study was to compare the \( \dot{V}O_2 \) kinetics response between older and young individuals of different training levels. Fifty-seven men were separated into two age groups: young (18 – 45 years) and older (65 – 75 years) and separated by activity level. A ramp incremental test on a cycle ergometer was performed on the first study visit to obtain \( \dot{V}O_2 \) peak, peak power output, and the gas exchange threshold. For the second visit, vascular occlusion of the tibialis anterior was performed with a pneumatic cuff while NIRS measurements were collected continuously throughout the procedure. The third visit consisted of a cycling test of transitions in power output. Indirect calorimetry and HHb of the vastus lateralis with NIRS was measured throughout the test. Across training status, there were no differences with age on \( \dot{V}O_2 \) kinetics, indicating that it may be fitness level and not age that determines these responses. When examining tissue saturation (StO₂) reperfusion, there was increased vascular responsiveness related to training status, independent of age. While this study examined endurance trained
individuals, these findings indicate that an active lifestyle that maintains muscle mass may prevent reductions in oxygen utilization typically seen with aging.


The purpose of this study\textsuperscript{60} was to compare NIRS-derived post-occlusion tissue oxygen saturation recovery kinetics between individuals less than 35 and older than 65 years of age using a NIRS approach with post-occlusive reactive hyperemia (PORH) to see if aging would prolong the recovery rate of muscle oxygen saturation compared to younger individuals. Young (n=24) and older (n=10) adults performed an MVC with a handgrip dynamometer. Skeletal muscle oxygenation of the flexor digitorum profundus was measured with a NIRS device to determine changes in O$_2$Hb and HHb and calculate StO$_2$ during the PORH. Baseline StO$_2$, desaturation rate during occlusion (an indirect measurement of skeletal muscle metabolic rate), StO$_2$ minimum as the lowest value achieved during ischemia, reperfusion rate (average upslope after cuff release), StO$_2$ maximum as the highest value reached after cuff release, and the reactive hyperemia AUC was calculated from cuff release to 1-, 2-, and 3 minutes post occlusion. Hyperemic reserve was calculated as the change in StO$_2$ above baseline (\%). These measurements were compared between young and older adults. The NIRS-derived markers of microvascular function using the PORH protocol were impaired in older adults. Baseline values were similar, but after the occlusion, the rate of StO$_2$ recovery was much slower and StO$_2$ maximum, AUC, and hyperemic reserve were all lower in older adults. Skeletal muscle metabolic rate was also slower in the older adults, leading to a much higher StO$_2$ min compared to younger adults. These findings indicate that NIRS can be used as a non-invasive, cost-efficient method to examine microvascular function.

The purpose of this study was to examine whether a vascular occlusion test with NIRS could detect differences in microvasculature after a hyperglycemic challenge between healthy obese and lean individuals. Lean (n=15) and obese (n=13) individuals were assessed for blood pressure, blood glucose concentrations, and vascular responsiveness with a vascular occlusion test while fasted. After glucose ingestion, blood glucose concentrations and vascular responsiveness was measured at 30, 60, 90, and 120 minutes after ingestion. Vascular responsiveness of the tibialis anterior was measured with NIRS for 5 minutes at baseline, 5 minutes occlusion, and 8 minutes following cuff release at each timepoint. Reperfusion rate and AUC was calculated. Obese individuals had higher blood glucose concentrations at 90 and 120 minutes. There were no differences in oxygen saturation at baseline, undershoot and overshoot values at each timepoint. Lean individuals had a greater reperfusion slope at 90 minutes and returned to baseline values at 120 minutes post glucose consumption than obese individuals. The AUC decreased at 90 minutes and returned to baseline values by 120 minutes in the lean group, whereas the obese group showed a decreased AUC at 90 and 120 minutes. These findings indicate the NIRS is capable of detecting differences in vascular responsiveness in distinct metabolic groups after a hyperglycemia challenge.


The purposes of this study were to investigate the role of resting muscle perfusion in both age-associated decline of mitochondrial oxidative capacity and whole-body aerobic capacity in healthy individuals, and to examine whether the association between muscle perfusion and aerobic capacity is mediated by mitochondrial oxidative capacity. Healthy adults from the Genetic and Epigenetic Signatures of Translational Aging Laboratory Testing Study (n=75) were screened for eligibility by having no genetic or autoimmune disease, cardiovascular, kidney,
liver, neurological diseases, diabetes, active cancer, hormonal dysfunction, chronic muscle pain, drug treatment, and capacity to perform normal activities of daily living. Aerobic capacity was measured with a modified Balke treadmill test to determine VO$_{2\text{peak}}$. Diffusion-weighted magnetic resonance imaging and phosphorus-31 magnetic resonance spectroscopy were performed to determine muscle perfusion of the thigh. Participants performed rapid, ballistic knee extensions for 25 – 50 s while phosphorus-31 magnetic resonance spectroscopy spectra was collected before, during, and after exercise. Maximum oxidative capacity was assessed during the recovery period after exercise with the rate of phosphocreatine (PCr) recovery reflecting maximal muscle oxidative ATP synthesis. Phosphocreatine recovery curves indicate a shorter recovery time in younger compared to older individuals. Resting muscle perfusion and VO$_{2\text{peak}}$ were also lower in older adults even when adjusting for BMI, sex, and race. This study indicates that muscle oxidative capacity and resting muscle perfusion decline with age, indicating that even with exercise, age may cause microvascular resistance in the muscle, limiting nutrient delivery.

2.2.14. Moro, Brightwell, Phalen, McKenna, Lane, Porter, Volpi, Rasmussen, and Fry. (2019)

The purpose of this study$^{58}$ was to examine if resistance training influences muscle capillarization in low active healthy older adults, and if basal muscle capillarization is predictive of the hypertrophy with resistance training. Healthy male (n=10) and female (n=9) older adults participated in 12 weeks of resistance training three times per week. Infusion of phenylalanine was used to measure muscle protein synthesis. Biopsies from the vastus lateralis were collected. Quantification of muscle capillarization density and capillary-to-fiber perimeter exchange index was performed. Fractional synthetic rate of muscle protein was determined. Findings from this study suggest that muscle fiber capillarization may be influential on the ability for skeletal
muscle to hypertrophy following resistance training. This may mean that those with less
capillarization experience more anabolic resistance and may have a diminished ability to gain
and/or maintain muscle mass as they age, possibly due to lack of blood flow to the muscle.

2.2.15. Alvares, de Oliveira, Soares, and Murias (2020)

The purpose of this study was to examine the relationship between the changes in
NIRS-derived measures of THb and StO₂ and Doppler ultrasound measures of blood flow in
response to fast- and slow-velocity shortening muscle contraction. Healthy, active males (n=12)
performed leg flexion and extension exercise with an isokinetic dynamometer at a slow-velocity
(30°·s⁻¹), and fast-velocity (180°·s⁻¹) during the extension phase and a velocity of 270°·s⁻¹ during
the flexion phase. The two velocities were separated by a 30 minute rest period to allow for
blood flow and NIRS measurements to return to resting levels. Near-infrared spectroscopy was
used to measure THb and StO₂ of the vastus lateralis to determine baseline StO₂ and THb,
amplitude of muscle oxygen desaturation, muscle oxygen desaturation rate, and blood volume
reperfusion rate. Ultrasound was used to examine femoral artery blood velocity and artery
diameter to calculate blood flow. Reperfusion slope of THb was significantly correlated with
peak blood flow during exercise-induced hyperemia after slow and fast velocity muscle
contractions, indicating the capability of using NIRS measurements of THb for interpretation of
changes in muscle blood flow resulting from hyperemia with exercise. Measurements of THb
with NIRS may reflect changes in blood perfusion to the muscle during recovery from exercise,
indicating it can be a non-invasive, indirect method to assess changes in local blood flow during
the reperfusion phase after exercise, which provides rationale to use it as a proxy for muscle
blood flow in older adults.
2.2.16. Meneses, Nam, Bailey, Anstey, Golledge, Keske, Greaves, and Askew (2020)

The purpose of this study is to compare skeletal muscle microvascular perfusion and whole-leg blood flow responses to occlusion and matched submaximal exercise between young and older adults. Secondary aims include exploring the relationship between microvascular perfusion and whole-leg blood flow responses and assess test-retest reliability of contrast-enhanced ultrasound skeletal muscle perfusion parameters. Healthy older (n=12) (mean age 68 ± 7 years) and young (n=12) (mean age 26 ± 3 years) were screened for cardiovascular and metabolic diseases. A maximal plantar-flexion force test was performed. Assessment of whole-leg blood flow was performed with strain-gauge plethysmography at rest, after thigh occlusion, and during a 5min bout of intermittent isometric plantar-flexion exercise. Muscle microvascular perfusion of the medial gastrocnemius was assessed with contrast-enhanced ultrasound before and after the five minute occlusion twice, separated by 15 minutes. Resting blood pressure and ankle brachial index was determined and anthropometrics including height, weight, BMI, and estimated calf muscle mass were measured. Older adults had lower MVC force than younger adults, even when adjusted for calf muscle mass. There were no differences in whole-leg blood flow, vascular conductance, muscle microvascular perfusion, and muscle microvascular blood volume between groups. However, estimated calf muscle mass was not different between older and younger adults, which may have been influential in the lack of difference in muscle perfusion responses. Examining the effect muscle mass and strength has on muscle perfusion may provide more insight on muscle perfusion with age.

2.3. Metabolic Flexibility and Muscle Metabolism with Aging

2.3.1. Bonadonna, Groop, Simonson, and DeFronzo (1994)
The purpose of this study was to quantify insulin sensitivity in a group of nondiabetic older adults and to verify whether the Randle cycle (characterized by increased FFA mobilization and FFA/lipid oxidation) is present in nondiabetic older adults. Healthy younger (n=7) and older (n=7) participants with no heart, liver, or kidney disease, normal blood pressure and were within 20% of desirable body weight were able to participate in this study. All younger participants and three older adults had normal glucose tolerance, and four older adults had a non-diagnostic OGTT. The study design had three different study portions. The first study consisted of determination of lean body mass by tritiated water dilution. The second two visits involved a sequential insulin clamp performed with a two-step or three-step protocol of different infusion rates in random order. Insulin was infused for 100 minutes at each stage and indirect calorimetry was performed during the equilibrium period and the final 60 minutes of each insulin infusion step for respiratory gas exchange. Blood and expired gasses were collected at 10 – 15 minute intervals during the last hour of the equilibrium period and during each insulin infusion step to determine plasma $[^{3}\text{H}]$ glucose, $[^{14}\text{C}]$FFA, and $^{14}\text{CO}_2$, and plasma insulin concentrations. Urine was also collected at baseline and during insulin infusion periods to analyze total nitrogen excretion to calculate protein oxidation. Whole body glucose metabolism, glucose oxidation rate and lipid oxidation rate were calculated from indirect calorimetry. Plasma FFA turnover and plasma FFA oxidation were calculated. There were no differences between age groups for glucose with any condition and insulin at baseline, but insulin was higher in older adults at the highest insulin infusion rates. Fat mass, plasma FFA (at all infusion rates), lipid oxidation were all greater in older adults. However, when corrected for fat mass, the differences disappeared. These findings indicate that in older adults with normal glucose tolerance but hyperinsulinemia, plasma FFA are comparable to younger participants, but in those with abnormal glucose
tolerance, plasma FFA was higher, indicating an increase in FFA turnover per kilogram lean body mass. These findings indicate an insulin resistant-like presence encompassing FFA/fat metabolism in nondiabetic older adults, evident by increased rates of FFA mobilization, fat oxidation, and impaired glucose metabolism pathways. This suggests with aging, excessive Randle cycle activity may cause a decrease in insulin-mediated glucose oxidation.

2.3.2. Calles-Escandón, Arciero, Gardner, Bauman, and Poehlman (1995)

The purpose of this study\(^{29}\) was to examine if whole body fat oxidation decreases with age in females and if this decrease is related to the age-associated decrease in the quantity of the FFM. Females (n=32) ranging in age and body composition and with normal OGTTs participated in this study. Body composition was assessed with underwater weighing and \(\dot{V}O_2\text{max}\) was determined with an incremental treadmill test. Fasting blood samples and basal fat oxidation (with indirect calorimetry) were obtained and analyzed for glucose, insulin, fatty acids, and rates of energy expenditure and substrate oxidation. Urinary nitrogen was collected to estimate protein oxidation rate. There was an age-related decline in FFM, as well as a positive relationship between rate of energy expenditure and FFM and \(\dot{V}O_2\text{max}\). Fat oxidation had a low, negative correlation with age and a positive, moderate correlation with FFM and \(\dot{V}O_2\text{max}\), but had no relationship with fat mass. However, when accounting for the effects of FFM on fat oxidation, there were no longer relationships between fat oxidation and \(\dot{V}O_2\text{max}\) and age. This indicates that in this population, the age-related decrease in fat oxidation is more related to the decrease in FFM with age and not necessarily with aging itself. These findings indicate that muscle loss with age may be a large contributor to changes in substrate utilization and metabolic dysfunction.
2.3.3. Rising, Tataranni, Snitker, and Ravussin (1996)

The purpose of this study\textsuperscript{278} was to examine longitudinal data on respiratory quotient (RQ) with age and to examine the cross-sectional relationship between 24 hour RQ and age. Seven men were followed longitudinally for seven years. Participants spent one day in a respiratory chamber to analyze 24 hour energy expenditure, basal metabolic rate, sleeping metabolic rate, and 24 hour RQ. The same procedures were repeated seven years later. Additionally, 24 hour RQ was determined in 131 men and was also adjusted for energy balance and percent body fat. Over the seven years, RQ increased from 0.84 to 0.86. Both unadjusted and adjusted 24 hour RQ was positively correlated with age. These older individuals displayed a lower ratio of net fat to carbohydrate oxidation, independent of energy balance and body fat. Basal metabolic rate also declined with age and percent body fat increased, suggesting that age is associated with metabolic changes, including lower fat oxidation.

2.3.4. Conley, Jubrias, and Esselman (2000).

The purpose of this study\textsuperscript{279} was to determine the oxidative capacity of muscle and how it is different between adults and older adults. Male (n=6) and female (n=3) adults and male (n=18) and female (n=22) older adults participated in a protocol using PCr and pH during stimulation to estimate glycolytic hydrogen production and measure ATP supply related to exercise to estimate oxidative capacity. Muscle stimulation of the quadriceps was performed to determine maximum electromyography response. Measurements of changes in PCr, ATP, inorganic phosphate and pH were taken during and after stimulation with magnetic resonance. Oxidative phosphorylation was calculated using linear models. Mitochondrial and muscle oxidative capacity were determined. Mitochondrial capacity was reduced in the older adults compared to the adult group. This indicates that muscles in older adults have lower oxidative capacity that may resultant from
decreased physical activity. It is possible that this reduced oxidative capacity may end up resulting in impaired nutrient delivery to the muscle and metabolic dysfunction in older adults with less muscle mass and strength.

2.3.5. Rizzo, Barbieri, Ragno, Grella, Provenzano, Villa, Esposito, Giugliano, and Paolisso (2005)

The purpose of this study was to examine the relationships between resting metabolic rate and RQ with human longevity and compared in adults and older adults. Eighty-one female adults less than 65 years (n=26), aged subjects from 66 – 94 years (n=27), and long-lived subjects older than 95 years (n=28) with a stable body weight, nonsmoking, and healthy were enrolled in this study. Anthropometrics and skinfold thickness measurements to estimate body fat percent were taken. Fasting blood samples were collected before calorimeter evaluation for analysis of glucose, total cholesterol, triglycerides, FFAs, total proteins, albumin, and blood cell count. A 24 hour urine collection was obtained for urea nitrogen. A calorimeter was used to assess resting metabolic rate for 60 minutes and to determine RQ. Age was found to be negatively related to resting metabolic rate and RQ. Additionally, RQ was negatively related to glucose, fat mass, and body fat percent. The long-lived participants had higher fasting RQ than the aged group, but lower than the adults. Body composition may have an influence on these findings that show metabolic declines with age that may be less prone in older subjects who maintained a lower waist hip circumference and BMI. This cross-sectional study suggests that in this population of females, while age was related to metabolic declines, those who were long-lived may be less prone to those declines due to maintaining a healthy body composition.
2.3.6. Short, Bigelow, Kahl, Singh, Coenen-Schlmke, Raghavakalmal, and Nair (2005)

The purpose of this study was to examine if muscle mitochondrial function declines with age and to determine causes of age-related changes in mitochondrial function. Healthy participants (n=146) ages 19 – 89 years old were included. Muscle biopsies of the vastus lateralis were taken for measurements of mitochondrial ATP production. A subgroup (n=10 young and n=10 older adults) were assessed for glucose tolerance before and after consumption of a meal (55% CHO, 30% fat, and 15% protein). Blood samples were collected at baseline and every 15 minutes for glucose, insulin, and fatty acids. The subgroup also provided muscle samples for protein identification. Measurements of DNA and RNA were quantified, DNA oxidation was determined, and maximal aerobic capacity was measured. The older individuals had reduced mitochondrial protein content and oxidative enzyme activity in skeletal muscle. The decline in mitochondrial ATP production in muscle may lead to decreased muscle function and/or performance and higher insulin sensitivity, indicating a potential relationship between metabolic inflexibility and muscle form and function.

2.3.7. Solomon, Marchetti, Krishnan, Gonzalez, and Kirwan (2008)

The purpose of this study was to examine if basal fat oxidation is decreased in older obese individuals as a function of age alone. Sedentary, obese, normal glucose tolerant males and females were divided into sex and BMI matched groups of younger (n=10) and older (n=10) individuals. Participants with heart, kidney, liver, intestinal, and pulmonary disease or taking medication for hypertension, diabetes, or other obesity-related conditions were excluded. Anthropometric and body composition were assessed with height, weight, waist circumference, and underwater weighting. Baseline metabolic measurements were taken for 30 minutes after a 12 hour fast to determine RQ, energy expenditure, and substrate oxidation rates. Urinary nitrogen
excretion was collected for estimation of protein oxidation rate. Maximal oxygen consumption ($\dot{V}O_2\text{max}$) was determined with an incremental treadmill exercise test. Fasting blood samples were obtained for determination of triglycerides, total cholesterol, glucose, insulin, and leptin. Two additional samples were collected at 10 minute intervals for glucose and insulin to calculate HOMA-IR. The older group had greater fat mass and lower FFM compared to the younger group. While there were no differences in RQ, CHO oxidation, protein oxidation, energy expenditure or any blood markers, the older group had lower fat oxidation when normalized to FFM and $\dot{V}O_2\text{max}$ than the younger counterparts. Additionally, while there was an inverse relationship seen with fat oxidation and age, this relationship remained significant even when controlling for FFM. These findings indicate that in an older (mean age of 60 years), glucose tolerant population, fat oxidation was reduced even when controlling for other variables that may influence metabolism. This indicates that muscle mass may not be the only factor influencing metabolic dysfunction with age. Future studies should examine the possible effects of inflammation, muscle perfusion and muscle strength on substrate utilization in an elderly population.


The purpose of this study was to examine if adipokines, muscle mass, and regional adiposity affect glucose disposal in older adults. Participants (n=539) from a larger study were assessed cross-sectionally for measurements of fasting adiponectin, leptin, OGTT, physical activity levels, anthropometrics, and computer tomography scans of the thigh. There were no relationships between low muscle mass and glucose disposal in individuals who were lean; however, those with both central and global adiposity were associated with having delayed glucose disposal rates during the OGTT. Additionally, there was a strong negative relationship
between adiponectin and glucose disposal rates after adjusting for adiposity, muscle mass, leptin, age, sex, and physical activity levels. This indicates that adiposity was related to worse glucose tolerance, regardless of muscle mass in older adults. Future studies should examine distinctly different groups of muscle and strength to see if glucose tolerance is affected.

2.3.9. Prior, Ryan, Stevenson, and Goldberg (2014)

The purpose of this study\textsuperscript{282} was to test if the ability to shift from fat to carbohydrate oxidation during submaximal exercise is lower in overweight-obese older adults with impaired glucose tolerance compared to those with normal glucose tolerance. Sedentary men and women (n=23) between the ages of 45 – 80 years old performed a graded \( \dot{V}O_2 \text{max} \) test on a treadmill. Indirect calorimetry was measured during the test. A 2 hour OGTT was performed after a 12 hour fast, and blood samples were drawn before and every 30 minutes after glucose consumption for two hours. Glucose and insulin were analyzed and used to calculate HOMA-IR, and participants were classified as normal glucose tolerant or impaired glucose tolerant. Resting RQ to determine resting substrate utilization was measured prior to undergoing a hyper-insulinemic-euglycemic clamp. Insulin-stimulated RQ was measured during the last 30 minutes of insulin infusion. During another visit, participants completed a submaximal exercise test of two continuous, 10 minute, steady-state treadmill exercise bouts at 50\% \( \dot{V}O_2 \text{max} \) and 60\% \( \dot{V}O_2 \text{max} \). Data collected during the last 5 minutes of each workload were analyzed. Energy utilization from CHO and fat and RQ were determined. Anthropometrics, body composition with DXA, and intra-abdominal and subcutaneous abdominal fat with computed tomography were determined. At rest, RQ was similar between impaired glucose tolerant and normal glucose tolerant individuals; however, during submaximal exercise at 50 and 60\%, the impaired glucose tolerant had a lower RQ than normal glucose tolerant, in which the impaired glucose tolerant saw little
change above resting. Both groups expended similar energy per kg lean body mass, yet the amount of energy from fat was greater in impaired glucose tolerant compared to normal glucose tolerant at both intensities. The impaired glucose tolerant were metabolically inflexible when stimulated with insulin infusion compared to normal glucose tolerant. While RER increased in both groups in response to insulin, the increase in impaired glucose tolerant was much less than the increase in normal glucose tolerant. These findings indicate that impaired glucose tolerant, obese, older adults showed metabolic inflexibility when transitioning from rest to steady-state aerobic exercise at 50 and 60% \( \dot{V}O_2 \) max, and during insulin infusion. Additionally, lower CHO utilization during exercise of increasing intensity was related to the amount of postprandial hyperglycemia. These impaired glucose tolerant adults showed a reduced ability to shift from fat to CHO oxidation when going from rest to submaximal exercise. This may limit the amount of energy that can be supplied to skeletal muscle during higher intensity activities.


The purpose of this study was to examine if fasting and 2 hour glucose levels after an OGTT were associated with decreased grip strength and if sex differences are apparent. Participants in the NIH Lipid Research Clinic Study were included in this analysis if they had some measurement of glucose and at least one measure of handgrip strength (n=1,420). An OGTT was administered and blood samples were obtained at baseline and 2 hours. Covariates such as demographics, medical history, anthropometrics, physical activity level, and comorbidities were reported. Grip strength was measured bilaterally. Grip strength was lowest in males with the highest fasting glucose after about age 65 years, and this relationship between increasing levels of glucose with lower grip strength remained even after accounting for potential confounders. However, grip strength was slightly higher in females in highest fasting glucose
until age 85 years. These findings indicate that metabolic flexibility may be related to muscle strength, and that sex may play a role in this relationship with older adults.

2.3.11. Siervo, Lara, Celis-Morales, Vacca, Oggioni, Battezzati, Leone, Tagliabue, Spadafranca, and Bertoli (2016)

The purpose of this study was to examine whether aging was correlated with adiposity indexes and fasting gas exchange, RQ, and fractional non-protein substrate oxidation of CHO and fat in males and females. Secondarily, an aim was to see if RQ was a significant predictor of subcutaneous fat and visceral fat deposition after adjusting for confounding factors. Additionally, adiposity indexes and basal substrate oxidation were tested to see if they were significant predictors of cardiovascular risk factors including blood pressure, glucose, high density lipoproteins, triglycerides, and development of metabolic syndrome. Males (n=894) and females (n=1,925) ages 18 – 81 years without medical conditions that may affect energy expenditure. Anthropometrics, BIA, and abdominal ultrasonography for visceral and subcutaneous adipose thickness were performed. Resting energy expenditure was measured with open-circuit ventilated-hood indirect calorimetry. Oxygen consumption and carbon dioxide production was measured every minute for 30 – 40 minutes. The average of the last 20 minutes was used to determine 24 hour resting energy expenditure and RQ. Fasting cholesterol, HDL, triglycerides, and glucose were measured. Presence of metabolic syndrome was determined based on having three or more of the following criteria: large waist circumference, low HDL cholesterol, high triglycerides, high blood pressure, and high glucose. Older subjects only made up 13% of the studied population. Gas exchanges and resting energy expenditure showed a decline with age, however basal RQ did not. Visceral adipose tissue was associated with age in males and females, and subcutaneous adipose in just females. In general, these results did not show an age-related
decline in RQ but did see associations with visceral and subcutaneous fat thickness. This indicates that even no association was found in this population, there is still support that body composition and muscle mass may be an influencing factor in substrate utilization in the aging process.


The purpose of this study was to examine differences in muscle mitochondrial function in pre-frail older adults with active older adults using phosphorus magnetic resonance spectroscopy and biological biomarkers in muscle biopsies of the vastus lateralis. Pre-frail (n=11) and active (n=11) ages 61 – 80 years performed muscle function tests including SPPB, handgrip strength, quadriceps strength, and postural stability, and participated in evaluation of mitochondrial function of the calf muscle. The main findings of this study showed an association between declining skeletal muscle mitochondrial function and pre-frailty, indicating that declines in muscle oxidative function may results in reduced functioning, and may exacerbate the development of sarcopenia. Interventions are necessary at this stage to prevent further loss of muscle mass, strength, and function.

2.4. Vitamin D Status with Aging and Sarcopenia

2.4.1. Verhaar, Samson, Jansen, de Vreede, Manten, and Duursma (2000)

The purpose of this study was examine if six months of alphacalcidol supplementation would improve muscle strength and functional mobility in vitamin D deficient females over 70 years old. Vitamin D deficient (< 20 nmol·L⁻¹) females greater than 70 years old (n=14, n=10 completed the study) were provided an oral drug (alphacalcidol) that quickly activated to the active form of vitamin D for six months. Age-matched controls (n=13) with normal vitamin D
status participated in the tests without vitamin D treatment. At baseline and after six months of treatment, participants were assessed for serum 25(OH)D levels, maximal voluntary knee extension strength, handgrip strength, walking test, and timed-up-and-go (TUG) test. After treatment, 25(OH)D levels increased but were still below normal (30 nmol·L⁻¹). The treatment group also experienced an increase in isometric knee extension strength over time compared to the control group. Distance during the walking test increased over time but was not different from the control group. However, no changes were observed in handgrip strength and TUG. These findings suggest that there is a link between vitamin D and muscle strength and function in older adults that may be observable in sarcopenic and non-sarcopenic older adults.

2.4.2. Dhesi, Bearne, Moniz, Hurley, Jackson, Swift, and Allain (2002)

The purpose of this study was to compare risk of falling in older adults categorized by vitamin D status, as well as to examine the relationship between vitamin D status and osteomalacia. Patients that had fallen at least once in the previous 8 weeks were enrolled into three groups based on 25(OH)D status: < 12 µg·L⁻¹ (n=20), 12 – 17 µg·L⁻¹ (n=20), and > 17 µg·L⁻¹ (n=20). Functional performance was determined with time to perform walking, ascending and descending stairs and standing up from a chair. Quadriceps strength and muscle activation was assessed using a strain gauge system and utilizing electrical stimulation during a voluntary isometric contraction. Postural stability was determined by examining body sway. Analyses of 25(OH)D, calcium, phosphate, alkaline phosphatase, and albumin were performed. Multiple regression analysis determined 25(OH)D as a significant and independent predictor for functional performance, quadriceps strength, and postural stability. These findings indicate that in individuals who fall, those with lower vitamin D have slower functional performance, weaker leg strength, and worse stability than those with higher vitamin D levels. This suggests that
vitamin D may have a role in the neuromuscular function necessary for strength and performance.

2.4.3. Visser, Deeg, and Lips (2003)

The purpose of this study\(^{143}\) was to examine if low serum 25(OH)D and high serum parathyroid hormone (PTH) concentrations were associated with loss of muscle strength and muscle mass over three years of follow up. Participants of the Longitudinal Aging Study Amsterdam (n=1509) aged 55 – 85 years were examined at baseline and three years later. Vitamin D and PTH status and change in grip strength and change in muscle mass were available in 1,008 and 331 participants, respectively. Sarcopenia was defined as a loss grip strength greater than 40% at follow up and a loss of appendicular skeletal muscle mass of greater than 3% at follow up. Fasted blood samples were collected for analysis of PTH and 25(OH)D. Data on potential confounding factors such as sex, age, height, BMI, physical activity level, creatinine, chronic disease, and season of data collection was determined. Sarcopenia based on grip strength and muscle mass was found in 136 and 52 individuals, respectively, at the three year follow up. Vitamin D deficiency (< 25 nmol·L\(^{-1}\)) were found in 9.6% of the population. Those with lower 25(OH)D levels were more likely to have a loss of grip strength and muscle mass over the three years. Even when adjusting for age and sex, those with vitamin D deficiency were more likely to have a reduction in grip strength. These findings indicate a relationship between lower 25(OH)D levels and an increased risk of sarcopenia, suggesting that vitamin D may have a role in maintenance of muscle mass and strength with age.

The purpose of this study\textsuperscript{162} was to examine if vitamin D + calcium supplementation would increase muscle strength to reduce the risk of falling by determining the number of falls and recurrent falls and changes in muscle function and markers of bone metabolism in older adults. Female older adults aged 60 years or older and had the ability to walk three meters with or without a walking aid were included. After a six week pre-treatment period, participants were randomized to two treatment protocols: a calcium group that received two tablets that were each 600 mg calcium carbonate daily and a vitamin D plus calcium group that received two tablets that were each 600 mg calcium and 400 IU of cholecalciferol daily. Comorbid conditions, cognitive impairments, number of drugs taken, and calcium intake were reported. Muscle function was assessed at baseline and after 12 weeks with the TUG test, leg extension and flexion strength, and grip strength. Fasted blood samples were analyzed for calcium, phosphate, albumin, alkaline phosphatase, 25(OH)D, 1,25-dihydroxyvitamin D, and PTH. Supplementation with vitamin D and calcium reduced falls by 49% in 3 months in older females with vitamin D deficiency. Additionally, compared to the calcium only group, the vitamin D and calcium group improved overall percent change in the tests of muscle function combined and also reported an improvement in bone metabolism, particularly in increased vitamin D levels. This indicates that vitamin D in addition to calcium has increased benefit for reducing falls, and might be at least partly explained by a potential role vitamin D has in improving muscle function.


The purpose of this study\textsuperscript{141} was to examine if there was a relationship between 25(OH)D concentrations and lower-extremity function in older adults, if this association differed based on activity level, and if there was a threshold with this association. This study examined participants (n=4,100) from the third National Health and Nutrition Examination Survey (NHANES III) who
completed the functional measurements and blood analysis. Lower-extremity function was examined with an eight foot walking speed test and a timed chair stand. Venous blood samples were taken and analyzed for 25(OH)D levels, which were divided into quintiles. Comorbidities, self-reported arthritis, activity level, use of a walking device, dietary intake, poverty-income ratio, and BMI were recorded for covariates. Vitamin D deficiency was prevalent in older adults and intake needed to reach adequate concentrations are higher than recommendations. Serum 25(OH)D concentrations and both tests of lower-extremity function were positively related and kept that relationship after adjustment of covariates. Concentrations at least 40 nmol·L⁻¹ and as high as 90 – 100 nmol·L⁻¹ may be beneficial for improving lower-extremity function.


The purpose of this study was to examine the effects of vitamin D supplementation on neuromuscular parameters known to be risk factors for falls and fractures. Patients aged 65 or older and participated in a fall clinic were recruited for this randomized, double-blind, placebo-controlled study. Recruited patients had to have at least one fall in the past eight weeks, 25(OH)D levels ≤ 12 μg·L⁻¹, and normal bone biochemistry. At baseline, medical history, mental score test, time spent outdoors, and BMI were recorded. Participants were instructed to record any falls over the intervention period. Functional performance was determined by time taken to complete a 50 foot walk, rising from a chair and walking 50 feet, and ascent and descent of 13 steps. These times were summed and calculated to determine the Aggregate Functional Performance Time. Psychomotor function was assessed by the four-choice reaction time. Postural sway was assessed with a balance test for 15 s to determine postural stability. Quadriceps strength was quantified by finding the MVC of the dominant leg using a strain gauge system. Blood samples were analyzed for calcium, phosphate, alkaline phosphatase, albumin,
PTH, and 25(OH)D. Participants were then randomized to receive active treatment of 600,000 IU ergocalciferol (n=61) or placebo (n=62) as an intramuscular injection. Baseline measurements were then repeated at six months following the intervention. The treatment group improved Aggregate Functional Performance Time by 2.0 second over six months, whereas the placebo group saw a 6.6 second decrease. Additionally, the treatment groups saw faster times for the four-choice reaction time and a 13% increase in postural stability compared to decreases in the placebo group. While both groups saw a decrease in strength, the placebo group had a greater amount of falls over the six months. This study emphasizes that vitamin D supplementation can improve neuromuscular function in older adults but did not have a positive effect on muscle strength. Perhaps vitamin D, combined with other nutrients important for muscle health is necessary to improve muscle strength in an older adult population.


The purpose of this study was to examine the effects of resistance training and vitamin D supplementation on physical performance in healthy older adults. Participants (n=96) aged ≥ 70 years and with 25(OH)D levels ≤ 16 ng·ml<sup>-1</sup> were randomized to a control or training group. Those groups were further randomized to receive either 800 mg of calcium or 800 mg calcium + 400 IU vitamin D daily for nine months. At baseline and at nine months, participants completed tests of body composition and bone mineral density, handgrip strength, maximal isometric quadriceps strength, endurance with a 12 minute walk, TUG and SPPB, body sway, and a fasting blood sample to analyze routine blood chemistry, insulin, serum thyroid stimulating hormone, PTH, and 25(OH)D. Those in the training group participated in session twice a week for 1.5 hours. While gait speed, muscle strength, and muscle function tests increased in trained subjects, TUG performance improved more in those also receiving vitamin D supplementation.
Additionally, those receiving vitamin D had a faster gait speed than those without supplementation, regardless of whether or not they were trained. These findings indicate that increasing vitamin D levels can have a beneficial effect on muscle function/performance with or without resistance training and may have important clinical outcomes for older adults that find it difficult to exercise.

2.4.8. Wicherts, van Schoor, Boeke, Visser, Deeg, Smit, Knol, and Lips (2007)

The purposes of this study\textsuperscript{138} include a) determine the association between vitamin D status and physical performance in a large group of older adults, b) examine whether lower vitamin D status increased the risk of a decline in physical performance over three years, c) evaluate which of the physical performance tests were most related to vitamin D status. Participants from a larger study were enrolled in this study and those who completed all tests were used for analysis (n=1234). The follow up three years later consisted of 979 of these participants. Physical performance was assessed by a walking test, chair stands, and balance tests. A decline in performance over the three-year follow was assessed by an index used to determine clinically significant change, the Edwards-Nunnally Index, and classified the change as improved, stable, or declined. Fasted blood samples were analyzed for 25(OH)D. Potential confounding factors such as age, sex, BMI, alcohol consumption, number of chronic diseases were collected. Levels of 25(OH)D below 10 ng·mL\textsuperscript{-1} were discovered in 10.9% and between 10 – 20 ng·mL\textsuperscript{-1} in 36.7% of the population. Those with lower vitamin D had lower performance scores, and were more likely to be women, older, have a higher BMI, and were less physically active. Those with the lowest vitamin D levels had the highest risk of physical performance decline at the three year follow up. The main findings of this study indicate that vitamin D status is related to physical performance not only cross-sectionally, but also with a decline over time.

The purpose of this study was to examine if 25(OH)D levels were associated with falls and physical performance in Japanese older adults. Older adult males (n=950) and females (n=2007) ≥ 65 years old with no history of malignant or other diseases affecting vitamin D regulation were interviewed to assess age, physical activity, chronic diseases, and fall experiences over the last year. Participants performed measurements of handgrip strength, stork walking (balance test), normal walking speed for five meters, and provided a nonfasted blood sample for analysis of 25(OH)D levels. Prevalence of falls was assessed by retrospectively asking about fall experiences within the previous year. When individuals with 25(OH)D levels < 20 ng·mL⁻¹ were compared to those with levels ≥ 20 ng·mL⁻¹, balance, walking speed and albumin were lower in those with low levels of 25(OH)D. Additionally, 25(OH)D was associated with all tests of physical performance and reduced risk of falls in women. These results indicate that low vitamin D status may be related to an increased risk of falls, which may be more prevalent in a sarcopenic population with reduced muscle mass and strength.

2.4.10. Moreira-Pfrimer, Pedrosa, Teixeira, and Lazaretti-Castro (2009)

The purpose of this study was to examine the 25(OH)D levels in different seasons and to compare the effects of seasonal and different levels of 25(OH)D supplementation on muscle strength in Brazilian institutionalized older adults. Older adults age 60 years and older were randomized into the calcium/placebo group (n=23) or the calcium/vitamin D group (n=23). Fasted blood samples for calcium and vitamin D were obtained at baseline and after 6 months of treatment. Muscle strength tests were performed to test maximum isometric strength of the hip flexors and knee extensors. At baseline, 10.7 and 3.6% of participants in the calcium/placebo and calcium/vitamin D group were deficient, respectively, while 53.7% and 67.9% were insufficient.
At 6 months, the vitamin D group increased levels by 84%. While there were no differences at baseline for strength tests, the vitamin D group had increased hip flexor and knee extensor strength by 16.4% and 24.7%, respectively. This indicates the potential for improving muscle strength with an improvement in vitamin D levels, suggesting a relationship between vitamin D levels and muscle strength maintenance.

2.4.11. Pfeifer, Begerow, Minne, Suppan, Fahrleitner-Pammer, and Dobnig (2009)

The purpose of this study was to examine the long-term effects and calcium and vitamin D on falls and parameters of muscle function in older adults 70 years or older. Participants (n=242) had serum 25(OH)D levels < 75 nmol·L⁻¹ and were randomly assigned to receive 500 mg calcium only (n=121) or 500 mg calcium + 400 IU vitamin D (n=121) twice daily for 20 months. Number of falls was recorded. Calcium and vitamin D intake was assessed by a food frequency questionnaire, and physical activity level, height, body sway, and TUG results were recorded. Maximum isometric leg extension was strength was determined. Fasted blood samples were collected for analysis of 25(OH)D, PTH, creatinine, and albumin. Supplementation of calcium + vitamin D for 20 months was effective in reducing the risk of falls. Additionally, by month 12 and to the end of the 20 month study, quadriceps strength was higher in the calcium + vitamin D group compared to baseline levels and to the calcium only group. These findings indicate that having adequate vitamin D levels may be an important contribution to reduced fall risk and/or the preservation or improvement of leg strength.


The purpose of this study was to compare the effects of calcium + vitamin D supplementation versus calcium only therapy on muscle strength, power, and functional mobility.
in vitamin D- insufficient female older adults. Female participants > 65 years old and had 25(OH)D levels between 25 and 50 nmol·L⁻¹ were randomly to receive 400 IU vitamin D + 500 mg calcium or 500 mg calcium + placebo daily for six months. At baseline and at six months, participants completed tests of isometric knee extension strength, handgrip strength, leg extension power, TUG, modified Cooper test of maximum walking in distance in two minutes and provided a non-fasting blood sample to be analyzed for 25(OH)D, 1,25 OHD, PTH, calcium, albumin, alkaline phosphatase, phosphate, and creatinine. Out of the 70 participants randomized, n=17 in the vitamin D + calcium and n=24 in the calcium + placebo groups were included in the per protocol analysis. At baseline, 25(OH)D levels were associated with knee extension and handgrip strength, leg extension power, TUG, and modified Cooper test; however, this relationship disappeared at six months, perhaps due to the increase in 25(OH)D levels in the vitamin D + calcium group. There were no differences between groups in any strength and function outcomes. These findings indicate while 25(OH)D levels are associated with strength and function, supplementation did not help to improve these outcomes.

2.4.13. Lips, Binkley, Pfeifer, Recker, Samanta, Cohn, Chandler, Rosenberg, and Papanicolaou (2010)

The purpose of this study was to examine whether vitamin D treatment of 8,400 IU once a week would improve body postural stability and lower-extremity function in elderly people with 25(OH)D levels ≤ 20 ng·mL⁻¹. Older adults ≥ 70 years old that met all inclusion criteria were enrolled in the 16-week randomized, double-blind, placebo-controlled, multicenter study and randomized to receive either a once-weekly dose of 8,400 IU vitamin D₃ or a placebo and were stratified according to baseline 25(OH)D concentrations ≤ 15 ng·mL⁻¹ or > 15 ng·mL⁻¹. Neuromuscular function was assessed by examining postural stability and SPPB. Postural
stability was measured with postural sway. The SPPB consists of assessments of balance, gait speed, and timed chair stand. Blood samples were analyzed for PTH and serum 25(OH)D concentrations. Postural sway or SPPB scores did not change at 16 weeks in either group. Serum 25(OH)D concentrations increased from approximately 14 to 26 ng·mL\(^{-1}\) over the 16 weeks in the treatment group, with no change in the placebo group. By week 16, there was a 13.0 ng·mL\(^{-1}\) difference between groups. Additionally, PTH decreased in the treatment group while increasing in the placebo group. While the intervention was enough to increase 25(OH)D levels, this increase did not have an effect on postural stability or SPPB scores in this older population.


The purpose of this study\(^{158}\) was to examine the association of 25(OH)D and 1,25(OH)\(_2\)D levels and skeletal muscle mass and strength in an age-stratified, random sample of adult males and females. Males (n=311) and females (n=356) between ages 21 – 97 years participated in anthropometric assessments of height and weight, body composition measurements of lean body mass, appendicular lean mass, and fat mass via DXA, and fasting blood samples, analyzed for serum 25(OH)D and 1,25(OH)\(_2\)D. Muscle strength was determined with handgrip force and isometric knee extension force. Those with lower 25(OH)D levels tended to have a higher BMI and fat mass; however, there were no associations with any measurements of muscle mass or strength. Lower levels of 1,25(OH)\(_2\)D were associated with lower muscle mass in both males and females, and with lower isometric knee extension force in females. Since this population was only modestly deficient in vitamin D, that may indicate levels were not severely low enough to contribute to sarcopenia. Therefore, future studies should examine if those with and without sarcopenia have differing levels of vitamin D.

2.4.15. Mastaglia, Seijo, Mozio, Somoza, Nunez, and Oliveri (2011)
The purpose of this study was to examine the relationship between vitamin D nutritional status and muscle function and strength in healthy female older adults. Fifty-four females over the age of 65 years were categorized into two groups: 25(OH)D levels ≥ 20 ng·mL⁻¹ (n=25) and 25(OH)D levels < 20 ng·mL⁻¹ (n=29). Occurrence of chronic diseases was determined, and sun exposure was evaluated. Dietary intakes of protein, calcium, vitamin D, and energy were determined with a food frequency questionnaire. Muscle function was determined with 8-foot walking speed, sit-to-stand, and balance tests. Muscle strength of hip flexors and abductors and leg extensors were assessed with a manual dynamometer, using the maximum value of three trials. Fasting blood samples were analyzed for calcium, phosphorus, and 25(OH)D. The calcium and creatinine ratio was determined from 24-hour urine samples. Lower limb lean mass was assessed by DXA, with sarcopenia determined using RSMI, calculated by dividing appendicular lean mass (kg) by height (m²). Compared to those with 25(OH)D ≥ 20 ng·mL⁻¹, those with lower 25(OH)D levels were more likely to have chronic diseases but did not have significant differences between weekly sun exposure or dietary intakes. While vitamin D levels were significantly different between groups, there were no differences in calcium, phosphorus, calcium/creatinine ratio, lean mass or presence of sarcopenia determined by RSMI were not different between groups. However, those with higher 25(OH)D had better performance scores on the muscle function tests (walking speed and sit-to-stand) and were stronger in the leg extensor and hip abductors tests, indicating the potential role adequate vitamin D status has on muscle strength and function.

2.4.16. Toffanello, Perissinotto, Sergi, Zambon, Musacchio, Maggi, Coin, Sartori, Corti, Baggio, Crepaldi, and Manzato (2012)
The purposes of this study\textsuperscript{92} include: 1) determining the relationship between vitamin D status and mobility in older adults by examining the relationship between 25(OH)D levels and physical performance tests that assess balance, gait speed, coordination, upper and lower limb strength, and aerobic capacity; and 2) identifying an adequate serum 25(OH)D level for skeletal muscle functions in older adults. Participants ≥ 65 years old in a large cohort in Italy that had 25(OH)D levels were included in this analysis (n=2,694). Physical performance measurements included the tandem test for balance, timed chair stand for coordination and strength, gait speed, 6-minute walking test for aerobic capacity, and handgrip and quadriceps strength for upper and lower limb muscle strength. Fasted blood samples were obtained for 25(OH)D levels. Participants were divided up into quintiles based on 25(OH)D concentrations. Levels of 25(OH)D were associated positively with four of the six performance tests (timed chair stand, gait speed, six minute walking test, and handgrip strength). Concentrations of 25(OH)D close to 100 nmol·L\textsuperscript{-1} were determined to be associated with greater benefit for skeletal muscle functioning in older adults, a level that is above the commonly reported optimal level of 75 nmol·L\textsuperscript{-1}, indicating that older adults may require additional vitamin D to reach optimal age-related levels for reduced risk of sarcopenia development.

2.4.17. Ceglia, Niramitmahapanya, da Silva Morais, Rivas, Harris, Bischoff-Ferrari, Fielding, and Hughes (2013)

The purpose of this study\textsuperscript{126} was to test if 4,000 IU of oral vitamin D\textsubscript{3} daily compared to a placebo effected total and/or subtype muscle fiber CSA and intramyonuclear VDR concentration over four months in mobility-limited females that were ≥ 65 years old and had moderately low vitamin D status. A secondary purpose of this study was to examine the effects of vitamin D on the proportion of type I and type II muscle fibers and urine nitrogen excretion as a marker of
muscle breakdown, and to confirmed effects of vitamin D₃ on muscle strength and function. Participants in this randomized, double-blind, placebo-controlled study were randomized to consume a supplement of 4,000 IU vitamin D (n=11) or a placebo (n=13) daily for four months. Participants had to meet all exclusion criteria including and SPPB score ≤ 9. Fasted blood samples were analyzed for serum 25(OH)D, and 24 hour urinary creatinine, urinary calcium and urinary nitrogen were measured. Muscle strength was determined by finding a one repetition maximum (RM) for leg extension of the non-biopsied leg at baseline and at four months. Muscle power (watts) was determined by performing five leg extension repetitions separated by 30 s as quickly as possible at 40% and 70% of 1RM at baseline and at four months. Muscle biopsies were taken from the vastus lateralis approximately one hour after a standardized meal and 24 hours after consumption of the last study capsule. Identification of fiber types within the muscle was performed and the relative proportion of type I, IIa, IIx, and hybrid muscle fibers were recorded, and changes in type I and II were analyzed. A subset of participants (n=14) contributed enough muscle tissue to analyze for VDR concentrations. The vitamin D group increased 25(OH) levels by 36.4 nmol·L⁻¹ while the placebo only saw a 4.2 nmol·L⁻¹ increase. However, there were no differences between the groups for urinary nitrogen, average power, and SPPB score. While there was a greater increase in total muscle fiber cross sectional area in the vitamin D group (10%), proportions of fiber types did not differ between groups. The vitamin D group saw a much larger percent change in VDR concentration compared to the placebo (29.7% versus 7.8%). This increase in VDR concentration after vitamin D supplementation indicates the potential effects vitamin D may have on muscle metabolism and function and indicates a possible role in increasing muscle fiber size through VDR.

The purpose of this study was to evaluate the influence of vitamin D status and PTH concentrations on changes in body composition, muscle strength, and indices of oral glucose tolerance in nondiabetic, older adults performing moderate intensity resistance training for 12 weeks to see if changes in body composition, muscle strength, and glucose tolerance would be lower in those with lower vitamin D status. Older adults (n=35) ranging from 50 – 80 years old that were nondiabetic were included in the retrospective analysis for this study. Measurements of body composition, muscle strength, oral glucose tolerance, blood concentrations, and skeletal muscle insulin signaling protein content were assessed at baseline and after a 12 week, three day per week full-body resistance training intervention. Additionally, participants consumed either a lower-protein (0.9 g·kg\(^{-1}\)·d\(^{-1}\)) or a higher protein (1.2 g·kg\(^{-1}\)·d\(^{-1}\)) diet during the 12 week intervention. Anthropometrics were measured and body composition was assessed with DXA. Muscle strength was assessed with 1RM tests for the seated row, leg extension, chest press, leg curl, and leg press. A whole-body strength summary was calculated as the sum of the five separate 1RM results. An OGTT was performed after a 10 hour fast using a 75 gram dextrose solution, with blood draws completed at baseline and 15, 30, 45, 60, 90, and 120 minutes after consumption. Homeostatic model assessment of insulin resistance (HOMA-IR) and insulin sensitivity index were calculated. Additionally, fasting blood samples were collected before and after the intervention to be analyzed for Hb A1c, 25(OH)D, and PTH. Muscle biopsies were taken from the vastus lateralis of the dominant leg to determine total insulin resistance, insulin resistance substrate-1, Akt, and protein kinase C. At baseline, seven participants had 25(OH)D concentrations < 50 ng·mL\(^{-1}\) but levels were not different between protein groups. Concentrations of 25(OH)D did not change over the intervention. Body composition and muscle strength improved over the intervention regardless of baseline vitamin D status. Glucose AUC
and two hour glucose decreased over the intervention, but there were no changes in fasting concentrations. While baseline vitamin D status did not influence changes in glucose tolerance from the resistance training intervention, it was found that those with low vitamin D status had higher glucose AUC and 2 hour glucose concentrations at both the pre- and post-intervention testing than those with normal levels, even after controlling for age and BMI. These findings indicate that although vitamin D levels had an effect on glucose tolerance, it was not associated with changes in muscle strength in this population. While more information is needed on the role vitamin D may have on muscle mass and strength, vitamin D did have a beneficial effect on glucose tolerance, indicating that higher levels may be important for maintaining metabolic flexibility with age.

2.4.19. Lagari, Gómez-Marín, and Levis (2013)

The purpose of this study was to examine the effectiveness of two doses of vitamin D₃ in improving measurements of physical performance in older adults, regardless of baseline 25(OH)D levels. Participants who were between 65 – 95 years old, ambulatory, and had a usual vitamin D intake that was less than 1,000 IU per day were randomized to receive either 400 IU or 2,000 IU of vitamin D₃ daily for six months. Levels of 25(OH)D, 4 meter gait speed, timed chair stand, single-leg balance, gallon-jug motor skill tests, and handgrip were used to evaluate physical performance at baseline and at six months. Body composition was assessed with DXA to determine changes in lean mass, lean mass of arms, lean mass of legs, and total fat mass. These variables were used to calculate total skeletal muscle mass, appendicular skeletal muscle index, and fat mass index. In both dosage groups, supplementation was more effective in those with low baseline values (< 30 ng·dL⁻¹), and that 400 IU may not be sufficient as a maintenance dose as individuals with sufficient baseline levels saw a decrease in 25(OH)D concentrations.
Neither group saw an increase in physical performance. However, increases in fat mass index was associated with declining levels of 25(OH)D, indicating that supplementation of higher doses may be necessary in those with higher fat mass.

2.4.20. Sinha, Hollingsworth, Ball, and Cheetham (2013)

The purpose of this study was to examine the effects of vitamin D₃ on skeletal mitochondrial oxidative function in participants who were vitamin D deficient. Participants (n=12) with severe vitamin deficiency (< 15 nmol·L⁻¹) and age-matched controls (n=15) (mean age 31 – 33 years) participated in phosphorus-31 magnetic resonance spectroscopy to quantify PCr, inorganic phosphate, and pH before and during an exercise protocol of the soleus and gastrocnemius muscles. Biochemistry assessments for the analysis of serum 25(OH)D at baseline and 10 – 12 weeks later. Mitochondrial oxidative phosphorylation rate was enhanced after vitamin D supplementation in those with severely low vitamin D levels. Additionally, increasing vitamin D levels all contributed to improvement of symptoms including myopathy and fatigue. These findings indicate a potential role of vitamin D in muscle metabolism.


The purpose of this study was to examine the association between 25(OH)D levels and vitamin D intake with muscle mass, strength, and physical performance in a pre-frail and frail elderly population. Pre-frail and frail participants (n=127) ≥ 65 years old who were free from cancer, chronic obstructive coronary disease, diabetes, or renal insufficiency provided fasted blood samples for analysis of 25(OH)D. Dietary intake was assessed with three-day dietary food records. Body composition, including lean body mass, appendicular lean mass, leg lean mass, and bone mineral content were determined with DXA scans. Muscle strength was evaluated with
IRM strength tests on the leg press and leg extension machines and by handgrip strength. Performance was assessed with the SPPB. Data including age, sex, height, weight, alcohol intake, physical activity level, season of data collection, education, serum creatinine, smoking, energy, and protein intake were collected as potential confounders. Levels of 25(OH)D was found to be related to appendicular skeletal mass, and both 25(OH)D levels and vitamin D intake were positively associated with SPPB score. These findings indicate the potential role vitamin D has on muscle health.


The purpose of this study was to examine the efficacy and safety of providing a nutritional supplement containing whey protein, leucine, and vitamin D compared to an isocaloric control supplement for improving characteristics of sarcopenia. Participants were enrolled in this 13 week, multi-center, randomized, controlled, double-blind, two parallel-group study if they met criteria for mild to moderate limitations in physical function, low skeletal muscle mass index, BMI between 20 – 30 kg·m⁻², no major cognitive impairment, and no comorbidities such as liver and kidney failure, anemia or acute inflammation determined as CRP > 10 mg·L⁻¹. Participants were randomized to the active (n=184) or control (n=196) product to be consumed twice daily. Handgrip strength was measured twice in both hands and the average of the maximum from both hands was used in analysis. The SPPB (gait speed, chair stand, and balance) was assessed. Appendicular muscle mass was assessed with DXA, and questionnaires were administered to obtain self-report of physical activity, activities of daily living, and health-related quality of life. Fasting glucose and insulin were measured at screening, and 25(OH)D and
IGF-1 were measured at baseline, week 7 and week 13. All participants had low muscle mass and a mean SPPB score of 7.5 out of 12. Handgrip strength increased by 0.79 kg in 13 weeks in the active group but did not significantly increase in the control. The SPPB scores increased in both groups, which chair stand time improving more in the active group. The active group also experienced a greater gain in appendicular muscle mass over time, with an estimated difference of 0.17 kg between groups. By week 13, 25(OH)D improved in the active group with an increase of 25.0 nmol·L⁻¹, whereas the concentrations in the control group had an average 6.0 nmol·L⁻¹ decrease. Serum IGF-1 also increase by 9.0 μg·L⁻¹ in the active group, with no changes in the control. These findings indicate the potential role of protein and vitamin D in improving muscle mass and strength, as well as concentrations of 25(OH)D and IGF-1 in older adults and shows a possible link between vitamin D levels and skeletal muscle.


The purpose of this study was to examine the effect of vitamin D supplementation alone on muscle function in younger postmenopausal females (aged 50 – 65 years). Participants were randomly assigned to receive a vitamin D₃ supplement of 1,000 IU (n=80) or a placebo (n=80) for nine months. Weight, height, waist circumference, and bone mineral density were assessed. Muscle strength was assessed via handgrip and the chair stand test. Lean body mass and body fat was assessed via DXA at baseline and after nine months. Plasma concentrations of 25(OH)D were measured at baseline and at nine months. Concentrations of 25(OH)D increased in the supplementation group and decreased in the placebo group. Additionally, the placebo group saw a decrease in lean muscle mass, whereas the vitamin D supplement group was able to preserve muscle mass over the nine months. Those who received the vitamin D supplement saw an increase in leg strength, whereas the placebo group saw no difference. These findings indicate
that vitamin D supplementation may prevent the development of sarcopenia and may be important for older adults to add in at the early stages of the aging process.


The purpose of this study was to examine the association between serum levels of 25(OH)D with mid-upper arm muscle circumference, handgrip strength, and length of hospital stay after hip fracture. Patients with a hip fracture not caused by pathological reasons and older than age 65 years (n=100) were evaluated. Anthropometrics (mid-upper arm circumference and triceps skinfold to calculate mid-upper arm muscle circumference), handgrip strength, and blood samples for serum biochemistry and 25(OH)D were collected. Additionally, age, sex, blood pressure and presence of diabetes were recorded. Patients were followed during the hospital stay and length of stay was recorded. While anthropometrics and laboratory values were not different between patients with normal versus low vitamin D, those with low vitamin D had lower handgrip strength and a higher mortality rate. Additionally, levels of vitamin D were associated with handgrip strength when adjusted for age and sex. This suggests a potential greater role in vitamin D in skeletal muscle strength compared to muscle mass. This is interesting in the progression of sarcopenia as strength tends to decrease sooner and at a quicker rate.

2.4.25. Iolascon, de Sire, Calafiore, Moretti, Gimigliano, and Gimigliano (2015)

The purpose of this study was to examine the association between serum levels of 25(OH)D, muscle strength of upper and lower limbs, and physical performance in post-menopausal women with low levels of 25(OH)D (< 30 ng·mL⁻¹) and normal levels (≥ 30 ng·mL⁻¹). Retrospective analysis of women ≥ 50 years old (n=80) who participated in outpatient
rehabilitation service for prevention and management of osteoporosis was performed. Upper limb strength was assessed with handgrip strength and lower limb strength was tested with the knee isometric extension strength test. The SPPB and 4 meter gait speed were performed to evaluate physical performance. Blood tests results were collected for levels of 25(OH)D, along with age, BMI, smoking and alcohol habits, sun exposure, physical activity, comorbidities, urinary calcium levels, pharmacological therapy, and history of fractures over 12 months as potential confounders. Low vitamin D status was found in 57.5% of the females analyzed. Those with low vitamin D status had lower upper and lower limb muscle strength and physical performance. These findings indicate that lower vitamin D levels may lead to increased risk for sarcopenia.

2.4.26. Verreijen, Verlaan, Engberink, Swinkels, de Vogel-van den Bosch, and Wijs (2015)

The purpose of this study was to compare a high whey protein-, leucine-, and vitamin D-enriched nutritional supplement with an isocaloric control over a 13 week weight-loss intervention that included a hypocaloric diet and resistance training on the effects of appendicular muscle mass preservation in obese older adults. Obese adults ≥ 55 years were randomized to receive the supplement (150 kcals, 20 g protein, 20 μg Vitamin D3, and 2.8 g leucine) (n=30) or the control product (150 kcals) (n=30). Body composition (appendicular muscle mass) was measured at baseline and at 13 weeks. Body weight, BMI, waist circumference muscle strength (handgrip), and physical functioning (400m walk, four meter gait speed, and chair stand test) were measured at baseline, week 7, and week 13. Participants followed a hypocaloric diet 600 kcals below estimated needs and participated in dietary counseling every two weeks. Three-day dietary recalls were completed at baseline, week 7, and week 13. All participants performed full-body resistance training three times per week for one
hour. While the weight loss intervention resulted in a decrease in body weight and fat mass in both groups, those in the experimental had a slight gain in muscle mass while the control group had a slight decrease. However, there were no differences in appendicular muscle mass for both groups over time. Both muscle strength and function improved over time in both groups. During weight loss in older adults, a protein and vitamin D supplement may be beneficial in preserving muscle mass but did not have additive benefits for muscle strength and function. However, adequate intake of protein and vitamin D may aid in the prevention of sarcopenia.

2.4.27. Cramer, Cruz-Jentoft, Landi, Hickson, Zamboni, Pereira, Hustead, and Mustad (2016)

The purpose of this study was to examine the effects of two high-quality oral nutritional supplements differing in amount and type of key nutrients in older adult males and females with combined malnutrition and sarcopenia. Participants were eligible to participate in this 24 week intervention study if they were 65 years or older and had both malnutrition and sarcopenia. Malnutrition was defined as a Subjective Global Assessment rating of B or C. Sarcopenia was defined as low grip strength (< 20 kg and < 30 kg in females and males, respectively) and/or low gait speed (<0.8 m·s⁻¹), as well as low skeletal muscle index. Participants were randomized to drink either two daily servings of a control nutritional supplement containing 14 g protein, 1 g fat, 44 g CHO, 147 IU vitamin D₃, plus additional micronutrients or an experimental nutritional supplement containing 20 g protein, 11 g fat, 36 g CHO, 1.5 g calcium β-methylbutyrate (CaHMB), 499 IU vitamin D₃, plus additional micronutrients. Additionally, participants were instructed to eat a diet with a minimum of 0.8 g·kg⁻¹·d⁻¹ of protein. At baseline and every 6 weeks for 24 weeks, participants visited the research facility to assess compliance, dietary intake, medication changes, and adverse events. At baseline, 12, and 24 weeks, a fasted blood draw for serum 25(OH)D, height, weight, body composition via DXA, leg strength with maximal
Voluntary isokinetic peak torque of leg extension, grip strength, and gait speed was assessed. Severe sarcopenia was defined as those with both low gait speed and low grip strength. Mild-moderate sarcopenia was defined as the sarcopenic participants who did not classify as severe. Peak torque improved in both groups at 12 and 24 weeks. Those taking the experimental supplement also saw an increase in 25(OH)D levels. Additionally, the severity of sarcopenia seemed to affect leg strength adaptations to supplementation, as those with mild-moderate sarcopenia were more responsive to the experimental supplement.

2.4.28. Rondanelli, Klersy, Terracol, Talluri, Maugeri, Guido, Faliva, Solerte, Fioravanti, Lukaski, and Perna (2016)

The purpose of this study was to examine if vitamin D supplementation would increase FFM compared to a placebo, while also improving strength, nutritional status, inflammation, and measures of quality of life and physical function. Eligible participants (n=130) from the geriatric division of a hospital were randomized into two groups. Body composition was measured by DXA, hydration status assessed by BIA, and the mini nutritional assessment evaluated nutritional status. A balanced diet was provided by the hospital kitchen. Handgrip strength was measured by a hand dynamometer. Fasting venous blood samples were collected and analyzed for cholesterol, triglycerides, total protein, bilirubin, iron, glucose, uric acid, creatinine, liver enzymes, CRP, Hb and other red blood cell indices, albumin, and IGF-I. Quality of life and daily functioning was assessed via questionnaires. All participants underwent a physical activity intervention five times per week for 12 weeks consisting of strength training, gait training, and balance. The intervention group was provided an essential amino acid, whey protein, and vitamin D (2.5 μg) mixture while the control was provided an isocaloric placebo. Fat-free mass, relative skeletal muscle mass, quality of life, handgrip strength, activities of daily living, mini nutritional
assessment, and IGF-I increased in the intervention group, while there was no change in the control. Additionally, the intervention group this increase in muscle mass and relative skeletal muscle mass advanced 68% of the participants in that group who were sarcopenic to being classified as non-sarcopenic. These findings indicate that a supplement of whey protein, essential amino acids, and vitamin D, along with strength training, can beneficially influence muscle mass in sarcopenic older adults. It is possible that supplementation effects on inflammation and IGF-I improved age-related loss of muscle mass, leading to the increased FFM seen in the intervention group with physical activity. These highlights the potential for adequate nutrition and vitamin D to be effective in reducing risk of inflammation and the development of sarcopenia.


The purpose of this study\textsuperscript{151} was to examine the association between 25(OH)D concentrations and muscle strength measured by handgrip strength and physical performance measured by the TUG in older adults \(\geq 85\) years. Participants from the Newcastle 85+ Study performed fasting blood samples for analysis of 25(OH)D and assessments of handgrip strength and the TUG test. Additional data including height, FFM, BMI, waist-hip ratio, number of chronic diseases, renal impairment, cognitive impairment, arthritis, requiring assistance to walk, physical activity, season of blood draw, vitamin D supplementation, and vitamin D medication were recorded as potential confounders. Participants were followed up at 1.5 years, three years, and five years. Older males with the lowest season-specific 25(OH)D concentrations showed a faster rate of decline in muscle strength over five years. However, the rate of decline in physical performance was not different across levels of 25(OH)D, indicating that muscle strength may be more influenced by vitamin D status than performance in older adults.
2.4.30. Kotlarczyk, Perera, Ferchak, Nace, Resnick, and Greenspan (2017)

The purpose of this study\(^{289}\) was to examine if supplementation of 800 IU per day was sufficient to maintain serum 25(OH)D levels above 20 ng·mL\(^{-1}\) in frail older adults and to examine associations between vitamin D deficiency with functional changes and falls over two years. Females age 65 or older (n=137) from long-term care facilities were followed for two years. Serum 25(OH)D levels were assessed at baseline, 12, and 24 months, with baseline values used to classify participants. A dose of 50,000 IU weekly were provided to those who were deficient to bring to levels > 20 ng·mL\(^{-1}\). All participants were provided vitamin D\(_3\) supplementation of 800 IU daily for 24 months. Functional tests assessing activities of daily living, physical performance (timed chair stand and gait speed over six meters), cognitive, and mental health were performed at baseline, 12, and 24 months. At baseline, 19% were deficient, 29% were insufficient, and 52% were sufficient. Those with insufficient or deficient levels indicated slower gait speed and lower activities of daily living score. Those with deficient levels also saw a greater decline in physical function over 12 months, even when levels were raised to above 20 ng·mL\(^{-1}\). This indicates that vitamin D levels may play a role in the development of reduced functional capacity and eventually sarcopenia.


The purpose of this study\(^{94}\) was to compare functional and nutritional status, body composition, and quality of life of older adults between age and sex-matched sarcopenic and non-sarcopenic older adults. A subsample of sarcopenic participants (n=66) from the PROVIDE study were recruited and matched with non-sarcopenic controls (n=66) for evaluation of baseline data. Body composition was assessed with DXA to determine appendicular muscle mass and fat
mass. Handgrip strength and SPPB were measured to assess muscle strength and function. Physical activity and quality of life were self-reported. Participants in both groups were categorized as non-frail, pre-frail, or frail. Malnutrition was assessed with the mini nutritional assessment short form and dietary intakes were estimated from a three day dietary intake record. Fasted serum samples were obtained and analyzed for 25(OH)D, vitamin B12, and folate. In general, there were no differences in malnutrition and intakes of energy, CHO, and fat, but sarcopenic individuals had lower protein intake relative to body weight than non-sarcopenic individuals. Vitamin D and vitamin B12 intakes were also lower in the sarcopenic group. Serum vitamin B12 was lower with sarcopenia, and there was a larger prevalence of vitamin B12 deficiency in sarcopenic compared to non-sarcopenic adults (26% vs. 11%). However, serum 25(OH)D concentrations were not different between groups, which may have been influenced by the larger number of blood draws in the wintertime in non-sarcopenic adults. Examining nutrients collectively and in association with sarcopenic characteristics may be useful in the future to determine parameters that may work together to prevent or treat sarcopenia.

2.4.32. Apaydin, Can, Kizilgul, Beysel, Kan, Caliskan, Demirici, Ozcelik, Ozbek, and Cakal (2018)

The purpose of this study was to examine and compare the effects and safety of a single high-dose with daily low-dose oral cholecalciferol on 25(OH)D levels in older adults with vitamin D deficiency or insufficiency. Postmenopausal women (n=60) ages 50 – 68 years old who had vitamin D levels below 20 ng·mL⁻¹ were randomized to receive either 800 IU vitamin D₃ daily (n=32) or a single oral dose of 300,000 IU (n=28) in a three month long clinical trial. Vitamin D concentrations and muscle strength of the quadriceps and hamstrings were measured with an isokinetic dynamometer at baseline, four weeks, and 12 weeks. At four and 12 weeks,
vitamin D levels increased in both groups but were higher in the single-dose than the daily dose group. While there were no differences between groups in muscle strength, the daily-dose group showed greater increases over time in both quadriceps and hamstring strength. These findings indicate that enhancing vitamin D levels in older females could have a positive effect on strength, and thus prevent or reduce risk of sarcopenia.


The purpose of this study is to examine the prevalence and predictors of vitamin D deficiency in frail older hospitalized patients. Participants (n=217) from a larger study were retrospectively analyzed to measure serum 25(OH)D levels of geriatric hospitalized patients. Concentrations below 20 ng·mL⁻¹ were considered deficient, 20 – 29.99 ng·mL⁻¹ were considered insufficient, and ≥ 30 ng·mL⁻¹ was adequate. Mean 25(OH)D levels was 12.7 ng·mL⁻¹ and 77.4% of patients were considered deficient. Almost 96% of patients had levels below 30 ng·mL⁻¹ indicating that older adults are at high risk for low vitamin D status, and that may be accentuated in frail or sarcopenic older adults.

2.4.34. Vaes, Tieland, Toussaint, Nilwik, Verdijk, van Loon, and de Groot (2018)

The purpose of this study was to examine the effect of supplementation with either vitamin D₃ and 25(OH)D₃ on muscle strength and physical performance in prefrail and frail, vitamin D-deficient older adults. Participants 65 years or older with serum 25(OH)D levels below 25 and 50 nmol·L⁻¹ and were frail or prefrail were randomized into one of three intervention groups to receive daily supplements of 10 µg 25(OH)D₃ (n=26), 20 µg vitamin D₃ (n=24) or placebo (n=25) for six months. Lower extremity strength was determined with
maximal leg-extension and leg-flexion torque. Upper extremity strength was determined by measuring handgrip strength. Physical performance was assessed with the TUG and SPPB. Postural sway was assessed. Fasting blood samples were analyzed for serum 25(OH)D concentrations. Muscle biopsies from the vastus lateralis were collected from a subgroup (n=35). Body composition was assessed by DXA to calculate appendicular lean mass. Dietary intakes of vitamin D and calcium were assessed with a food frequency questionnaire. While supplementation was effective in increasing 25(OH)D levels to 99 and 72 nmol·L\(^{-1}\) for the 10 μg 25(OH)D\(_3\) and 20 μg vitamin D\(_3\), respectively, there were no effects on muscle strength or physical performance. It is thought that levels higher than observed are necessary to improve muscle function. Most of the older adults in this study were considered pre-frail, so examining if there are differences in baseline 25(OH)D levels between very distinct sarcopenic and non-sarcopenic groups may provide more insight on relationships between vitamin D levels and muscle strength and function.

2.4.35. Verlaan, Maier, Bauer, Bautmans, Brandt, Donini, Maggio, McMurdo, Mets, Seal, Wijers, Sieber, Boirie, and Cederholm (2018)

The purpose of this study\(^{137}\) was to see if baseline nutritional status could influence the efficacy of a vitamin D and protein intervention compared to an isocaloric control in sarcopenic older adults in the PROVIDE study. Participants from the larger PROVIDE study were assessed for eligibility to enroll (SPPB score 4 – 9, class I or II sarcopenia, and BMI of 20 – 30 kg·m\(^{-2}\)). Participants were randomized to an active or control group, and received a product containing 20 g whey protein, 3 g leucine, a mixture of carbohydrates and fat providing 150 kcals, 800 IU vitamin D, and a mixture of fibers and micronutrients or an isocaloric control product with no protein or micronutrients as two servings per day for 13 weeks. Questionnaires assessing self-
reported physical activity, nutritional status, mental state, and depression were assessed at baseline. Appendicular muscle mass was measured at baseline and at 13 weeks via DXA. The SPPB was assessed, and maximum handgrip strength was determined from the average of the highest measurement from each hand. Fasted blood samples were analyzed for serum 25(OH)D, which was used as a dichotomous variable (cut-off was considered 50 nmol·L$^{-1}$). A three day food record was conducted at baseline and week 13. At baseline, individuals with 25(OH)D < 50 nmol·L$^{-1}$ tended to have lower scores for physical activity, mental state, and nutritional status, and higher scores for depression, and lower body weight, appendicular muscle mass, muscle strength and function compared with those with higher baseline 25(OH)D levels. Additionally, mean fat and lean body mass and dietary intakes of vitamin D and protein were not different between 25(OH)D groups at baseline. When examining changes over time between the active and control group, the 25(OH)D concentrations increased in the active group compared to baseline, with a greater increase observed in those with lower 25(OH)D concentrations (38.5 nmol·L$^{-1}$) than those with higher concentrations (25.3 nmol·L$^{-1}$). Those with higher baseline 25(OH)D also saw greater increases in muscle mass than those with lower concentrations; however, there was no difference in chair-stand time between these groups based on the intervention. Those with higher baseline protein intake also saw a higher increase in muscle mass, but there was no difference in chair stand time based on baseline protein intake. In this study, sarcopenic participants with higher 25(OH)D levels and a higher baseline protein intake saw greater improvement in muscle mass in response to the vitamin D and leucine protein supplement, but no effect on lower-extremity function measured by the chair-stand. This indicates that adequate dietary intakes of vitamin D and protein-rich foods may be influential in the treatment or prevention of sarcopenia.
2.4.36. Aspell, Laird, Healy, Lawlor, and O’Sullivan (2019)

The purpose of this study was to examine the prevalence of impaired muscle function with handgrip strength and SPPB performance and to determine the association between handgrip strength and SPPB with serum vitamin D status, as well as the association between vitamin D status and falls, in older adults from the English Longitudinal Study of Aging. Participants were included if they completed the 25(OH)D measurement, were ≥ 60 years old, and performed measurements of handgrip strength and SPPB. Fasted blood samples were obtained for analysis of 25(OH)D. Vitamin D deficiency was defined as < 30 nmol·L\(^{-1}\) and insufficiency as < 50 nmol·L\(^{-1}\). Socio-demographic variables, health status, BMI, waist circumference, physical activity, alcohol intake and vitamin D supplement usage was recorded as potential confounders. Vitamin D insufficiency and deficiency was prevalent in 53.7% and 21.8% of the population. When separated into quintiles by 25(OH)D levels, there were a greater amount of older adults with impaired handgrip strength and SPPB performance in the lowest quintile compared to the others; however, rate of falls was not different between levels. Additionally, after adjusting for confounding factors, vitamin D deficiency was associated with low SPPB performance and was a predictor of low handgrip strength. These findings indicate that preventing vitamin D deficiency may be important for maintaining muscle function in older adults.


The purpose of this study was to use cross-sectional and prospective data from a population-based study to examine associations of baseline 25(OH)D levels with the prevalence and incidence of sarcopenia, and to changes in muscle parameters in German older adults. Participants who completed the baseline and follow up assessments after three years (n=702)
were included in this analysis. Muscle mass was estimated with BIA values to determine muscle mass index. Handgrip strength, gait speed, and TUG tests were measured. Non-fasting blood samples were obtained to analyzed serum 25(OH)D concentrations. Information including sex, age, nutrition score, physical activity, BMI, and use of vitamin D supplements were collected as confounding variables. Low levels of 25(OH)D were associated with changes in muscle mass and TUG score, indicating that low vitamin D status may a risk factor for development of sarcopenia.

2.4.38. Hajj, Fares, Chardigny, Boirie, and Walrand (2019)

The purpose of this study was to examine the effect of vitamin D supplementation on handgrip strength and appendicular skeletal muscle mass in pre-sarcopenic older subjects in a six month randomized, controlled, double-blind study and if the effect was different in normal-weight compared to obese pre-sarcopenic individuals. Participants who were determined as pre-sarcopenic based on Skeletal Muscle Index, deficient in vitamin D (25(OH)D < 20 ng·mL\(^{-1}\)), and no history of type 2 diabetes (n=128) were randomized to receive either vitamin D supplementation of 10,000 IU of cholecalciferol or a placebo and a total of 115 completed the study. Assessments of handgrip strength, appendicular muscle mass from BIA, and 25(OH)D concentrations were analyzed at baseline and six months later. Additionally, weight, fat mass, and muscle mass were also determined from BIA. Dietary intake was assessed with a questionnaire at baseline. In the pre-sarcopenic participants, 51% were normal weight and 49% were obese. Serum 25(OH)D levels increased from approximately 10 to 28 ng·mL\(^{-1}\) in the vitamin D supplement group (195% change). While the placebo group also increased (11 to 16 ng·mL\(^{-1}\)), the percent change was significantly different between groups. Additionally, the vitamin D supplement group experienced an increase in handgrip strength and appendicular
skeletal muscle mass at six months, while the placebo group showed no change. While there was a difference between groups for muscle mass, values for muscle strength were not different between groups. Fat mass, body weight, and waist circumference decreased after vitamin D supplementation, indicating that increasing vitamin D concentrations have a beneficial effect on fat mass and muscle mass and may be preventative for sarcopenia.


The purpose of this study was to examine if 13 weeks of nutritional supplementation affected circulating inflammatory markers in older sarcopenic adults enrolled in the PROVIDE study. Participants from the larger PROVIDE study were assessed for eligibility to enroll (SPPB score 4 – 9, class I or II sarcopenia, and BMI of 20 – 30 kg·m²). Participants were randomized to an active or control group, stratified for SPPB categories and study center. To be included in analysis, participants needed to provide inflammatory markers at baseline, leaving n=137 in the active group and n=151 in the control group. Participants received a product containing 20 g whey protein, 3 g leucine, a mixture of carbohydrates and fat providing 150 kcals, 800 IU vitamin D, and a mixture of fibers and micronutrients or an isocaloric control product with no protein or micronutrients as 2 servings per day for 13 weeks. At baseline, demographics, cognitive function, and pre-existing medical conditions were assessed. At baseline, seven weeks, and 13 weeks, assessments of handgrip strength, body composition (DXA), physical performance and activity (Physical Activity Scale for the Elderly), dietary intake (three day dietary record), and blood samples (IL-8, IL-1Ra, sTNFR1, IL-6, CRP, pre-albumin, 25(OH)D) occurred. A higher intake of vitamin D and 25(OH)D levels were related to lower levels of IL-8, and a lower SPPB was related to higher baseline cytokine levels. IL-6 and IL-1ra increased over
the 13 weeks, in which IL-6 only increased in the control group. Linear regression indicated that changes in pre-albumin over the 13 weeks, but not serum 25(OH)D, were associated with changes in IL-6. This study indicates that a whey protein supplement with leucine and vitamin D may help prevent increases in inflammatory cytokines in sarcopenic older adults.

2.4.40. Orces, Lorenzo, and Guarneros (2019)

The purpose of this study was to examine nationwide prevalence and causes of 25(OH)D inadequacy, and the effect of vitamin D supplements on 25(OH)D2 and 25(OH)D3 concentrations in older adults. Participants aged 60 years or older who completed the NHANES survey from 2007 – 2014 and had data on BMI, dietary vitamin D intake, and 25(OH)D concentrations were included in this analysis (n=6,261). A 24 hour dietary recall was used to collect vitamin D intake and supplementation. Vitamin D intake was divided into three categories: <400 IU, 400 – 800 IU, and > 800 IU·day⁻¹. Concentrations of 25(OH)D were measured with liquid chromatography-tandem mass spectrometry. Mean daily intake was 4.7 μg and 17.9 μg from food and supplements, respectively. Hispanics and non-Hispanic blacks, lower poverty level, smokers, physical inactivity, and older adults had lower 25(OH)D concentrations. Prevalence of 25(OH)D deficiency and inadequacy was 4% and 17.4%, respectively. Those who were obese, non-Hispanic blacks, and Hispanics were more likely to have 25(OH)D inadequacy. Those who had a total vitamin D intake of < 400 IU·day⁻¹ were 6.5 times more likely to have low vitamin D status than those between 400 – 800 IU·day⁻¹. The findings of this study indicate that vitamin D inadequacy is prevalent in U.S. older adults. Additionally, despite the fact that high/adequate 25(OH)D levels were observed in those who took vitamin D supplements, only about half of the participants were taking supplements. These findings indicate the low vitamin D status is a problem for older adults and may be preventable with dietary intake or
supplementation. This may not only improve vitamin D status but may also have other beneficial effects.


The purpose of this study\textsuperscript{291} was to determine the association of 25(OH)D concentrations with physical performance and frailty status. Participants (n=756) 65 years or older that attended a screening visit for a larger study were assessed for 25(OH)D status and frailty criteria (gait speed, handgrip strength, physical activity, weight loss, and self-reported exhaustion). Additional measurements of muscle strength (leg extension strength) and physical function (gait speed and TUG test) were available for 494 of the participants. Frailty status was assessed, as well as covariates including BMI, age, sex, ethnicity, physical activity, vitamin d supplement use, smoking status, alcohol intake, and number of chronic diseases. Those with lower 25(OH)D levels performed worse on the gait speed and TUG test, but there were no relationships seen with handgrip or leg extension strength. Those with 25(OH)D levels < 50 nmol·L\textsuperscript{-1} were about two time more likely to be categorized as frail. Additionally, higher levels of 25(OH)D than recommended may be necessary in older adults for adequate muscle function, thus supporting the idea that 25(OH)D levels may be different in older adults with adequate muscle mass and function compared to those who have developed sarcopenia.


The purpose of this study\textsuperscript{156} was to examine the relationship between vitamin D and handgrip strength in adults stratified by 50 years old. Participants from the Tianjin Chronic Low-grade Systemic Inflammation and Health Cohort (n=5102) who participated in analysis of serum
25(OH)D and measurement of handgrip strength were included in this study. In addition to assessing handgrip strength and fasting 25(OH)D concentrations, height, weight, waist circumference, demographic and socioeconomic variables, physical activity, alcohol and smoking status, sleep duration, depressive symptoms, a food frequency questionnaire, and medical history/comorbidities were also collected as potential confounders. There were positive relationships discovered between 25(OH)D and handgrip strength in males above age 50 years, but not in males below age 50. This relationship was not present in females. This study indicates that in males older than 50 years, those with lower 25(OH)D levels had weaker handgrip strength than those with higher 25(OH)D levels, suggesting that vitamin D may be important for strength in aging males. It is unclear why this relationship was not present in females. Future studies in older adults should re-examine sex differences and also see if there are differences in older adults with low and normal muscle mass.

2.4.43. Bischoff-Ferrari, Vellas, Rizzoli, Kressig, da Silva, Blauth, Felson, McCloskey, Watzl, Hofbauer, Felsenberg, Willett, Dawson-Hughes, Manson, Siebert, Theiler, Staehelin, Molino, Chocano-Bedoya, Abderhalden, Egli, Kanis, and Orav (2020)

The purpose of this study is to examine if vitamin D, omega-3s, and a strength training program, alone or in combination, would improve cardiovascular health, bone health, muscle health, brain health, and immunity in older adults without major co-morbidities. Participants (n=2157) age 70 years or older with no major health events in the past five years and taking no more than 800 IU of vitamin D were randomized into one of eight treatment groups. Participants were followed for three years with visits at baseline, 12, 24, and 36 months and telephone calls every three months. Measured outcomes included systolic and diastolic blood pressure, non-vertebral fractures, SPPB, the Montreal Cognitive Assessment, and infections. None of the
treatments were effective in improving any of the six outcomes. However, 83% of the participants were already engaging in moderate to high physical activity. Determining the vitamin D status of non-active sarcopenic and non-sarcopenic adults may provide more insight on the associations between vitamin D and muscle mass, strength, and performance.

2.4.4. Lengele, Moehlinger, Bruyère, Locquet, Reginster, and Beaudart (2020)

The purpose of this study was to examine the effect of variations of macro- and micronutrient intakes on muscle strength and physical performance in an observational cohort study of 534 older adults. Data was analyzed in participants who completed a food frequency questionnaire twice: after the second (T1) and last year of follow up (T2) (n=238). Daily amount of nutrients was calculated (frequency x portion size) for energy intake, protein, lipids, saturated fatty acids, polyunsaturated fatty acids, omega-3 and 6 fatty acids, monounsaturated fatty acids, CHO, sodium, potassium, magnesium, phosphorus, iron, calcium, zinc, and vitamins D, A, E, C, and K. Physical performance was assessed with gait speed over four meters. Muscle strength was assessed with handgrip strength. Data such as age, sex, BMI, smoking status, number of comorbidities, number of drugs consumed, and level of physical activity were collected as possible confounding factors. Overall, participants had lower energy and CHO intake and higher saturated fatty acids at T2 compared to T1. Additionally, there were lower intakes of sodium, magnesium, iron, calcium, and zinc at T2. When examining a cross-sectional with baseline data, muscle strength was associated with energy intake, omega-3 fatty acids, potassium, and vitamins D, A, and K. However, there was no relationship between changes in nutritional intakes and changes in muscle strength and performance over time. This study findings indicate that higher intake of nutrients, including vitamin D are associated with greater muscle strength. However, in this population, changes in muscle strength did not seem to be related to the decline in nutrient
intakes over time. This population was healthy, non-sarcopenic adults, so future studies should examine how nutrient intakes such as vitamin D impact strength in sarcopenic adults.

2.5. Vitamin D and Inflammation and Immune Function

2.5.1. Barnes, Horigan, Cashman, Hill, Forsythe, Lucey, McSorley, Kiely, Bonham, Magee, Strain, and Wallace (2011)

The purpose of this study was to examine the effect of a cholecalciferol (D₃) intervention of 5, 10, and 15 μg·d⁻¹ compared to a placebo on pro-inflammatory cytokines at wintertime in apparently healthy younger and older adults. This double-blind, placebo-controlled vitamin D intervention included young adults (n=239) and older adults (n=222) that were randomly assigned to receive a dose of D₃ (or placebo) daily for 22 weeks. Height and weight were recorded to calculate BMI. Fasting blood samples were obtained and analyzed for 25(OH)D, CRP, IL-6, IL-10, TNF-α, and fibrinogen. Incidence and severity of respiratory tract infections were determined every two weeks in the older adults. Younger participants had higher concentrations of 25(OH)D and lower concentrations of inflammatory markers than observed in the older adults. Vitamin D insufficiency was more prevalent in older adults and levels were negatively correlated with IL-6. Older adults saw an increase in 25(OH)D in the 10 and 15 μg·d⁻¹ groups and a decrease in the control groups. However, there were no changes in cytokine concentrations in any of the groups for the younger or older adults. It is possible that a higher dose of vitamin D₃ may be necessary to elicit a response in cytokine concentrations. While there were no significant improvements, these findings provide evidence that lower levels of vitamin D are related to higher levels of inflammation, which may be contributory to inflammaging.
2.5.2. Barker, Martins, Hill, Kjeldsberg, Henriksen, Dixon, Schneider, Dern, and Weaver (2012)

The purpose of this study\textsuperscript{115} was to examine the influence of different doses of supplemental vitamin D on inflammatory cytokines and muscular strength in young adults. Participants (aged 18 – 45 years old) who were recreationally active were randomized to receive either 200 IU vitamin D (n=10), 4,000 IU vitamin D (n=10), or a placebo (n=10) for 28 days. Fasted blood draws were performed at baseline and 7, 14, 21, and 28 days after supplementation and analyzed for 25(OH)D levels and cytokine concentrations (IFN-$\gamma$, IL-5, and IL-10) that are important for immunity and regulated by vitamin D. Leg strength and power tests were performed at baseline and at 28 days post supplementation. Levels of 25(OH)D improved in both the 400 IU and 2,000 IU groups, with a greater increase in the 4,000 IU group. While IFN-$\gamma$ and IL-10 were not different, there was a decrease in IL-5 in the placebo group and an increase in the 200 IU group that was not present in the 4,000 IU group. These findings indicate that even a moderate increase in vitamin D intake can help maintain 25(OH)D and IL-5 concentrations in this population. While there were no changes seen in muscle strength and power, these findings have important implications for the role vitamin D may have on immune function, and therefore, inflammaging.

2.5.3. Shab-Bidar, Neyestani, Djazayery, Eshraghian, Houshiarrad, Kalayi, Shariatzadeh, Khalaji, and Gharavi (2012)

The purpose of this study\textsuperscript{112} was to examine the effect of vitamin D intake on both glycemic status and inflammatory markers in Iranian individuals with type 2 diabetes. Participants (n=100) were randomized into two groups receiving either a yoghurt drink (placebo) (n=50) or vitamin D-fortified yoghurt drink (treatment) (n=50) supplying 1,000 IU vitamin D$_3$ per day for 12 weeks. Blood samples were obtained to evaluate glycemic status, insulin
resistance, 25(OH)D concentrations, and inflammatory status (CRP, serum amyloid A, IL-2, IL-6, IL-10, and TNF-α). Fat mass was also determined. Serum 25(OH)D increased in the treatment group, along with an improvement in fat mass and insulin resistance. While the placebo group showed increases in IL-6 and CRP and a decrease in IL-10, the treatment group showed decreases in CRP and serum amyloid A. Additionally, improvement in vitamin D status in the treatment group compared to the placebo group saw simultaneous decreases in IL-6, TNF-α, CRP, and serum amyloid A and an increase in IL-19. These findings indicate that an increase in 25(OH)D resulted in a decrease in inflammatory markers, suggesting that vitamin D levels have a role in reducing inflammation that may be mediated by insulinemia and glycemia status. This suggests a potential relationship between vitamin D levels and presence of metabolic flexibility, mediated by inflammation.

2.5.4. Wamberg, Kampmann, Stødkilde-Jørgensen, Rejnmark, Pedersen, and Richelsen (2013)

The purpose of this study was to examine if an increase in 25(OH)D levels would reduce ectopic lipid accumulation and have beneficial effects on chronic low-grade inflammation, insulin resistance, hypertension, and dyslipidemia. Obese individuals with low levels of 25(OH)D (< 50 nmol·L⁻¹) and no history of diabetes, impaired kidney function, hepatic function, osteomalacia, or substance abuse were recruited with 43 participants available for analysis. Participants were randomized to 26 weeks of treatment of either a daily dose of 7,000 IU vitamin D₃ or similar placebo tablets. Assessment of medical history, smoking, and physical activity level were determined. Height, weight, waist circumference, and blood pressure were measured. Body composition was assessed by DXA (total fat mass, total lean body mass, percent body fat) and MRI (abdominal subcutaneous and visceral adipose tissue, intrahepatic lipids, intramyocellular lipids). Fasted blood samples were analyzed for cholesterol, triglycerides,
25(OH)D, glucose, insulin, CRP, IL-6, MCP-1, adiponectin, leptin, MMP-9, PAI-1, and osteopontin, and HOMA-IR was calculated. During the first two weeks, a three day dietary record was completed for assessment of vitamin D and calcium intake. Levels of 25(OH)D in the vitamin D group increased from 33 to 110 nmol·L⁻¹ and from 34 to 47 nmol·L⁻¹ in the placebo group. However, there were no differences in body composition, ectopic fat accumulation, or metabolic and inflammatory risk factors between groups in obese population. This indicates that vitamin D treatment alone may be unable to influence changes; however, vitamin D along with improvements in other nutritional and metabolic factors may show results in populations prone to chronic inflammation.

2.5.5. De Vita, Lauretani, Bauer, Bautmans, Shardell, Cherubini, Bondi, Zuliani, Bandinelli, Pedrazzoni, Dall’Aglio, Ceda, and Maggio (2014)

The purpose of this study was to evaluate whether serum 25(OH)D levels are inversely associated with high-sensitivity CRP, TNF-α, sTNFR1 and sTNFR2, IL-1ra, IL-1β, IL-10, IL-18, IL-6, and IL-6 soluble receptors (sIL-6r and sgp130). Participants (n=867) were part of the larger InCHIANTI study that had blood analysis data and did not potential indicate signs of having active inflammation. Fasting blood samples were analyzed for 25(OH)D, PTH, CRP, TNF-α, sTNFR1 and sTNFR2, IL-1ra, IL-1β, IL-10, IL-18, IL-6, sIL-6r and sgp130. Potential confounders such as obesity, physical activity, smoking habits, dietary intake, disability, cognitive function, depressive status, chronic disease, vitamin D supplementation, number of medications, and season of blood collection were assessed. Participants were divided into three tertiles based on 25(OH)D levels. Higher levels of IL-6, sIL-6r, hsCRP, sgp130, TNF-α, sTNFR1, and sTNFR2 were associated with the lowest tertile (<31.4 nmol·L⁻¹). There were also inverse associations between 25(OH)D and IL-6, hsCRP, and sgp130 and positive correlations
with sIL6r. These relationships were still present after accounting for confounding factors. These findings emphasize a relationship between 25(OH)D levels and inflammatory markers that affect immune system function in an elderly population and indicate the need to see if this relationship differs in older adults with low versus normal skeletal muscle strength and mass.


The purpose of this study\textsuperscript{116} was to examine the association between vitamin D status and immune markers of inflammation in older adults. Participants (n=957) from a larger study who were ≥ 60 years old and met all the inclusion criteria were assessed for demographical and anthropometric measurements. A non-fasting blood sample was collected for analysis of 25(OH)D\textsubscript{2}, 25(OH)D\textsubscript{3}, and total 25(OH)D, CRP, TNF-\textgreek{a}, IL-6, and IL-10. Those with sufficient vitamin D status tended to be older and leaner. A higher vitamin D status was also concomitantly observed with lower IL-6, CRP, and ratios of IL6:IL-10 and CRP:IL-10, after adjustment for age, sex, and BMI. A negative relationship was present between 25(OH)D levels and IL-6, CRP, and IL-6:IL-10. Individuals who were vitamin D deficient were more likely to have a large IL-6:IL-10 ratio. The findings of this study indicated that low vitamin D status was associated with a more pro-inflammatory state and has potential to influence the inflammatory response in an older adult population.

2.5.7. Han, Jones, Tangpricha, Brown, Hao, Hebbar, Jeong Lee, Liu, Brown, Ziegler, and Martin (2016)

The purpose of this study\textsuperscript{122} was to examine the safety and efficacy of 250,000 and 500,000 IU vitamin D\textsubscript{3} given in doses divided over 5 consecutive days to increase 25(OH)D
concentrations to levels > 30 ng·mL⁻¹ and increase LL-37, an anti-microbial peptide, in adult ventilated patients in the intensive care unit. Patients (n=31) were randomized into either a placebo, a 250,000 IU, or a 500,000 IU group. Sequential organ failure assessment scores, laboratory values, and other clinical data were collected daily. Venous blood was collected at baseline and at seven and 14 days to analyze 25(OH)D and LL-37 levels. Vitamin D deficiency (<20 ng·mL⁻¹) was present in 43% of participants and 40% were insufficient (20 – 30 ng·mL⁻¹). Concentrations of 25(OH)D increased in both treatment groups by day seven, while there was no change in the placebo. While LL-37 levels did not change significantly with 25(OH)D concentrations, the length of stay was much shorter for the groups that received vitamin D supplementation with the length of stay 36 days in the placebo group, 25 days in the 250,000 IU group, and 18 days in the 500,000 IU. Additionally, the high-dose supplementation was well-tolerated and did not result in significant adverse events. These findings suggest that increasing vitamin D levels in critically ill patients who have low vitamin D status may have a positive impact on decreasing the length of stay necessary in an intensive care unit. This finding emphasizes the importance vitamin D levels may have on inflammatory status and immune function.

2.5.8. Han, Alvarez, Jones, Tangpricha, Brown, Hao, Brown, Martin, and Ziegler (2017)

The purpose of this study was to examine the impact of high-dose vitamin D₃ supplementation on free 25(OH)D concentrations, the relationship between free 25(OH)D and antimicrobial proteins (LL-37 and hBD-2), and relationships between free 25(OH)D and the above mentioned anti-microbial proteins to alveolar function in critically ill adults with respiratory failure. Patients (n=30) were randomized into a placebo group or an intervention group receiving either 250,000 IU or 500,000 IU of vitamin D₃ over 5 consecutive days. Blood
was collected at baseline and at seven and 14 days to determine free and total 25(OH)D, LL-37, hBD-2, and mRNA expression of hCAP18. Broncho alveolar lavage collection was performed. In the placebo group, 25(OH)D levels did not change, whereas both vitamin D supplementation groups showed an increase in 25(OH)D levels from baseline by day 7, with a further increase seen by day 14 in the 500,000 IU group. There were no changes over time for LL-37, or hBD-2, and there was no correlation present between 25(OH)D and LL-37 or hBD-2 concentrations. There was also no correlation between 25(OH)D and alveolar function indices. However, the percent increase in 25(OH)D was associated with the percent increase in hCAP18 mRNA expression at days 7 and 14. These findings indicate vitamin D levels can increase in critically ill patients in response to high-dose supplementation and that the changes in vitamin D status may have beneficial effects on infections and inflammatory outcomes.

2.5.9. Alves, Ishimura, Duarte, and Bueno (2018)

The purpose of this study is to examine changes in certain immune system markers in individuals 80 – 100 years old and if there is a relationship with vitamin D levels. Young (n=10; male, n=5 and female, n=5) and old (n=12, male, n=6 and female, n=6) were evaluated as part of a larger epidemiologic survey. Fasted blood samples were collected and analyzed for metabolic data, cytomegalovirus IgM and IgG, and 25(OH)D. Cell cultures were obtained and evaluated for T lymphocytes. Culture supernatants were assayed for cytokines (IL-1, IL-2α, IL-6, IFN-γ, and TNF-α). Older participants were considered reasonably healthy but still indicated signs of immunosenescence (increased MDSC, decreased leukocytes, reduced CD8+ and CD8+ naïve T cells). Approximately half of the older adults were vitamin D deficient. Additionally, vitamin D levels were positively related to CDH8+ T cells, indicating that vitamin D could be helpful in
preventing the decrease in these cells, and that vitamin D may have a direct effect on immunity, and may be related to the presence and/or severity of inflammaging and immunosenescence.

2.5.10. Goncalves-Mendes, Talvas, Dualé, Gutman, Corbin, Marceau, Sapin, Brachet, Evrard, Laurichesse, and Vasson (2019)

The purpose of this study was to examine the effect of vitamin D supplementation on the immune response to influenza vaccination in vitamin D deficient older adults by evaluating cathelicidin status and antibody response to vaccine, cytokine production, and ROS production. Participants aged 65 years or older with vitamin D levels < 30 ng·mL$^{-1}$ were randomly assigned to either a supplementation group receiving six vitamin D doses over three months (100,000 IU) before receiving an influenza vaccination or a placebo group that received a placebo for three months followed by an influenza vaccination. 25(OH)D, cathelicidin, serum antibodies, cytokines (IL-5, IL-6, IL-10, IL-13, IL-17A, IFNγ, TNF-α, IL23, and transforming growth factor (TGF)-β), lymphocytes and ROS production were assessed at baseline, after 3 months of supplementation, and one month after vaccination. A total of 38 vitamin D deficient individuals were analyzed in the vitamin D group (n=19) and placebo group (n=19). While the placebo group had no changes in 25(OH)D, the vitamin D group had a mean increase of 20.7 to 44.3 ng·mL$^{-1}$. One month after the end of supplementation, 25(OH)D levels did not significantly change. In this population, there was no correlation between vitamin D and cathelicidin, despite the multiple studies examining the antimicrobial properties of vitamin D. There was also no effect on vaccine response. However, vitamin D did increase TGF-β levels in these older adults, as well as decreased levels of IL-6 and TNF-α, indicating that sufficient levels of vitamin D may have an anti-inflammatory role.
The purpose of this study was to examine the effect of different doses of cholecalciferol for 24 weeks on parameters of microcirculation, peripheral neuropathy, and inflammatory markers in patients with type 2 diabetes. Participants (n=62) were randomized to receive either 5,000 IU of cholecalciferol once weekly or 40,000 IU of cholecalciferol once weekly for 24 weeks. Demographics including sex, age, anthropometrics, blood pressure, diabetes information, and medications were assessed at baseline. Neuropathy was determined using questionnaires. Fasting blood samples were analyzed for cholesterol, CRP, HbA1c, 25(OH)D, IL-1β, IL-6, IL-10, and TNF-α. Skin microcirculation was determined at baseline and 24 weeks by laser Doppler flowmetry. At baseline, 80% and 77% from the 5,000 and 40,000 IU groups, respectively had low vitamin D status. Both groups saw an increase in 25(OH)D after 24 weeks, with all participants in the 40,000 IU group reaching levels $\geq 30$ ng·mL$^{-1}$ while only 15 participants from the 5,000 IU group achieved this level. Additionally, those with the higher dosage also saw decreases in BMI, HbA1c, IL-6, and an increase in IL-10. The data at the end of the 24 weeks indicated a negative correlation between 25(OH)D and HbA1c. These findings indicate that individuals with impaired glucose regulation and metabolic flexibility had a high prevalence of low vitamin D levels that improved with vitamin D supplementation. Additionally, improved levels of vitamin D also were associated with improved skin microcirculation and inflammatory status (decreased IL-6 and increased IL-10), indicating that restoring vitamin D levels to a sufficient level may improve complications related to diabetes and other similar metabolic profiles such as an aging population.
2.6. Iron Status and Anemia with Aging and Sarcopenia


The purpose of this study was to test physiological measurements (IL-6 and Hb) as potential correlates of frailty, with the hypothesis that chronic inflammation not related to a specific disease and weakness and fatigue associated with anemia would influence frailty development. Older adults aged 74 years or older were screened to be categorized as frail (n=11) and non-frail (n=19). Screening criteria included unintentional weight loss, low grip strength, slow walking speed over 15 feet, subjective exhaustion, and low levels of physical activity. If three or more criteria were met, the participant was categorized as frail and if zero were met, the participant was categorized as non-frail. The frail participants had higher serum IL-6 and lower Hb and hematocrit concentrations than the non-frail participants. There was an inverse correlation observed between IL-6 and Hb (r = -0.46) and IL-6 and hematocrit (r = -0.48) in the frail group but not in the non-frail group. However, the frail group did have more chronic diseases than the non-frail group. This study provides insight on the relationships between inflammation, anemia, and frailty in older adults that should be tested in a healthy population with no chronic diseases.


The purpose of this study was to examine if anemia and Hb levels were associated with muscle mass, fat mass, and muscle strength in older adults. Participants (n=909) aged 65 – 102 years from the InCHIANTI study completed blood sample collection for Hb concentrations, calf
peripheral quantitative computed tomography for total, muscle and fat mass of the calf, and assessment of ankle extension strength with a handheld dynamometer. Data including sociodemographic variables, BMI, comorbidities, physical activity level, and biological parameters of albumin, creatinine, cholesterol, and triglycerides were determined as covariates. Prevalence of anemia was 10.3% in this population. Anemic individuals were older, had a higher prevalence of stroke and gastric ulcer, used more medications, had increased creatinine levels, and a lower BMI and levels of albumin, total cholesterol, and triglycerides. Muscle density and strength were also lower in anemic individuals. Muscle mass measurements were associated with Hb levels, but there was no relationship between muscle strength and Hb levels. However, when regression analyses were run with anemia as a dichotomous variable, anemic individuals had significantly lower muscle strength and muscle density. This study indicates that older adults with lower Hb levels also have lower muscle strength and density, independent of disease status. This indicates the possibility of Hb levels influencing age-related changes in muscle mass and strength.


The purpose of this study was to evaluate iron status in healthy participants 80 years old and to determine the relationship between iron status and food composition, dietary, and supplemental iron intake. Participants were part of a larger longitudinal study and recruited to participate. Blood samples were obtained in 358 participants that participated in the iron status survey, and 232 of the subjects completed the nutritional status assessment. Blood samples were obtained non-fasting and analyzed for Hb, ferritin, and CRP. Ferritin cutoffs used to indicate small or absent iron stores (< 13 μg·L⁻¹, < 16 μg·L⁻¹, and < 32 μg·L⁻¹). Iron overload was considered moderate at ferritin concentrations of 301 – 700 μg·L⁻¹ and heavy at > 700 μg·L⁻¹.
Hemoglobin cutoffs for anemia were based off WHO recommendations of < 130 g·L$^{-1}$ for males and < 120 g·L$^{-1}$ for females, with cutoffs of < 8.0 mmol·L$^{-1}$ (128.92 g·L$^{-1}$) and < 7.5 mmol·L$^{-1}$ (120.86 g·L$^{-1}$) for males and females, respectively. In iron-replete subjects, 5th percentiles for Hb were utilized, and for classification of iron deficiency anemia, ferritin < 13 μg·L$^{-1}$ and below the 5th percentile for Hb. Diet history was obtained via a three day estimated food record and a frequency checklist of commonly eaten foods over the last month. Dietary supplements and medications were recorded. Those with elevated CRP had higher ferritin but lower Hb concentrations than those with normal CRP. Males had higher ferritin concentrations than females, mainly due higher energy, iron and meat intake. Ferritin < 32 μg·L$^{-1}$ was found in 7.1% and 12.9% of males and females, respectively. The findings of this study indicate higher ferritin levels may be associated with nutritional intake in older adults; however, presence of inflammation is associated with higher ferritin levels, an acute phase protein, and lower hemoglobin levels. Nutritional status may be inter-related with inflammatory status in older adults and may provide insight on inflammaging with sarcopenia.


The purpose of this study was to use data from a larger prospective study to examine whether anemia was associated with disability and lower performance and muscle strength. Additionally, the study aimed to examine whether associations were independent of inflammatory markers. A total of 1,156 participants aged 65 and older were included for analysis. Fasted blood samples were analyzed for Hb, serum ferritin, and sTfR (to determine the ratio of serum transferrin receptor and log ferritin for iron deficiency). Disability was self-reported and the short physical performance battery assessed walking speed, balance, and the ability to rise from a chair. Isometric knee extension strength and hand grip strength was
assessed with a hand-held dynamometer. Serum levels of inflammatory markers (IL-6, TNF-α, and CRP) were analyzed as covariates. Those with anemia had more disabilities, performed worse on the SPPB, and had lower knee extensor and handgrip strength than those without anemia, independently of iron deficiency. Those with anemia also had higher levels of serum CRP, IL-6, and TNF-α. However, the associations between anemia and disability, performance and strength remained significant, independent of inflammatory markers. It is possible that poor muscle oxygenation will have an influence in these adverse health outcomes of low performance and strength resulting from anemia.

2.6.5. Milman, Pedersen, Ovesen, and Schroll (2008)

The purpose of this study was to examine age- and sex-related reference intervals for Hb and to evaluate the associations between Hb, muscle strength, physical performance, and mortality. Participants were part of a larger longitudinal study and recruited to participate. Blood samples were obtained in 358 participants. Non-fasting blood samples were collected and analyzed for Hb, red blood cell count (RBC), packed red cell volume, mean red cell Hb, mean red cell Hb concentration, and serum ferritin. Ferritin levels < 15 μg·L⁻¹ were considered iron depletion and < 12 μg·L⁻¹ were considered absent iron stores. Hemoglobin cutoffs for anemia were based off World Health Organization (WHO) recommendations of < 130 g·L⁻¹ for males and < 120 g·L⁻¹ for females, with cutoffs of < 8.0 mmol·L⁻¹ (128.92 g·L⁻¹) and < 7.5 mmol·L⁻¹ (120.86 g·L⁻¹) for males and females, respectively. Serum CRP was measured to detect presence of an inflammatory disorder, and creatinine, plasma aspartate aminotransferase and alkaline phosphatase were measured. For anthropometrics and physical performance measurements, BMI was calculated and muscle strength was determined from maximal isometric strength from handgrip, elbow flexion, and knee extension of the dominant side, as well as maximal isometric
body flexion and extension while standing. The highest result of three trials was recorded. Criteria for normal Hb concentrations of 80 year-olds were established by evaluating values in the entire Danish community, values in a subset sampled from the general population, values with healthy individuals with normal ferritin, CRP, renal and hepatic function, values from participants with a good physical performance score, and values from participants with good survival of greater than 10 years. Prevalence of anemia was 7% in males and 4% in females. Iron deficiency anemia occurred in 0.83% of participants. Hb was significantly correlated (low correlations) with muscle strength: handgrip (r=0.28), knee extension (r=0.19), elbow flexion (r=0.22), and body extension (r=0.24). There was no correlation between Hb and physical performance score. There was a low (r=0.21) correlation between Hb and meat intake in males, but not females. There was also a significant association between anemia and risk of mortality within 10 years. This study supports a high prevalence of anemia in older adults and a small association with Hb levels and parameters of strength, indicating that low Hb levels may be influential on muscle strength maintenance with age.

2.6.5. Thein, Ershler, Artz, Tecson, Robinson, Rothstein, Liede, Gylvs-Colwell, Lu, and Robbins (2009)

The purpose of this study was to evaluate the association with measures of health-related quality of life and functional status. This study was a multi-center, cross-sectional survey of older patients in an out-patient setting. A total of 328 subjects met all eligibility criteria. Performance assessments were performed to assess health-related quality of life and functional status outcomes. Disability and depression were assessed via questionnaires. Physical performance was determined by measuring handgrip strength three times and using the mean value. Blood analysis included Hb, hematocrit, WBC, platelet count, RBC indices, serum iron,
total iron binding capacity (TIBC), erythropoietin, vitamin B12, and serum creatinine. Anemia was defined based on WHO criteria. Anemia was associated with a lower quality of life and is a risk factor for depression and disability. There was also a significant relationship between anemia and declines in health-related quality of life, functional status, and physical strength. Interestingly, these negative outcomes may occur at higher Hb levels than the criteria established for anemia by the WHO. These findings indicate that low Hb may contribute or be related to declining muscle function leading to sarcopenia.


The purposes of this study were to examine: 1) the prevalence of anemia in Spanish older adult nursing home residents, 2) iron nutritional status and 3) relationships between nutritional status and age, sex, BMI, dietary intake, iron supplements, functional status, and diseases. Male (n=101) and female (n=151) participants ages 65 – 96 years were examined cross-sectionally. All participants had at least one disease or disorder. Self-reported ADLs were collected, and food intake was assessed with a four day weighed food record. Daily intake was estimated for energy, protein, iron, folate, vitamin B12, and vitamin C. Height and weight were measured to calculate BMI. Fasting blood samples were obtained for analysis of RBC, mean cell volume, Hb, Hct, iron, TIBC, folate, vitamin B12, CRP, ferritin, and transferrin saturation (%). The Recommended Dietary Intake (RDI) was met for all nutrients except folate. Age was negatively related to energy, protein, iron, folate, vitamin B12, vitamin C, and animal sources. Concentrations of Hb (and other iron status indices) were positively related to intakes of energy, protein, iron, folate, vitamin B12, and animal sources. Concentrations of CRP were considered high in 41% of the population and was significantly related to ferritin, but not Hb concentrations. Prevalence of anemia was 25.4% and age was negatively related to all iron status measurements,
while functional status (ADLs) and BMI were positively related. The findings in this study indicate that in this older adult population, markers of iron status were related to age and dietary intakes, indicating lower intakes with age may be influential to nutritional status.


The purpose of this study\(^{297}\) was to examine the prevalence of anemia among older adults and to explore relationships between hemoglobin and the phenotype of frailty. Participants were randomly selected from the Study on Aging and Dementia in Mexico to estimated prevalence of frailty. Eligible participants (n=1,933) were interviewed and underwent physical examination for sociodemographic data, height, weight grip strength, gait speed, diabetes mellitus and other chronic conditions and comorbidities. Fasting venous blood sample were collected and analyzed for hemoglobin concentration. Frailty was determined based on criteria including weight loss, exhaustion, grip strength, and walking speed. Functional status, ability in home, depression, cognitive function, and comorbidity were assessed with questionnaires. Anemia was present in 160 participants (8.3%) and was 2.8% in frail and 2.7% in pre-frail participants. There was no difference in anemia by age group (60-74 y, 75-84 y, 85+ y). Lower Hb quintiles were associated with all frailty criteria, and those in the low quintile were older, had a greater prevalence of comorbid conditions, a low BMI, and more cognitive problems. Therefore, a greater risk for frailty occurs in those with lower Hb concentrations, independent of confounding factors.


The purpose of this study\(^{185}\) was to examine the relationship between serum ferritin levels and sarcopenia in South Korean older adults. Participants from the KNHANES IV (n=2,332) that
were ≥ 60 years old, completed the assessment of body composition via DXA, and were free from liver disease, renal disease, or hemochromatosis, and had not received treatment for anemia within the last three months, were included in this analysis. Height, weight, blood pressure, and waist circumference were measured. Fasted blood samples were obtained for analysis of glucose, ferritin, and insulin. Fasting insulin and glucose values were used to calculate HOMA-IR. Data on alcohol consumption, smoking, comorbidities, and physical activity were determined to be used as potential confounding factors. Sarcopenia was determined solely on body composition, using appendicular skeletal muscle mass as a percentage of body weight. Those with sarcopenia were more likely to be > 80 years old, obese and have more comorbidities. Vitamin D concentrations were lower and HOMA-IR was higher in the sarcopenic group, and in females, serum ferritin was higher in the sarcopenic group compared to the non-sarcopenic group. This study also reported a weak correlation between ferritin levels and body composition (r= -0.096 and -0.126) for males and females, respectively. Since ferritin is an acute phase protein and is increased in inflammatory conditions, it is possible that this weak relationship is governed by an inflammatory state in the older, more obese, sarcopenic population in this study, as no correction for inflammation was performed and no other measurements of iron status were measured. Future studies should include biomarkers of iron status that are not influenced by inflammation, as well as observe if there are differences in inflammation between sarcopenic and non-sarcopenic groups to provide more insight ferritin and iron status with sarcopenia.


The purpose of this study was to examine the relationships between Hb concentration and frailty status, associations between Hb concentration and frailty status, associations between anemia, Hb, and each frailty criterion, and associations between Hb concentration and the
number of frailty criteria in older adults living in Sao Paulo, Brazil. This study was a cross-sectional study and part of a larger longitudinal survey. Data analysis was performed on 1,256 participants who completed blood counts. Participants were assessed for living conditions and health status, anthropometrics, physical performance, and dental examination, blood and urine samples, and accelerometer data. Fasting blood samples were obtained for analysis of Hb. Frailty syndrome was evaluated based on standardized criteria, and were considered frail if they had three or more of the following criterion: unintentional weight loss, exhaustion, weakness, slow walking speed, and low physical activity. Covariates such as sex, age, clinical characteristics, BMI, and cognitive status was assessed. Prevalence of frailty was 8.0%. Low physical activity was the most prevalent criterion. Those who were frail had lower Hb than older adults who were not frail (13.3 vs. 14.3 g·L⁻¹). Overall, 7.7% had anemia, with the prevalence of anemia much higher in those who were frail (24.2% vs. 3.8%). The associations found between anemia, Hb concentration, and frailty were independent from other possibly confounding health conditions, indicating that low Hb may be contributory to development of sarcopenia.


The purpose of this study was to examine the relationship between low muscle mass and anemia in Korean men ≥ 65 years. Participants (n=1,464) from the KNHANES were included in analysis based on age, lack of chronic liver or kidney disease or anemia, and participation in a DXA scan. Behaviors such as smoking status, alcohol consumption, and exercise habits were recorded. A 24 hour recall was used to estimate dietary intakes of energy, iron, and calcium. Height and weight were measured, and DXA scans were performed. Fasting blood samples were obtained for analysis of Hb. Appendicular muscle mass divided by height squared was used to define low or normal muscle mass. Analysis was compared to a group of
younger men aged 20 – 39 years (n=2,385). In this population, low muscle mass was related to anemia, independent of any confounding variables, indicating that nutritional status such as hemoglobin and iron status may be influential to maintenance of muscle mass and function, especially with age.

2.6.11. Beaudart, Locquet, Touvier, Reginster, and Bruyère (2019)

The purpose of this study\(^{180}\) was to examine the association between dietary intake and sarcopenia by assessing the difference in micronutrient and macronutrient consumption between sarcopenic and non-sarcopenic individuals with a food frequency questionnaire, and by observing the proportion of individuals with insufficient intake of these nutrients in both populations. This study examined participants in the SarcoPhAge study that were included in the two year follow up and had complete data (n=331). Participants completed a food frequency questionnaire and were assessed for sarcopenia by muscle mass determination by DXA, muscle strength determined by handgrip, and physical performance determined by SPPB. Total energy intake, protein, fat, CHO, sodium, potassium, magnesium, phosphorus, iron, calcium, and vitamins D, E, A, C, and K were determined. Covariate information was collected including BMI, number of comorbidities, cognitive function, level of depression, and level of physical activity. Sarcopenic adults had lower strength, lower muscle mass, lower physical activity scores, and were older. Sarcopenic individuals also consumed lower amounts of protein and fat, as well as many micronutrients including iron and vitamin D compared to those who were non-sarcopenic. There was also higher prevalence of insufficient intakes in sarcopenic adults for iron. In particular, low intakes of protein, iron, and vitamin D may be related to strength and performance, indicating that deficiencies in these nutrients may be contributory to the adverse effects that are associated with sarcopenia.

The purpose of this study was to examine the association between anemia and frailty in community-dwelling adults aged 50 years and older from the World Health Organization Study on global AGEing and adult health in China Wave 1. Participants in this study were part of a longitudinal cohort study of ageing and older adults in low- and middle-income countries and were included if they responded to the questionnaire (n=13,175). Blood Hb concentrations were obtained from dry blood spot samples. Frailty was determined by using the deficit accumulation approach. A Frailty Index was made based on self-rated health, nine medically diagnosed conditions, four medical symptoms, 13 functional activity assessments, 10 activities of daily living, BMI, grip strength, and gait speed. The index ranged from 0 to 1 with 0 being no deficits and 1 representing having the highest level of deficits in all variables. The cut-off of 0.2 was defined as approaching a frail state. Sociodemographic and behavioral risk factors were collected as covariates. Participants were categorized into four age groups: 50 – 59, 60 – 69, 70 – 79, and 80 years or older. Overall prevalence of anemia was 31.0% (males=31.7%, females=30.3%). Anemia prevalence was also higher in older age groups. Frailty prevalence was 14.7% overall (males=11.9%, females=17.4%), with the 80+ age group having the highest prevalence. Presence of anemia was significantly associated with frailty, with this effect only slightly attenuated after adjusting for confounding factors. For each 1 g·L⁻¹ decrease in Hb, there was a 4% increase in the odds of frailty after adjusting for confounding factors, indicating that lower Hb concentrations are related to frailty in older adults.


The purpose of this study was to examine the association between handgrip strength and anemia based on the data from the 6th and 7th Korean National Health and Nutrition
Examination Survey (KNHANES). Analyses were performed on 16,638 participants that were not missing data on anemia, handgrip strength, were under 19 years, or had other missing data. Handgrip strength was measured three times per hand. The average of the three measurements for either hand was utilized. Participants were categorized as weak (< 26 kg for males and < 16 kg for females) and strong (≥ 26 kg for males and ≥ 16 kg for females). Hemoglobin concentrations were measured to determine level of anemia. The WHO standards were used to define anemia. Sociodemographic and lifestyle habits were determined to look at confounding factors. Nutritional information was determined with a food frequency questionnaire and a 24-hour recall. Overall, 7.7% of the population had anemia, and 6.7 and 15.8% were in the strong and weak handgrip strength groups, respectively. The mean age of participants with anemia was higher than those without anemia. The prevalence of anemia was 2.5% higher in those with comorbid diseases and was 3.5% greater in those with insufficient iron intake. The findings of this study indicate that regardless of age and sex, there was a significant relationship between handgrip strength and anemia that was more pronounced in males than in females and in those older than 65 years.


The purpose of this study\textsuperscript{173} was to examine if iron deficiency is an independent risk factor for low skeletal muscle function, functional impairment, fatigue, and rehabilitation progress in older hospitalized patients. Hospitalized patients (n=224) age ≥ 65 years participated in this longitudinal, observational study. Blood samples were collected for analysis of ferritin, transferrin, iron, and Hb. Patients were categorized as iron deficient (ferritin < 30 ng·L\textsuperscript{-1}, transferrin saturation < 16%) or anemic (<13 mg·dL\textsuperscript{-1} for males and < 12 mg·dL\textsuperscript{-1} for females) and having iron deficiency anemia if were low in all. Functional iron deficiency was determined
as ferritin \( \geq 30 \text{ ng}\cdot\text{L}^{-1} \) and transferrin saturation < 16%. Concentrations of CRP were determined, and a level > 3.0 mg\cdot\text{dL}^{-1} was considered as inflammation. Assessments of ADLs, frailty, and risk of sarcopenia with the SARC-F questionnaire were performed. Handgrip strength, physical performance with the SPPB, and isometric leg extension strength were determined. Iron deficiency was present in 41%, in which 86% of those were classified as functionally iron deficient. Absolute iron deficiency with anemia was present in 13% of patients, and absolute iron deficiency without anemia was diagnosed in 1% of patients. Functional iron deficiency with and without anemia was prevalent in 72% and 14% of patients, respectively. In those with iron deficiency, frailty and fatigue scores were higher, Hb levels were lower, and CRP levels were higher. This indicates the iron deficiency, which may result in anemia, is a risk factor for fatigue and frailty, indicating a role they may have in the development and/or presence of sarcopenia.

2.7. Interrelationship between Iron Status, Vitamin D Status, and Inflammation

2.7.1. Heldenberg, Tenebaum, and Weisman (1992)

The purpose of this study\(^{300}\) was to examine the prevalence of iron-deficiency anemia and vitamin D deficiency in randomly selected infant patients with iron deficiency. Infants aged 6 – 24 months (n=25) with anemia underwent blood draws for analysis of Hb, hematocrit, mean corpuscular volume, red cell distribution width, iron, TIBC, \textit{25(OH)D}, \textit{24,25(OH)\textsubscript{2}D}, and a comprehensive metabolic panel. Twenty infants were considered to have iron deficiency anemia and were included in analysis. Intramuscular iron was given to all infants. Blood analyses were repeated after eight weeks of treatment. Besides comparing pre- and post-treatment biomarkers, patients were categorized and analyzed based on vitamin D metabolite concentrations. Before supplementation, those with low vitamin D status also had lower Hb and transferrin saturation. These lower levels were no longer different when compared to the other groups after iron
supplementation. These findings suggest a relationship between iron deficiency and vitamin D levels that should be explored in other vulnerable populations, such as older adults.

2.7.2. Sim, Lac, Liu, Meguerditchian, Kumar, Kujubu, and Rasgon (2010)

The purpose of this study was to examine if there is an association between 25(OH)D deficiency and anemia in individuals with and without chronic kidney disease by using a large database to assess prevalence and risk of anemia in those with documented 25(OH)D deficiency and normal levels. Eligible participants had to have at least one 25(OH)D and one Hb level available to analyze. Age, sex, creatinine, estimated glomerular filtration rate, calcium, phosphorus, serum albumin, erythropoietin stimulating agent use, iron saturation, and ferritin were collected if available. Conditions that may be associated with inflammation or malnutrition such as diabetes mellitus, lupus, ulcerative colitis, and Crohn’s disease were identified in participants. Those with IDA, folic acid deficiency, vitamin B12 deficiency, bacteremia, and gastrointestinal bleed diagnosed within six months were excluded. Of the 554 individuals who had both Hb and 25(OH)D levels, 43% were 25(OH)D deficiency and 57% had normal levels. Those with 25(OH)D deficient levels (< 30 ng·mL⁻¹) had greater serum ferritin levels, but lower TIBC than those with normal 25(OH)D levels (≥ 30 ng·mL⁻¹). The odds ratio for anemia when controlling for confounding factors were higher in those with 25(OH)D deficiency. Additionally, the prevalence of anemia in the deficient group was 48% compared to 36% in those with normal levels. Those with 25(OH)D deficiency also had lower albumin levels and were more likely to have diabetes. These findings indicate that there is a greater prevalence of anemia in those with 25(OH)D deficiency compared to those with normal levels. Interestingly, those with lower 25(OH)D levels also had lower Hb and TIBC, but higher ferritin and iron saturation, indicating
that these individuals may have been iron-deficient but were masked by a possible inflammatory state. This indicates the need to examine inflammatory status in those at risk for vitamin D and iron deficiency and/or anemia.

2.7.3. Perlstein, Pande, Beliner, and Vanasse (2011)

The purpose of this study was to examine the relationship between vitamin D status and subtypes of anemia seen in older adults. Participants from two phases of NHANES (n=4,103) age 60 years or older were included in this analysis. Blood samples were analyzed for Hb, mean corpuscular volume, red cell distribution width, WBC, ferritin, iron, TIBC, erythrocyte protoporphyrin, folate, vitamin B12, and 25(OH)D concentrations. Anemia was classified based on nutritional deficiency, chronic kidney disease, or inflammation. Transferrin saturation was determined, and iron deficiency was determined as meeting two or more of the following criteria: ferritin < 12 ng·ml⁻¹, transferrin saturation < 15%, and erythrocyte protoporphyrin > 1.2 µM. Prevalence of vitamin D deficiency was 34 – 46% and of anemia was 9%. There was a higher prevalence of anemia in those with vitamin D deficiency compared to those with normal vitamin D levels. An association between anemia and vitamin D deficiency remained even after adjustment for age, sex, and ethnicity. An increased risk of anemia was shown to begin at 25(OH)D levels < 24 ng·ml⁻¹. When examining vitamin D deficiency within specific sub-types of anemia, the prevalence was 56% in anemia of inflammation, 47% in anemia of nutrient deficiency, and 37% in anemia of chronic kidney disease, compared to those without anemia (33%). The findings of this study indicate the association vitamin D deficiency has with anemia caused by both inflammation and nutrient deficiencies. A deeper look at other markers that are related to anemia from nutrient deficiencies such as markers of iron status, and inflammatory markers would provide more insight into this relationship in older adults.
2.7.4. Blanco-Rojo, Pérez-Granados, Toxqui, Zazo, de la Piedra, and Vaquero (2013)

The purpose of this study\textsuperscript{301} was to examine if there was a relationship between 25(OH)D levels and iron status biomarkers, if consumption of an iron-fortified fruit juice that improves iron status causes a change in bone remodeling, and if the recovery of iron status by consuming iron-fortified food varies depending on baseline vitamin D status. Iron deficient females (n=123) provided fasting blood samples to analyze for RBC, hematocrit, mean corpuscular volume, Hb, iron, ferritin, transferrin, 25(OH)D concentrations, and markers of bone turnover and formation. A subset was randomly selected to participate in a 16 week randomized, double-blind, placebo-controlled study and were randomized to consume 500 mL daily of a placebo fruit juice (n=18) or an iron-fortified fruit juice (n=23) supplying 18 mg of iron. Blood samples and 24 hour urine samples were collected at baseline, eight, and 16 weeks. In these iron deficient females, 42% were vitamin D deficient, 50% were insufficient, and 8% were sufficient. There was a positive relationship between 25(OH)D levels and transferrin saturation, but no other correlations present between vitamin D status and markers of iron status. Those taking the iron-fortified drink saw an increase in Hb, ferritin, and transferrin saturation and a decrease in transferrin levels, and compared to the placebo, had higher levels of Hb and ferritin at week 16. Concentrations of 25(OH)D decreased from baseline in both groups. However, transferrin saturation was higher in those in the iron-fortified group who had 25(OH)D levels ≥ 50 nmol·L\textsuperscript{-1} compared to those with levels < 50 nmol·L\textsuperscript{-1} at eight and 16 weeks. While there was no increase in 25(OH)D levels with iron supplementation, these results indicate a relationship between transferrin saturation and vitamin D, indicating that this relationship between iron status and vitamin D status may mainly influence iron supply to the tissues, and potentially be impactful for muscle function.

2.7.5. Han, Kim, Kim, Lee, Oh, Lee, Kim, Joo, Lim, Kim and Kim (2013)
The purpose of this study was to examine the relationship between serum 25(OH)D levels and Hb levels in a representative Korean adult population. Participants from data sets of the KNHANES V of 2010 – 2011 were cross-sectionally analyzed if they were ≥ 20 years old and had 25(OH)D and Hb levels available (n=11,206). Potential confounding variables including age, sex, smoking status, socioeconomic status, menstrual cycle, exercise habits, chronic conditions, BMI, and iron intake were reported. Fasted blood samples were collected and analyzed for Hb, 25(OH)D, ferritin, iron, TIBC, cholesterol, triglycerides, and creatinine. A non-linear relationship was observed between 25(OH)D levels and Hb. Additionally, Hb levels tended to decrease below a 25(OH)D threshold of 26.4 ng·mL\(^{-1}\) independent of any confounding factors. Risk of anemia also increased as 25(OH)D levels decreased, particularly in females. These findings indicate a potential relationship between 25(OH)D and Hb, indicating that adequate vitamin D status may be important for decreased risk of anemia, particularly in older adults vulnerable to anemia and malnutrition.

2.7.6. Shin and Shim (2013)

The purpose of this study was to examine the influence vitamin D levels had on Hb levels in a Korean adult population. Participants (n=5786) from the KNHANES were included. Data was collected for lifestyle factors, menopausal status, and chronic kidney disease. Fasted blood samples were analyzed for Hb, hematocrit, ferritin, 25(OH)D, serum iron, TIBC, and creatinine. A total of 3.6% of males and 13.6% of females were classified as anemia, with iron deficiency determined as the main cause for 76.2% of pre-menopausal females and anemia of inflammation as a cause for 22.3% of post-menopausal women. In females, those with iron deficiency anemia had the lowest vitamin D levels, but in males, those with anemia of chronic inflammation had the lowest vitamin D levels. Vitamin D was related to Hb levels in all groups.
In females, those with low levels of vitamin D had an increased risk of anemia, iron deficiency anemia, and anemia from chronic inflammation. These findings suggest that in this population, those who had low vitamin D status, particularly females, had higher risk of anemia independent of confounding factors.

2.7.7. Toxqui, Pérez-Granados, Blanco-Rojo, Wright, González-Vizcayno, and Vaquero (2013)

The purposes of this study were to examine the influence of consumption of an iron-fortified flavored skim milk on iron metabolism in iron-deficient menstruating women, and to see if the supplementation of vitamin D to the iron-fortified product would result in a great effect on iron metabolism. Females (n=109) with low iron stores (ferritin < 30 ng·mL\(^{-1}\) and Hb ≥ 11 g·dL\(^{-1}\)) and without any health problems that may influence iron status were analyzed for this study. Participants were randomized into two groups: one received 500 mL·d\(^{-1}\) of an iron-fortified (15 mg) dairy product (n=54) and the other received 500 mL·d\(^{-1}\) of an iron and vitamin D-fortified (15 mg + 5 µg) dairy product (n=55) for 16 weeks. Dietary intake was evaluated at baseline, eight, and 16 weeks. Body weight and height were measured monthly. Fasting blood samples were collected at baseline and every four weeks for 16 weeks and analyzed for RBC, Hematocrit, mean corpuscular volume, red cell distribution width, Hb, iron, ferritin, transferrin, sTfR, and 25(OH)D concentrations. Mean corpuscular Hb, TIBC and transferrin saturation were calculated. Participants were 24.8 ± 4.1 and 24.7 ± 4.6 years old in the iron and iron + vitamin D group, respectively. There were no differences at baseline. Vitamin D intake increased in the iron + vitamin D group compared to baseline and to the iron group. Levels of 25(OH)D increased more in the iron + vitamin D group compared to the iron group. Ferritin increased during the first four weeks and came to baseline values by week 16, and serum transferrin and TIBC increased from week eight to week 16 in both groups, but there was no change in serum iron, transferrin
saturation, or sTfR. The iron + vitamin D group showed higher values of erythrocytes and hematocrit at week eight compared to the iron group. This study showed that supplementing with iron + vitamin D sufficiently increased 25(OH)D levels and had a slightly beneficial effect on erythropoiesis and iron status compared to the iron group. This indicates that vitamin D may have a role in promoting iron metabolism. It is possible that the inhibiting effects of calcium and milk proteins on iron absorption contributed to these results, so examining interventions with vitamin D and iron at separate feeding times may produce a greater enhancement of iron stores.


The purpose of this study\textsuperscript{198} was to examine the role of vitamin D in the regulation of hepcidin expression \textit{in vitro} and \textit{in vivo}. For the \textit{in vivo} experiment, THP-1 macrophage-like monocytic cells were differentiated into macrophage phenotype upon vitamin D exposure. Inflammation was induced upon the cells with lipopolysaccharide exposure. RNA was isolate for gene expression analysis. Cytokines IL-6 and IL-1β were quantified. Hepcidin-25 was measured. For the \textit{in vitro} experiment, a clinical pilot study was performed with individuals with early stage chronic kidney disease. Participants were randomized to take 50,000 IU of oral vitamin D\textsubscript{3} weekly for 12 weeks, followed by 50,000 IU every other week for 40 weeks or a matching placebo for one year. Serum measurements of 25(OH)D and PTH were obtained. To be eligible for this analysis, participants also needed a hepcidin measurement at baseline and at a three month follow up (n=38). Concentrations of 25(OH)D suppressed hepcidin in the presence of inflammation, indicating that vitamin D can help regulate iron transport in inflammatory states. Additionally, vitamin D decreased levels of IL-6 and IL-1β, which have a role in the cytokine stimulating hepcidin release. In the pilot study, the percent change in 25(OH)D concentrations was inversely associated with the percent change in serum hepcidin concentrations, suggesting
that vitamin D can help lower hepcidin expression either directly or indirectly by decreasing cytokines that increase hepcidin. These findings indicate the potential role vitamin D has in iron transport during inflammatory states.


The purpose of this study\textsuperscript{305} to examine and compare the relationships of 25(OH)D and 1,25 dihydroxyvitamin D (1,25D) status with Hb levels in older males. Australian males aged 70 years or older (n = 1666) were analyzed at baseline and at a two year and five year follow up. Fasting blood samples were collected for analysis for serum 25(OH)D and 1,25D levels, Hb, albumin, and WBC. Data regarding socioeconomic status, lifestyle factors (smoking, physical activity), height, weight, vitamin D supplement use, season of blood sampling, comorbidities, frailty, and renal function was determined. Vitamin D deficiency and insufficiency was present in 10.5\% and 44.7\% of participants, respectively. Prevalence of anemia was 14\%, and lower concentrations of 25(OH)D and 1,25D were found in those who were anemic compared to those who were not anemic. Anemic prevalence was higher in the lowest categories of both 25(OH)D and 1,25D. Longitudinally, 1,25D was associated with changes in Hb from baseline to two year follow up to five year follow up, but there was no relationship with 25(OH)D. This association may be influenced by the effect of chronic inflammation on the development of anemia in older adults, which may be modulated by 1,25D levels. This indicates a potential relationship between vitamin D levels and iron deficiency anemia in older adults and leads to the possibility of vitamin D supplementation improving anemic status in older adults with iron deficiency anemia.

2.7.10. Monlezun, Camargo, Mullen, and Quraishi (2015)
The purpose of this study was to examine the association of 25(OH)D levels with the risk of anemia in a large, nationally representative, community-dwelling sample in the United States. NHANES survey data from 2001 – 2006 and included participants that had values for 25(OH)D and Hb. Data including age, sex, race, BMI, poverty-to-income ratio, presence of chronic kidney disease, and levels of CRP, ferritin, iron, vitamin B12, and folic acid were extracted from the databases as possible confounding factors. Linear regression indicated that for every 1 ng·mL\(^{-1}\) increase in 25(OH)D, there was a 0.001 g·dL\(^{-1}\) increase in Hb levels and a 3% lower likelihood of anemia. These findings indicate the low levels of 25(OH)D may be related to anemia. It is hypothesized that insufficient vitamin D status may contribute to anemia by either influencing low erythropoietin production or by promoting an inflammatory state resulting an increase in hepcidin production, indicating a potential relationship between vitamin D status, iron status, and inflammation that may be contributory to inflamming in sarcopenic older adults.

2.7.11. Sharma, Jain, Dabla (2015)

The purpose of this study was to examine the prevalence and risk factors for vitamin D deficiency and its association with iron deficiency anemia in children of Northern India. Children (n=263) between the age of three months and 12 years that were attending an outpatient hospital for acute disorders were included in this one year study. Clinical examination was performed, followed by blood tests for CBC, ferritin, iron, TIBC, transferrin saturation, vitamin D, calcium, phosphorus, and alkaline phosphatase. Participants were classified as iron deficient and vitamin D deficient based on established criteria. Patients with low Hb levels made up 66% of the population in the vitamin D deficient group and while only 35% of vitamin D sufficient group had low Hb levels, indicating that iron deficiency anemia was more prevalent in those with vitamin D deficiency. Out of all participants, 86% were vitamin D deficient or insufficient, and
mean Hb, iron, and transferrin saturation were lower in the vitamin D deficient group compared
to the insufficient and sufficient groups, whereas TIBC was higher. These findings indicate that
not only are vitamin D deficiency and iron deficiency anemia prevalent in children, but the
commonly occur together, indicating these nutrient deficiencies may be interrelated. Further
research should be performed to examine this relationship.


The purpose of this study was to examine the effect of vitamin D on Hb concentration
in otherwise healthy subjects with iron deficiency anemia and vitamin D deficiency. Participants
aged 15 – 60 years old, diagnosed with iron deficiency anemia (ferritin < 15 µg·ml\(^{-1}\), and normal
vitamin B12 and folate levels) and 25(OH)D levels < 20 ng·ml\(^{-1}\) (n=3) were randomized into
either a placebo group or a vitamin D therapy group. Iron was administered to all patients, and
those in the vitamin D group were provided 60,000 IU of vitamin D\(_3\) intramuscularly while those
in the placebo group received a similar volume of saline. Levels of vitamin B12, RBC folate,
ferritin, PTH, and 25(OH)D were measured. After 12 weeks, 25(OH)D levels were higher in the
vitamin D group than the placebo and ferritin and Hb levels increased in both groups with no
difference between groups. This study indicated that vitamin D supplementation does not have
an additive effect on an increase in Hb concentrations in those with iron deficiency anemia,
despite the potential relationship vitamin D has on erythropoiesis. It is possible that vitamin D
will be more influential in those with iron deficiency anemia who are also in an inflamed state.


The purpose of this study was to determine an optimal serum ferritin level to
differentiate between older adults with and without iron deficiency anemia with greater
sensitivity and specificity. A secondary purpose of this study was to determine diagnostic properties of varying levels of ferritin in the diagnosis of iron deficiency anemia in older adults aged 60 years or older. Participants with iron deficiency anemia (n=80) and without iron deficiency anemia (n=160) that were part of a larger study were included in this analysis. Data examined included serum iron, percent transferrin saturation, and ferritin, as well as presence of comorbidities/chronic medical conditions. In this sample of older adults, a ferritin cutoff of 100 ng·mL⁻¹ had the highest sensitivity and specificity for the diagnosis of iron deficiency anemia. Ferritin levels also had less diagnostic ability compared to transferrin saturation. These findings indicate that ferritin levels may be high in older adults with iron deficiency anemia and may therefore not be a reliable measurement when examining iron deficiency, especially in populations prone to chronic inflammation. Examining multiple iron indices in this population and utilizing a higher ferritin cutoff may be necessary in order to identify iron deficiency.


The purpose of this study was to examine the acute effect of vitamin D3 supplementation on hepcidin, inflammatory cytokines, and ferritin concentrations in healthy adults to better understand the mechanism by which vitamin D may affect iron recycling. Healthy adults (n=28) were randomized to take a single bolus oral dose of 250,000 IU of vitamin D₃ (n=14) or a matching placebo (n=14). Samples were taken at baseline (n=28) and approximately one week later for both the vitamin D₃ group (n=13) and the placebo group (n=11). Plasma 25(OH)D, IL-1β, IL-6, IL-8, MCP-1, ferritin, and hepcidin were determined. Across both groups, over 70% of participants had 25(OH)D levels < 20 ng·mL⁻¹. In the vitamin D group, 25(OH)D concentrations increased 150% compared to baseline after one week, with no changes in the placebo group. There were no effects on the pro-inflammatory cytokine
concentrations after one week of supplementation. However, after one week, hepcidin concentrations were 73% lower in the vitamin D group while ferritin concentrations showed no change in either group. These findings suggest that vitamin D may act directly on hepcidin in its role on iron recycling, independent of inflammatory conditions.

2.7.15. Syed, Michalski, Tangpricha, Chesdachai, Kumar, Prince, Ziegler, Suchdev, and Kugathasan (2017)

The purpose of this study was to examine the associations of vitamin D with markers of inflammation and hepcidin and to determine if vitamin D status was associated with Hb and anemia in children with inflammatory bowel disease. Children ages 5 – 18 years with inflammatory bowel disease were included in this study and stratified based on inflammatory status and presence of iron deficiency and/or anemia. Blood samples were collected for analysis of ferritin, CRP, α-1-acid glycoprotein, sTfR, retinol binding protein, hepcidin, and 25(OH)D concentrations. Participants were classified as iron deficient, anemic, or vitamin D deficient and/or insufficient based on established criteria. Prevalence of vitamin D deficiency and insufficiency were 38% and 77%, respectively. Iron deficiency was found in 67% of participants, while 28% had iron deficiency anemia. Those with lower 25(OH)D levels had higher hepcidin and lower Hb, but did not have a relationship with inflammation. These results indicate a relationship between iron and vitamin D status, suggesting that having optimal vitamin D levels may result in improved hemoglobin levels, and thus improved anemic or iron status, possibly through the role vitamin D has on mediation hepcidin response. Examining this relationship in other populations while also examining multiple inflammatory markers may provide some insight on the interrelationship between inflammation, vitamin D, and iron status.
The purpose of this study was to examine if deficiencies of vitamin D are associated with reduced iron status and if progressive iron deficiency occurred with inferior vitamin D status. Female professional athletes (n=219) were analyzed for this study. Fasted blood samples were collected and analyzed for blood morphology indices (including Hb, hematocrit, and RBC), 25(OH)D, and iron status biomarkers including ferritin, iron, and sTfR. Stages of iron deficiency were determined based on ferritin, sTfR, Calculation of TIBC was performed using concentrations of iron and unsaturated binding capacity, and Hb, Hct, and RBC. Inflammatory status was determined with concentrations of CRP. Insufficient vitamin D status was found in 54% of the athletes, but only 2% were deficient. Odds ratios indicated that vitamin D deficiency was found to be influenced by iron deficiency, and iron deficiency was associated with vitamin D deficiency. These findings indicate that in female athletes, low vitamin D status significantly increases the risk of iron deficiency, potentially related the role vitamin D has on iron availability and absorption via hepcidin regulation. These results indicate an association between these nutrients in athletes, and should be investigated in older adults.
CHAPTER III: METHODS

Study Design

This study was an extension of a larger clinical trial (NCT03701878) that was a single-center, cross-sectional pilot study aimed to evaluate metabolic flexibility during post-prandial rest and exercise in sarcopenic and non-sarcopenic men and women (under review, June 2020). The present study was a retrospective analysis expanding on the data acquired in the previous clinical trial to examine the role of inflammaging and nutritional status on the presence of sarcopenia (Figure 2). Participants were pre-screened, deemed eligible or ineligible to participate, and if eligible, were enrolled into the study. The study included two laboratory visits: Screening Visit and Test visit, separated by four to ten days. During the Screening Visit, measurements of anthropometrics were performed, body composition was assessed with DXA, and B-mode ultrasound quantified muscle cross-sectional area and subcutaneous adipose thickness. During the Test Visit, resting metabolism and post-prandial carbohydrate and fat oxidation were measured continuously. Vastus lateralis muscle tissue oxygenation was simultaneously monitored using continuous-wave NIRS. Venous blood samples were obtained fasted and periodically post-prandial.

Participants

Recruitment took place from a previous study database, local community organizations, and retirement or older adult living communities. Three hundred fifty-six individuals were pre-screened for eligibility, with a total of 22 participants deemed eligible and were enrolled into the study. Five male subjects (mean ± standard deviation [SD]; age = 71.8 ± 5.7 y; stature = 172.2 ± 6.0 cm; body weight = 80.2 ± 9.5 kg) and six female subjects
(age = 75.0 ± 6.4 y; stature = 160.6 ± 7.8 cm; body weight = 62.2 ± 7.2 kg) were categorized as non-sarcopenic. Six male subjects (age = 87.0 ± 9.4 y; stature = 167.1 ± 4.3 cm; body mass = 69.0 ± 8.9 kg) and five female subjects (age = 74.2 ± 7.3 y; stature = 157.8 ± 8.5 cm; body mass = 71.5 ± 6.6 kg) were categorized as sarcopenic. One female, non-sarcopenic subject was not included in the present analysis due to inability to obtain a blood sample. Prior to any study participation, participants reviewed, signed, and dated an Informed Consent Form approved by the University of Nebraska-Lincoln Institutional Review Board (IRB) for the protection of human subjects (Project Title: A pilot study to explore muscle energy metabolism and metabolic flexibility in older men and women; IRB Project # 18543; IRB Approval Date: September 6, 2018). A signed and dated copy was provided to the participant prior to any study participation. This study was registered in clinicaltrials.gov (NCT03701867).

Inclusion Criteria

Participants were eligible for the study if they met the following criteria during pre-screening and the Screening Visit: 1) Participant is 65 years of age or older at the time of screening; 2) Participant’s BMI is ≥ 18.0 and ≤ 39.0 kg·m\(^{-2}\); 3) Participant is ambulatory (able to walk without assistance); 4) Participant is not a current smoker (within past 10 years); 5) Participant is classified as low OR moderate risk as defined by the American College of Sports Medicine (ACSM) Guidelines for Exercise Testing & Prescription\(^{309}\) based on the responses from AHA/ACSM Health/Fitness Facility Preparticipation Screening Questionnaire; 6) Participant has normal muscle mass and strength/performance (normal grip strength [≥ 30.0 kg (men); ≥ 20.0 kg (women)]) OR low muscle mass and strength/performance (low grip strength [< 30 kg (men); < 20 kg (women)])\(^{310}\); 7) If
participant is on thyroid medication or hormone replacement therapy, states he/she has been on a constant dosage for at least two months prior to Screening Visit; 8) Participant states he/she is willing to follow protocol as described; 9) Participant has voluntarily signed the informed consent form approved by the University of Nebraska-Lincoln IRB prior to any participation in the study.

**Exclusion Criteria**

Participants were excluded from the study if they met any of the following criteria during pre-screening, Screening Visit, or Test Visit: 1) Participant states he/she has a history of metabolic/endocrine (diabetes), hepatic, or renal disease, myocardial infarction, peripheral vascular disease, respiratory or neuromuscular disease; 2) Participant states he/she regularly participates in a resistance exercise program; 3) Participant states he/she has had poor appetite with recent unexplained weight loss (e.g., 10 pounds [4.5 kilograms]) over the past 6 months; 4) Participant states he/she has a current infection (requiring medication or which might be expected to require hospitalization), has had inpatient surgery, or corticosteroid treatment (excluding topical creams) in the last three months or antibiotics in the last three weeks prior to the Screening Visit; 5) Participant states that he/she has an active malignancy, excluding carcinoma in-situ of the cervix, cutaneous malignancies (basal cell carcinoma, squamous cell carcinoma, except melanoma); 6) Participant states that he/she has a chronic, contagious, infectious disease, such as active tuberculosis, Hepatitis A, B, or C, or HIV; 7) Participant reports currently taking medications/dietary supplements or substances that could profoundly modulate metabolism in the opinion of the principal investigator (PI) or study physician, e.g. progestational agents, steroids, growth hormone, dronabinol, marijuana, CaHMB, free amino acid supplements, dietary supplements to aid weight loss or gain. Exceptions included use of
multi-vitamin/mineral supplement, topical or optical steroids and short-term use (less than two weeks) of dexamethasone; 8) Participant is known to be allergic or intolerant to any foods; 9) Participant states he/she has had history of gastrointestinal disease (e.g., Crohn’s, colitis, celiac), or surgeries (including gastric balloon), gastroparesis, or taking medications that are known in the opinion of the PI or study physician (e.g., cholinergic agonists, prokinetic agents, opioid antagonists, antidiarrheals, and antibiotics) to interfere with consumption/digestion/absorption of nutrients; 10) Participant states he/she has an eating disorder, severe dementia or delirium, history of significant neurological or psychiatric disorder, alcoholism, substance abuse, or other conditions that may interfere with compliance with study protocol procedures in the opinion of the PI or study physician; 11) Participant states that he/she is a participant in a concomitant trial or trial of a non-registered drug (or is within the 30 day follow-up period for such a trial).

Screening Visit

Demographic information was collected, and assessment of inclusion and exclusion criteria were performed. If the participant remained eligible, anthropometry was assessed. Height (cm) and body mass (kg) were measured using a calibrated digital scale and stadiometer (Seca 769, Hamburg, Germany). Values were used to calculate BMI.

Strength and Function Assessments

Handgrip strength (kg) was measured with a handheld dynamometer (Jamar® Hydraulic Hand Dynamometer, Patterson Medical, Warrenville, IL). The dynamometer was standardized and adjusted to the second joint of the finger just below the handle. Participants were asked to squeeze the dynamometer handle with their right hand as forcefully as possible for three to five seconds while the arm remained in 90° flexion at the elbow. The average of three
trials were used as the final strength value.

The SPPB\textsuperscript{312} consisted of three tests evaluating balance, gait speed at four meters, and the ability to stand up from a chair and return to the seated position five times. Each test was scored based on level of completion and summed together at the end. The total score ranged from 0 – 12, where 0 was the lowest and 12 was the highest level of functionality. Gait speed was assessed by instructing participants to walk four meters at their normal walking speed. The gait speed assessment was performed twice, and if there was a difference greater than 10\% between the two trials, a third trial was performed.

\textit{Dual X-Ray Absorptiometry}

Whole-body DXA (Lunar iDXA, GE Healthcare, Madison, WI) assessed fat mass (FM) and FFM. The DXA was calibrated daily using a quality assurance phantom that consisted of varying bone mineral density and percent fat standards (Lunar iDXA User Manual, GE Healthcare, Madison, WI). Participants were instructed to lie supine on the scanner table with their hands pronated and legs adducted. Participants were categorized as having “normal” or “low” skeletal muscle mass based on RSMI (\%) from Kim et al.\textsuperscript{313} and Janssen et al.\textsuperscript{314}

\textit{B-mode Ultrasonography}

Ultrasound images were taken using a portable brightness mode (B-mode) ultrasound-imaging device (GE Logiqe, USA) and a multi-frequency linear-array probe (12L-RS; 5–13 MHz; 38.4 mm field-of-view).\textsuperscript{315} Participants were instructed to lie supine on a plinth with legs extended and relaxed. Transverse images were taken at the site of the NIRS device at 66\% of the distance the distance from the anterior superior iliac spine to the superior border of the patella. Panoramic images of the quadriceps were also taken at the site of NIRS placement from the most lateral aspect to the most medial aspect of the quadriceps and were used to quantify muscle CSA.
A generous amount of water soluble transmission gel was applied to the skin to enhance acoustic coupling and reduce near field artifacts. Equipment settings were set at a gain of 58 dB and a frequency of 12 MHz to optimize image quality and were held constant across participants. Image depth was adjusted based on size of the leg and then held constant across all images. Images were taken until three images of acceptable quality were obtained. Images with the highest visual contrast were analyzed with Image-J Software (National Institutes of Health, USA, version 1.52v). Prior to analysis, images were scaled with pixels to cm using the Image-J straight-line function. The polygon function was used to quantify quadriceps CSA (cm$^2$) by selecting the maximal region of interest that included as much of the quadriceps muscles as possible while excluding the surrounding fascia.$^{315}$ Subcutaneous fat (cm) was quantified using the straight-line function over the site of NIRS placement by including as much of the subcutaneous fat as possible while excluding the vastus lateralis.

**Near-infrared Spectroscopy (NIRS)**

A portable, continuous wavelength NIRS device (PortaMon MKII, Artinis, Einsteinweg 17, Netherlands) was placed upon the right vastus lateralis at 66% of the distance from the ASIS to the superior border of the patella for assessment of muscle tissue oxygenation. In preparation for placement, the local skin area was lightly abraded and cleaned with isopropyl alcohol. The NIRS device was covered in a thin, transparent plastic wrap as protection from fluids. The device was placed on the muscle with a black cloth covering it and was then secured to the skin with a dark-colored, opaque self-adherent Coban™ wrap (3M™, Maplewood, MN, USA), in order to limit the effects of peripheral light disrupting the sensor.
NIRS continually measured relative concentration changes from baseline in O$_2$Hb and HHb and used to calculate THb. Spatially resolved spectroscopy measured StO$_2$ (%) to obtain absolute values. Three light emitting diodes each transmit two wavelengths (760 and 850 nm) of light through the skin, which scatters back and is received by the receiver. The transmitting diodes and receiver are designed spatially to provide three source-detector distances of 30 mm, 35 mm, and 40 mm, respectively, to allow for a depth sensitivity of ~1.5 cm. The NIRS device recorded muscle tissue oxygenation at rest both before and post-prandial a CHO-rich meal to examine responsiveness to a hyperglycemic challenge.

Submaximal Estimation of Leg Extension Strength

Leg extension strength was estimated sub-maximally with a unilateral, dynamic constant external resistance 5RM test performed during the screening visit. Leg extensions were completed on a plate-loaded leg extension machine (Hammer Strength Plate-Loaded, Iso-Lateral Leg Extension Machine; LifeFitness, Rosemont, IL, USA) that was custom mounted to a Biodex chair (Biodex Medical Systems, Inc., Shirley, NY, USA) by the researchers and custom fitted with a load cell (Omegadyne, model LCHD-500, 0-500 lb; Stamford, CT, USA). Participants were seated on the Biodex chair and secured with restraining straps over the pelvis, trunk, and contralateral thigh. They were asked to sit upright with their back against the chair and to tightly hold the handles located near their hips. The lateral epicondyle of the right femur was aligned with the axis of rotation of the leg extension machine.

A brief warm-up set of 10 repetitions with 4.5 kg external resistance was performed first to familiarize the subjects with the leg extension movement. After a two-minute rest period following the warm-up set, an appropriate amount of weight was added for a first attempt to find a 5RM. A minimum of two minutes and a maximum of five minutes rest was allowed between
attempts. The set was determined as a successful 5RM if the subject was able to fully complete the five repetitions through their full range of motion, but not able to complete a sixth repetition. Once the 5RM was determined, the external resistance added was recorded and used to estimate the 1RM using the following equation (r=0.994):\footnote{317}

\[
1RM = 1.0970 \times (5RM \text{ loaded resistance (kg)}) + 14.2546
\]

**Test Visit**

*Dietary Food Recall*

For the second visit, participants arrived at after an 8 – 16 hour fast with a completed three-day dietary food recall. Participants were instructed to complete the recall for the three days prior to the scheduled test visit. Prior to receiving the food recall form, a Registered Dietitian explained how to complete the form with each participant. Participants were also instructed to consume a minimum of 150 grams of CHO each day for the three days prior to the test visit. This was confirmed visually at each test visit by a Registered Dietitian and was later analyzed in a software program (MyFitnessPal, Under Armour, Inc., 2005). Mean ± SD of energy intake (kcal·day\(^{-1}\)) and macronutrient intake (g·d\(^{-1}\)) were later analyzed for each day.

*Indirect Calorimetry and Resting Metabolism*

Resting metabolism was measured for 30 minutes prior to and 180 minutes post-consumption of a standard high CHO meal consisting of 51 g CHO, 9 g fat, and 6 g protein. The \(\dot{V}O_2\) and rate of carbon dioxide production (\(\dot{V}CO_2\)) were measured continuously with calibrated metabolic carts (Parvo Medics TrueOne® 2400 Metabolic Measurement System, Sandy, Utah). The oxygen and carbon dioxide gas analyzers were calibrated with a gas mixture of 4% CO\(_2\),
16% O₂, and 80% N. The spirometer was calibrated with a 3 L syringe (Series 5530, Hans Rudolf, Inc., Shawnee, KS). A ventilated metabolic hood (Parvo Medics TrueOne® 2400 Canopy System, Sandy, Utah) was placed over the head while the subject was lying supine on a bed. The metabolic hood was secured to form an airtight seal around the head. During the exercise bouts, face masks (7450 V2, Hans Rudolph, Inc., Shawnee, KS, size small - large), headgear (7450 V2, Hans Rudolph, Inc., Shawnee, KS), with attached two-way non-rebreathing valves (2700, Hans Rudolph, Inc., Shawnee, KS) were secured around the nose and mouth of each participant to form an air tight seal on the face. Flow was controlled by personnel at the initiation of metabolic measurements and was held constant throughout the assessment. Participants were asked to remain as still as possible and were asked to refrain from talking during metabolic testing. Metabolic measurements including \( \dot{V}O_2, \dot{V}CO_2, RQ \), and energy expenditure were saved and exported for signal processing. Metabolic measurements were averaged over 10 minute windows within the 15 or 30 minutes timepoints during the resting period and over 2 minute windows during the exercise bouts.

The CHO oxidation rate (g·min\(^{-1}\)) and fat oxidation rate (g·min\(^{-1}\)) were calculated using standard stoichiometric equations\(^{318,319}\), where CHO and fat oxidation is based on a \( \dot{V}O_2 \) in standard temperature pressure and dry (STPD) conditions of 2.500 L·min\(^{-1}\) and a \( \dot{V}CO_2 \) (STPD) of 2.250 L·min\(^{-1}\) and negligible protein oxidation (n = 0):\(^{319}\)

CHO Oxidation Rate \( (g \cdot min^{-1}) = 4.55 (\dot{V}CO_2) - 3.21 (\dot{V}O_2) - 2.87 (n) \)

Fat Oxidation Rate \( (g \cdot min^{-1}) = 1.67 (\dot{V}O_2) - 1.67 (\dot{V}CO_2) - 1.92 (n) \)

Venous Blood Samples
A fasting blood sample was collected at time “0” and every 15 ± 5 minutes afterwards for the first 90 minutes, and then approximately every 30 ± 5 minutes up to 180 minutes (Figure 1). Approximately 12 mL of venous blood was collected at each time point and separated into serum and plasma vacutainer tubes (Fisher Scientific). Blood samples were centrifuged at 2000 g for 15 minutes and then aliquoted into microtubes. Samples were stored in a -80°C freezer for later analysis. For the present study, the fasting plasma samples were analyzed with Multi-Analyte Profile (MAP) to determine concentrations of a wide range of biomarkers including inflammatory markers, immune markers, and cytokines and growth factors, referred to from this point forth as inflammatory markers. A total of 37 markers were measured using the multiplexed immunoassay array (Custom Discovery MAP®v.1.1 Myriad; Myriad-RBM, Texas) and are reported in Table 1. Of the inflammatory biomarkers, 17 were excluded from statistical evaluation due to results being below assay detection levels in ≥ 30% of subjects; (Table 1) however, individual results that were within detectable ranges were reported (Tables 2 and 3). Enzyme-linked immunoassays (ELISA) determined fasting concentrations of nutritional biomarkers including plasma 25(OH)D, plasma ferritin, plasma transferrin, plasma Hb, serum iron and TIBC, and plasma IGF-1. The coefficient of variation for assays were all ≤ 10%.

Statistical Analyses

Means, standard deviations (SD), and 95% confidence intervals were calculated in a spreadsheet software program for all baseline demographic measurements and dependent variables (Microsoft Excel, version 16.10). Inflammatory and nutritional markers were expressed in absolute terms and normalized to FM to examine the influence of adipose tissue on differences between groups. Normal distribution of the data was confirmed by the Shapiro-Wilk test, and non-normal data was log-transformed. Separate two-way factorial analyses of variance
(ANOVA) (sex [male vs. female] x sarcopenic status [non-sarcopenic vs. sarcopenic]) were used to analyze inflammatory and nutritional markers as absolute values and normalized to fat mass. Follow-up statistical tests were performed with Bonferroni-corrected independent samples t-tests to examine sarcopenic status and sex differences.

Equality of variances was tested using Levene’s Test for Equality of Variances. If the homogeneity of variances assumption was not met, the error term and degrees of freedom were be adjusted using the Welch-Satterhwaite method. All statistical analyses were performed with IBM SPSS v. 25 (Chicago, IL, USA). An alpha of $p \leq 0.05$ was considered statistically significant for all comparisons.
CHAPTER IV: RESULTS

Anthropometrics, Body Composition, and Muscle Strength

Baseline characteristics (means ± SD) of the 21 participants separated by sarcopenic status and sex are displayed in Table 4. There were significant sex x sarcopenic status interactions for age, weight, BMI, FFM, 5RM leg extension strength, and repetitions to exhaustion (p=0.014 – 0.034, Table 4). When comparing by sarcopenic status, sarcopenic individuals were older, had greater percent body fat, and lower FFM, RSMI, handgrip strength, gait speed, SPPD scores, estimated \( \dot{V}O_2 \) max, leg extension strength, and repetitions to exhaustion compared to non-sarcopenic individuals (p<0.001 – 0.038, Table 4). When comparing by sex, males were taller, had lower percent body fat and fat mass, and greater FFM, RSMI, handgrip strength, estimated \( \dot{V}O_2 \) max, leg extension strength, and repetitions to exhaustion compared to females (p<0.001 – 0.003, Table 4).

Inflammatory Biomarkers

There was a significant sarcopenic status x sex interaction for VDBP (p=0.038), that when normalized to FM, was higher in males than females (p=0.004) (Tables 5 and 6). Specifically, non-sarcopenic males had higher VDBP normalized to FM compared non-sarcopenic females (p=0.008) (Figure 3). There were no other significant interactions (p=0.309-0.974). Sarcopenic individuals had 45% greater concentrations of IL-1ra and 89% greater concentrations of MIP-1β compared to non-sarcopenic individuals (p=0.038 – 0.042) when collapsed by sex (Figures 4 and 5). However, these differences were eliminated when normalized to FM (p=0.098-0.296). Factor VII was greater in females compared to males, (p=0.044) (Tables 5), with sarcopenic females having 54% greater levels than sarcopenic males (p=0.04) (Figure 6),
but this difference was no longer present when normalized to FM. While there were no significant differences in raw values, when normalized to FM, intercellular adhesion molecule 1 (ICAM-1) concentrations were higher in non-sarcopenic individuals compared to sarcopenic individuals (p=0.039) (Figure 7), and alpha-1-antitrypsin (AAT), IgM, and vascular endothelial growth factor (VEGF) were greater in males than females regardless of sarcopenic status (p=0.004 – 0.048) (Table 6) (Figures 8, 9, and 10).

When examining the biomarkers that were excluded from statistical analysis, characteristics of participants who presented concentrations within the detectable range were explored. Out of the 17 biomarkers excluded, individual participants had detectable concentrations in eight biomarkers (Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF), IL-6, IL-7, IL-8, IL-10, IL-17, MIP-1α, and Matrix Metalloproteinase-9 (MMP-9)), that fell within the detectable range. Besides two non-sarcopenic individuals with detectable concentrations for MMP-9, all other participants were sarcopenic that had higher concentrations than the lower limit of quantitation for GM-CSF (n=1), IL-6 (n=1), IL-7 (n=1), IL-8 (n=3), IL-10 (n=4), IL-17 (n=1), MIP-1α (n=2), and MMP-9 (non-sarcopenic, n=2; sarcopenic, n=3). Tables 2 and 3 report biomarker values and characteristics of these individuals.

Nutritional Biomarkers

There was a significant sarcopenic status x sex interaction for ferritin (p=0.049), in which non-sarcopenic males were greater than non-sarcopenic females (p=0.025) (Table 7, Figure 11). There were no other significant interactions. There was a significant main effect for IGF-1 for sarcopenic status (p=0.011) in which non-sarcopenic individuals had 31% greater concentrations of IGF-1 than sarcopenic individuals (Figure 12). Specifically, non-sarcopenic males had 70% greater levels than sarcopenic males (p=0.033). There was a significant sex (p=0.024) for IGF-1
concentrations in which males were greater than females (p=0.024) (Figure 12). Any sarcopenic status or sex differences in IGF-1 were no longer present when normalized to FM (Tables 7 and 8). Table 7 reports the number of individuals in each group with low concentrations of each nutritional biomarker.
CHAPTER V: DISCUSSION

The primary results of this study demonstrated that in this population of non-sarcopenic and sarcopenic older adults, there were differences between groups based on sarcopenic status for specific inflammatory biomarkers, including IL-1ra, MIP-1β, and ICAM-1 and nutritional markers ferritin and IGF-1. However, the differences observed between non-sarcopenic and sarcopenic individuals for IL-1ra, MIP-1β, ferritin, and IGF-1 were no longer present after normalizing for FM, while the difference seen between groups for ICAM-1 was only present when normalizing for FM, indicating that adipose tissue quantity has an effect on inflammatory status in older adults. Additionally, the differences observed between males and females for factor VII and IGF-1 were no longer significant after normalizing for FM, while differences between sexes for AAT, IgM, VEGF, and VDBP were only significant when normalizing for FM. Since females had greater FM than males, adipose tissue may have been a contributor to the differences in inflammation and nutritional biomarkers observed. Despite distinct metabolic and muscle perfusion differences between non-sarcopenic and sarcopenic individuals, this study population overall did not demonstrate high inflammatory status as evidenced by many inflammatory biomarkers levels being below the detectable assay range. However, these study findings indicate some inflammatory and nutritional differences in iron status and IGF-1 between non-sarcopenic and sarcopenic older adults. Sarcopenic older adults also had a higher prevalence of low vitamin D and iron status compared to non-sarcopenic older adults. The findings of the present study aid in understanding the physiological differences between non-sarcopenic and sarcopenic older adults that may help with understanding mechanisms behind the development of inflammaging and sarcopenia in older adults.
Inflammation

Chronic inflammation is commonly present in the aging population, related to immunosenescence, decreased physical activity, increased adipose tissue, and malnutrition. The development of chronic inflammation with age, known as inflammaging does not have specific criteria for inflammatory biomarkers to quantify this phenomenon. However, chronic inflammation, typically examined with pro-inflammatory biomarkers such as IL-6 and TNF-α has been associated with sarcopenia, and more commonly found in frail older adults and older adults with chronic disease. While the present study examined the presence of inflammaging in older adults with well-established differences in skeletal muscle mass, strength, and function, levels of pro-inflammatory cytokines were within normal ranges, in general, indicating that chronic inflammation was not present in this population. Previous studies have reported that high levels of pro-inflammatory markers are related to impaired skeletal muscle mass, strength, and/or function in older adults. For example, older adults with higher concentrations of IL-6 and TNF-α had 3.3 – 6.5% lower muscle mass and 5.5 – 8.85% lower muscle strength compared to older adults with lower inflammatory cytokine concentrations. Bian et al. found that levels of IL-6 and TNF-α were higher in older adults with sarcopenia (n=79) compared to levels in the control group (n=362). Additionally, BMI and visceral adipose tissue were independent risk factors for inflammation, suggesting that FM is a major contributor to increased inflammation.

In the present study, sarcopenic older adults demonstrated 45% higher concentrations of IL-1ra compared to non-sarcopenic older adults (Table 5, Figure 4). Previously, concentrations of IL-1ra were reported to be inversely associated with physical performance and predictive of mortality in older adults. It has been hypothesized that the balance between IL-1 and IL-1ra is
highly influential towards pathophysiological outcomes such as inflammatory bowel disease, cancer, osteoporosis, diabetes, and coronary artery disease.\textsuperscript{322} This imbalanced ratio of IL-1 to IL-1ra may be present in this population of older adults since the levels of IL-1 were low enough to be beyond the detectable range (Table 1). Additionally, it is suggested that IL-1ra be considered as predictive biomarker of aging and influential in the array of cytokines involved in inflammaging.\textsuperscript{321,323} Since sarcopenic individuals had higher concentrations of IL-1ra when compared to non-sarcopenic individuals (185.2 ± 65.5 vs. 127.7 ± 23.3), it is possible that this biomarker could be used as an early detector for inflammaging.

Non-sarcopenic older adults had 89\% higher concentrations of MIP-1β than the sarcopenic older adults in the present study. The pro-inflammatory biomarker MIP-1β plays a role in the development and modulation of inflammatory responses during infection and infectious disease.\textsuperscript{324} This biomarker is a pro-inflammatory macrophage that increases not only with inflammation but is also elevated within skeletal disease. Higher levels of MIP-1β have been associated with pathogenesis of skeletal disease in patients with multiple myeloma\textsuperscript{325} and type 1 Gaucher patients.\textsuperscript{326} This relationship with skeletal system impairment, including suppression of osteoblastic bone formation, may also be associated with skeletal muscle, since in the present study, participants with lower muscle mass and strength had higher values (361.0 ± 237.6) in comparison to those with normal muscle mass and strength (191.3 ± 65.1).

Body composition is a known contributor to the chronic inflammatory environment associated with inflammaging, with a disproportional amount of muscle and adipose tissue resulting in an imbalance of pro- and anti-inflammatory cytokines.\textsuperscript{73,238,244} Specifically, adipose tissue produces pro-inflammatory cytokines, resulting in an increased inflammatory state, whereas skeletal muscle produces anti-inflammatory cytokines.\textsuperscript{238,244} This indicates that the
maintenance of skeletal muscle mass and prevention of excess adipose tissue may be essential to prevention inflammaging, and thus, further exacerbating sarcopenia development.

Body fat percentage was higher in sarcopenic males and females (42% and 27%, respectively) compared to non-sarcopenic males and females (36% and 23%, respectively) in the current study (Table 2). This indicates that the higher adipose tissue quantity in sarcopenic individuals were contributory to the higher levels of pro-inflammatory biomarkers. For both IL-1ra and MIP-1β, the differences observed between non-sarcopenic and sarcopenic individuals disappeared after normalizing to FM (Table 6), indicating when taking adipose tissue content into account, there were no longer differences in inflammation. Similarly, Brinkley et al. discovered that when adjusting for FM, relationships between skeletal muscle function tests and inflammatory markers (CRP and IL-6) were no longer present. Concentrations of IL-1ra and MIP-1β have been elevated with obesity. For example, in non-diabetic, obese individuals, IL-1ra concentrations were 6.5 times greater than lean individuals. Additionally, lean body mass and insulin resistance were predictors of IL-1ra concentrations, suggesting that the lower muscle mass and presence of metabolic inflexibility in the sarcopenic individuals also contributed to the higher IL-1ra concentrations. Additionally, MIP-1β, along with five other inflammatory markers, were identified has being oversecreted by adipose tissue in human obesity and may be connected to the obesity-related development of cardiovascular disease and metabolic impairments such as skeletal muscle insulin resistance. These finding emphasizes the importance of maintaining a healthy body composition with age to prevent inflammaging and further progression of sarcopenia.

Factor VII is a coagulation factor that has an important role in fibrin formation and the conversion of prothrombin to thrombin. Elevated factor VII concentrations is a risk factor for
venous thrombosis and ischemic heart disease. In the present study, sarcopenic females had 54% higher factor VII concentrations than sarcopenic males, with no other differences between groups (Figure 6). Markers of coagulation, including factor VII, have been associated with aging and functional decline. Increased biomarkers of coagulation are associated with impaired lower-extremity function and activities of daily living. Typically, inflammatory and coagulation pathways increase with age, usually in those who are considered frail, and in conjunction, may provide early signs of inflammaging. Additionally, as noted previously, adipose tissue largely expresses pro-inflammatory cytokines, as well as coagulation factors; thus the greater FM accumulation commonly seen with age, particularly in older adults with low skeletal muscle mass, strength, and function may contribute to higher coagulation levels. The higher body fat percentage of sarcopenic females again may a large contributor to an increased inflammatory and prothrombic state with age.

Interestingly, certain biomarkers displayed differences between sarcopenic status (ICAM-1) and sex (IgM, VEGF, and VDBP) only when FM was accounted for, yet there were no differences when examining raw values (Tables 5 and 6). When accounting for FM, non-sarcopenic individuals had 23% higher concentrations of ICAM-1 compared to sarcopenic individuals. This biomarker is an adhesion molecule that coordinates leukocyte delivery in response to inflammation. Typically, higher concentrations of ICAM-1 is associated with increased cardiovascular and mortality risk in older adults, endothelial dysfunction, and obesity. Before normalizing to FM, sarcopenic females had the highest ICAM-1 concentrations, although not statistically different from the other groups (Table 3), along with the highest body fat percentage. Non-sarcopenic individuals had lower body fat percentage than their sarcopenic counterparts (Table 2), so when normalizing by FM, higher values were present in
those with lower adiposity. In individuals with type 2 diabetes, ICAM-1 concentrations correlated with measurements of adiposity (BMI and waist circumference), insulin resistance, and endothelial dysfunction. Therefore, it is likely that adipose tissue is also a large contributor to differences in ICAM-1, specifically between the non-sarcopenic and sarcopenic individuals in the present study.

Furthermore, sex differences were observed in AAT, IgM, VEGF, and VDBP when normalized to FM, in which males had greater concentrations compared to females. Alpha-1 antitrypsin has protective functions within the lung, along with an anti-inflammatory role. Deficiencies in AAT are associated with inflammation and immune dysfunction and thought to accelerate aging. Higher concentrations in the males that were no longer significant when normalized to FM, may indicate that body composition differences between males and females may contribute to early aging through immunosenescence and inflammation.

Concentrations of IgM can help detect immune response and infection and typically decrease with age in both males and females. In contrast to the present study, Carballo et al. found higher levels in females compared to males. However, BMI was negatively associated with IgM concentrations, suggesting that the female older adults with greater FM in the present study would have lower IgM concentrations. Additionally, Pereira et al. recently reported that oral nutritional supplementation improved IgM concentrations in malnourished, sarcopenic older adults, suggesting immune function in older adults can be improved.

Vascular endothelial growth factor (VEGF) has an essential role in vascular function. Decreased VEGF with age leads to vascular aging that contributes to a multitude of age-associated developments. In mice, VEGF treatment decreased age-associated weight and adipose tissue gain, improved metabolic flexibility, protected against loss of muscle and bone, reduced
inflammaging, and improved muscle tissue perfusion and oxygenation.\textsuperscript{348} Males, regardless of sarcopenic status, had higher VEGF concentrations than females, potentially contributing to the body composition differences observed. Although this population of non-sarcopenic and sarcopenic older adults had distinct differences in metabolic flexibility\textsuperscript{31} and muscle perfusion (unpublished data), differences in VEGF due to sarcopenic status were not observed. Future studies in non-sarcopenic and sarcopenic older adults may see differences in VEGF concentrations related to metabolic flexibility and muscle perfusion.

Vitamin D-binding protein (VDBP) performs a variety of functions involving vitamin D metabolite transport, bone development, and modulating immune and inflammatory actions.\textsuperscript{349} Zhu et al\textsuperscript{350} reported an inverse relationship between concentrations of VDBP with age in older adults, suggesting that older adults, in general are at risk for lower VDBP levels. Additionally, VDBP was inverse correlated with BMI.\textsuperscript{350} This aligns with the higher levels observed in the males of the present study due to lower adiposity compared to females (Table 4).

While sarcopenia demonstrated higher concentrations in a few select biomarkers (IL-1ra and MIP-1β), overall both non-sarcopenic and sarcopenic individuals in the present study were within normal ranges or below detectable levels for most of the 37 inflammatory cytokines analyzed (Table 1 and 3). While not different between groups, the older adults had higher than normal levels of eotaxin-1, IL-18, and MCP-1.\textsuperscript{351–355} These biomarkers are associated with an increased inflammatory state and aging-related cognitive function, suggesting that these older adults, in general, may be showing early signs of developing inflammaging and cognitive decline, yet still very healthy. Since inflammaging is typically associated with the development of chronic disease,\textsuperscript{260,261} is it possible that this population of non-sarcopenic and sarcopenic older adults were not experiencing a chronic inflammatory environment due to the strict health
inclusion and exclusion criteria utilized for the study screening. Marcos-Perez et al. examined frail and non-frail older adults to see if frailty was related to inflammatory and immune function biomarkers. Frail participants had lower immune function markers and higher inflammatory markers compared to non-frail participants, indicating that inflammaging may be related to frailty status in older adults, thus, frail older adults may more vulnerable to developing inflammaging. In contrast to the present study, in the study by Marcos-Perez et al., most participants had co-morbidities (15% of non-frail and 40% of frail individuals), that include conditions that may influence inflammation such as diabetes and cardiovascular disease.

**Nutritional Status**

In the present study, ferritin concentrations were greater in non-sarcopenic males compared to non-sarcopenic females; however, there were no statistical differences in biomarkers of iron status or vitamin D status between non-sarcopenic and sarcopenic older adults. Additionally, non-sarcopenic individuals had greater concentrations of IGF-1 that were no longer significant after normalizing for FM. In the total population of older adults, means ± SD for ferritin, hemoglobin, serum iron, TIBC, transferrin saturation, and IGF-1 were within normal ranges (Table 7). Sarcopenic females were considered vitamin D deficient with a mean concentration of 18.7 ± 4.7 ng·mL⁻¹. Non-sarcopenic females and sarcopenic males were considered vitamin D insufficient (46.6 ± 32.0 and 36.8 ± 26.1 ng·mL⁻¹, respectively), and non-sarcopenic males were vitamin D sufficient (50.7 ± 49.2 ng·mL⁻¹) (Table 7). However, individual examination of biomarkers highlighted nutritional deficiencies, particularly in the sarcopenic population (Table 7). These findings indicate sarcopenia-related and sex differences in nutritional status, suggesting that nutrition may be a key factor in preventing mechanistic factors contributing to sarcopenia.
Ferritin concentrations were 242% higher in non-sarcopenic males compared to non-sarcopenic females, while there were no differences when sex was collapsed across sarcopenic status. Previously, higher ferritin concentrations were reported in males compared to females as seen in the non-sarcopenic group (Figure 11), and these higher levels were associated with dietary intake.\textsuperscript{295} Kim et al\textsuperscript{185} reported higher ferritin concentrations in female sarcopenic older adults compared to female non-sarcopenic older adults. Since ferritin is an acute phase protein and increases in a pro-inflammatory state,\textsuperscript{186} it is possible this difference is due to inflammation in the study population. No measurements of inflammation or other markers of iron status were obtained in the study conducted by Kim et al\textsuperscript{185} in contrast to the present study, suggesting that the higher ferritin values signify greater inflammation. In the present study, inflammatory markers were within normal ranges (Table 5), indicating that ferritin values were not affected by inflammatory status. In the non-sarcopenic individuals, only one female was considered iron deficient based on ferritin concentrations $> 35$ ng·mL$^{-1}$, and two sarcopenic males and two sarcopenic females had low concentrations.\textsuperscript{356} This suggests that in an older adult population, sarcopenic individuals may be more at risk for iron deficiency. Iron deficiency has been associated with low SPPB, fatigue, and muscle strength in older adults.\textsuperscript{173,357} These findings, along with the higher prevalence of iron deficiency in the sarcopenic participants of the present study, suggest that iron deficiency may contribute to a decline in muscle strength and function in older adults.

Anabolic hormones such as IGF-1 are important for the growth of muscle cells, but typically decrease with age. Additionally, age-related decreases in IGF-1 have been associated with sarcopenia.\textsuperscript{358} Previously, higher IGF-1 concentrations have demonstrated higher muscle mass and strength and adequate nutritional status,\textsuperscript{359–362} indicating that higher IGF-1
concentrations may contribute to maintaining muscle mass and strength with age. Non-sarcopenic older adults had 46% greater IGF-1 concentrations than sarcopenic older adults in the present study (Figure 12). While neither group had levels that were considered deficient for older adults, the difference observed in IGF-1 (144.97 vs. 99.02 ng·mL⁻¹ in non-sarcopenic and sarcopenic groups, respectively) may be a large enough difference to contribute to impaired muscle mass and strength as observed with sarcopenia. Previously, Bian et al. reported a similar difference in IGF-1 concentrations in non-sarcopenic and sarcopenic older adults (136.31 vs 98.53 ng·mL⁻¹), suggesting that the concentrations observed in sarcopenic older adults are low enough to impact muscle mass, strength, and function.

For both ferritin and IGF-1, the differences observed between groups were eliminated when normalized to adipose tissue (Table 8). Body fat content and obesity are known to affect iron metabolism, causing impaired iron absorption and lower iron stores. This relationship is commonly a result of an increase in inflammation coinciding with obesity. Increased inflammation, primarily IL-6 concentrations, result in an increase in hepcidin production, and thus, iron sequestration, lower ferritin concentrations, and impaired iron absorption. While IL-6 levels, and most other measured inflammatory cytokines were low in the present study population, it is possible that the higher adiposity present in females, predominantly sarcopenic females, is promoting an environment that leads to inflamaging development and nutritional deficiencies. Additionally, IGF-1 has been associated with BMI and bodyweight, leading to hypotheses that the decline in IGF-1 concentrations with age relates to changes in body composition. Therefore, body composition may also be influential to IGF-1 concentrations with age.
While there were no differences between groups for vitamin D status, vitamin D deficiency (< 20 ng·mL⁻¹) was more prevalent in sarcopenic females, with four of the five sarcopenic females vitamin D deficient (Table 7). Low vitamin D status has been associated with lower muscle mass, strength, and function in older adults. Additionally, vitamin D deficiency is thought to be associated with higher inflammation, indicating that deficiencies in this nutrient may be compound inflammaging within sarcopenic individuals. Although prevalence of vitamin D deficiency was higher in sarcopenic females, there were no differences observed between groups based on sarcopenic status. Therefore, it is likely that the lower vitamin D concentrations observed in females was primarily due to higher adipose tissue. Obesity is associated with lower vitamin D levels indicating that adverse changes in body composition that occur with sarcopenia may be contributory to the development of nutritional deficiencies such as vitamin D and iron.

Besides for ferritin, none of the other markers of iron status showed differences between groups. However, low TIBC and Hb were present in sarcopenic individuals. Specifically, four sarcopenic males had low concentrations of Hb and low TIBC was present in three sarcopenic males and three sarcopenic females (Table 7). Anemia and iron deficiency have been well documented in sarcopenic older adults and have been associated with impaired muscle strength and function. Therefore, the higher prevalence of deficiencies observed with sarcopenia emphasize that nutrition is important for maintenance of muscle mass and strength with age.

Nutrition plays a large role on skeletal muscle and body composition, metabolic health, and inflammatory status. Malnutrition and nutritional deficiencies are common in sarcopenic older adults and are related to chronic inflammation, skeletal muscle decline,
increased adiposity, and metabolic impairment and other chronic diseases. In this population of older adults, differences in metabolic flexibility, skeletal muscle mass and strength, and body fat were apparent. These differences are likely main contributors to the demonstrated differences in inflammatory and nutritional markers, therefore targeted nutritional strategies may be imperative to prevent inflammaging and sarcopenia. These findings suggest that adequate overall nutrition, with a focus on specific nutrients such as iron and vitamin D, along with regular physical activity to maintain a healthy body composition, may be a necessary component to preventing or delaying the progression of inflammaging with sarcopenia.

Conclusions

Overall, the present results demonstrated sarcopenic status and sex-specific differences in certain inflammatory and nutritional markers in non-sarcopenic and sarcopenic older adults. Previously, this study population demonstrated distinct differences in metabolic flexibility and energy metabolism and muscle perfusion. Therefore, these distinguishing factors, along with the differences reported in inflammation and nutritional status may all be contributory to early development of inflammaging with sarcopenia.

This study explored an array of biomarkers with the intent of distinguishing if there were differences in inflammation and nutritional status between non-sarcopenic males and females and sarcopenic males and females. Sarcopenic individuals had 45% higher concentrations of IL-1ra and 89% higher concentration so MIP-1β compared to non-sarcopenic individuals. However, these differences were no longer significant when normalizing to FM. Additionally, sex differences were observed for factor VII, IgM, VEGF, and VDBP, in which males had lower factor VII and higher IgM, VEGF, and VDBP concentrations compared to females. However, levels of AAT, IgM, VDBP, and VEGF were higher in males compared to females only when
normalized to FM. This finding may be more related to the particular study population of the
males in this study. Anecdotally, the males in the study population were previously manual
laborers and continued to stay active despite their age. Additionally, the final group to be filled
were the sarcopenic males, in which a majority were found within the same assisted living
community in which members were very well-taken care of, which may have influenced results
in biomarkers.

Ferritin concentrations were higher in non-sarcopenic males compared to non-sarcopenic
females, suggesting lower iron stores in females, potentially due to dietary intake. Additionally,
sarcopenic individuals had a higher prevalence of iron deficiency in this population, as well as
vitamin D deficiency. Sarcopenic individuals also demonstrated lower IGF-1 levels. However,
differences between groups were again no longer present when normalized to FM, which re-
emphasizes the fact that adipose is highly influential on inflammation and nutritional status with
sarcopenia. Collectively, these findings indicate that sarcopenic individuals are more likely to
have nutritional deficiencies, which are influential to sarcopenia progression and increase
vulnerability for developing inflammaging.

Interestingly, all of these biomarkers are influenced by adipose tissue, signifying that
body fat content is the main contributor in differences in inflammatory cytokine markers in this
population of older adults. Adipose tissue is a primary driver of inflammation, metabolic disease,
and immune dysfunction with aging. With the sarcopenia-related and sex differences observed in
this population, evidence suggests that the higher adipose tissue in both sarcopenic individuals
and females may be the influential factor in the development of inflammaging with sarcopenia.
Therefore, muscle disuse, along with inadequate nutrition, lead to the body composition
paradigm of decreased muscle mass and increased adiposity, compounding the risk for increased
inflammation, metabolic inflexibility, decreased muscle perfusion and vascular function, and impaired nutritional status, emphasizing the need to research strategies to improve exercise and nutrition in older adults.

**Strength and Limitations**

Strengths of this study included a population of older adults with distinct differences in metabolism and muscle tissue oxygenation. These older adults were meticulously screened to exclude older adults with co-morbidities that may have affected inflammatory and nutritional status. Additionally, a variety of biomarkers were chosen to explore in order to provide support for biomarkers useful to target in future studies. One limitation of the present study was the lack of longitudinal data. Previous studies have used similar cross-sectional designs to examine inflammatory status and nutritional status in older adults. However, we are unaware of any previous studies that examine differences in an array of biomarkers related to inflammaging in a population of non-sarcopenic and sarcopenic males and females that were otherwise healthy in order to decrease the influence of chronic disease on inflammation. An additional limitation was the small sample size in this study. However, this study was an exploratory study to determine mechanistic differences in sarcopenic status by examining metabolism, muscle tissue oxygenation, inflammation, and nutritional status in this population in order to direct future studies.

**Future Directions**

Although the present results contribute unique information to the literature regarding how inflammaging may be related to metabolic flexibility, muscle tissue oxygenation, and nutrition in older adults, additional studies are required to better understand these underlying mechanisms.
linking inflammation to declines in muscle mass, strength, and function. Other contributory mechanisms such as targeted nutritional and exercise strategies to improve nutritive flow should be examined and compared to better understand how to prevent and/or delay the progression of sarcopenia and inflammaging. Additionally, due to the large influence adipose tissue and skeletal muscle have on inflammatory and nutritional status, examining sex-specific, sarcopenic status-related outcomes on metabolism, muscle perfusion and oxygenation, and how it relates to inflammatory and nutritional status would benefit the literature in prevent and delaying the progression of inflammaging and sarcopenia in older adults, particularly focusing on sarcopenic obesity.
Figure 1. Sarcopenia is a multi-factorial condition that is influenced by integrating, contributing factors. Examining multiple factors is causing or exacerbating the risk of developing sarcopenia may provide insight on strategies for prolonging the progression of sarcopenia.
**Figure 2.** Study design and main outcomes of the original clinical trial (BL39: A pilot study to explore muscle energy metabolism and metabolic flexibility in older men and women, NCT03701878) and how the variables tested retrospectively may relate to the previous findings.
**Figure 3.** Means ± SD for vitamin D binding protein (VDBP) in non-sarcopenic males, non-sarcopenic females, sarcopenic males, and sarcopenic females. * indicates higher VDBP in males compared to females.
**Figure 4.** Means ± SD for interleukin-1 receptor antagonist (IL-1ra) in non-sarcopenic males, non-sarcopenic females, sarcopenic males, and sarcopenic females. * indicates higher IL-1ra in sarcopenic individuals compared to non-sarcopenic individuals.
Figure 5. Means ± SD for macrophage inflammatory protein-1 beta (MIP-1β) in non-sarcopenic males, non-sarcopenic females, sarcopenic males, and sarcopenic females. * indicates higher MIP-1β in sarcopenic individuals compared to non-sarcopenic individuals.
Figure 6. Means ± SD for Factor VII in non-sarcopenic males, non-sarcopenic females, sarcopenic males, and sarcopenic females. * indicates higher Factor VII concentrations in sarcopenic females compared to sarcopenic males.
Figure 7. Means ± SD for intercellular adhesion molecule 1 (ICAM-1) normalized to fat mass (FM) in non-sarcopenic males, non-sarcopenic females, sarcopenic males, and sarcopenic females. * indicates higher ICAM-1 normalized to FM concentrations in non-sarcopenic individuals compared to sarcopenic individuals regardless of sex.
**Figure 8.** Means ± SD for alpha-1-antitrypsin (AAT) normalized to fat mass (FM) in non-sarcopenic males, non-sarcopenic females, sarcopenic males, and sarcopenic females. * indicates higher AAT normalized to FM concentrations in males compared to females regardless of sarcopenic status.
**Figure 9.** Means ± SD for immunoglobulin (IgM) normalized to fat mass (FM) in non-sarcopenic males, non-sarcopenic females, sarcopenic males, and sarcopenic females. * indicates higher IgM normalized to FM concentrations in males compared to females regardless of sarcopenic status.
Figure 10. Means ± SD for vascular endothelial growth factor (VEGF) normalized to fat mass (FM) in non-sarcopenic males, non-sarcopenic females, sarcopenic males, and sarcopenic females. * indicates higher VEGF normalized to FM concentrations in males compared to females regardless of sarcopenic status.
Figure 11. Means ± SD for ferritin in non-sarcopenic males, non-sarcopenic females, sarcopenic males, and sarcopenic females. * indicates higher ferritin concentrations in non-sarcopenic males compared to non-sarcopenic females.
Figure 12. Means ± SD for insulin growth factor 1 (IGF-1) in non-sarcopenic males, non-sarcopenic females, sarcopenic males, and sarcopenic females. * indicates a main effect for sarcopenic status, in which IGF-1 concentrations were reported in non-sarcopenic individuals compared to sarcopenic individuals. ** indicates a main effect for sex, in which non-sarcopenic males had greater IGF-1 concentrations compared to non-sarcopenic females.
Table 1. Inflammatory (37) and nutritional (5) biomarkers analyzed in non-sarcopenic and sarcopenic older adults

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha-1-Antitrypsin (AAT)</td>
<td>mg·mL⁻¹</td>
</tr>
<tr>
<td>Brain-Derived Neurotrophic Factor (BDNF)</td>
<td>ng·mL⁻¹</td>
</tr>
<tr>
<td>Complement C3 (C3)</td>
<td>mg·mL⁻¹</td>
</tr>
<tr>
<td>Eotaxin-1</td>
<td>pg·mL⁻¹</td>
</tr>
<tr>
<td>Factor VII</td>
<td>ng·mL⁻¹</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>mg·mL⁻¹</td>
</tr>
<tr>
<td>Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF)</td>
<td>pg·mL⁻¹</td>
</tr>
<tr>
<td>Haptoglobin</td>
<td>mg·mL⁻¹</td>
</tr>
<tr>
<td>Immunoglobulin A (IgA)</td>
<td>mg·mL⁻¹</td>
</tr>
<tr>
<td>Immunoglobulin M (IgM)</td>
<td>mg·mL⁻¹</td>
</tr>
<tr>
<td>Intercellular Adhesion Molecule 1 (ICAM-1)</td>
<td>ng·mL⁻¹</td>
</tr>
<tr>
<td>Interferon gamma (IFN-γ)</td>
<td>pg·mL⁻¹</td>
</tr>
<tr>
<td>Interleukin-1 alpha (IL-1α)</td>
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<tr>
<td>Interleukin-1 alpha (IL-1β)</td>
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</tr>
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<td>Interleukin-1 receptor antagonist (IL-1ra)</td>
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<tr>
<td>Interleukin-3 (IL-3)</td>
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<td>Interleukin-4 (IL-4)</td>
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<td>Interleukin-5 (IL-5)</td>
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</tr>
<tr>
<td>Interleukin-6 (IL-6)</td>
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</tr>
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<td>Biomarker</td>
<td>Unit</td>
</tr>
<tr>
<td>--------------------------------------------------------------------------</td>
<td>--------</td>
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<tr>
<td>Interleukin-7 (IL-7)</td>
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<td>Interleukin-8 (IL-8)</td>
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<td>Interleukin-10 (IL-10)</td>
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<td>Interleukin-12 Subunit p40 (IL-12p40)</td>
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<td>Interleukin-12 Subunit p70 (IL-12p70)</td>
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<td>Interleukin-17 (IL-17)</td>
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<td>Interleukin-18 (IL-18)</td>
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<td>Macrophage Inflammatory Protein-1 alpha (MIP-1α)</td>
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<td>Macrophage Inflammatory Protein-1 beta (MIP-1β)</td>
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<td>Matrix Metalloproteinase-3 (MMP-3)</td>
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<td>Matrix Metalloproteinase-9 (MMP-9)</td>
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<td>Stem Cell Factor (SCF)</td>
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<tr>
<td>Tumor Necrosis Factor alpha (TNF-α)</td>
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<tr>
<td>Tumor Necrosis Factor beta (TNF-β)</td>
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<tr>
<td>Vascular Endothelial Growth Factor (VEGF)</td>
<td>pg·mL⁻¹</td>
</tr>
<tr>
<td>Vitamin D-Binding Protein (VDBP)</td>
<td>μg·mL⁻¹</td>
</tr>
</tbody>
</table>

*Indicates biomarkers that were excluded from statistical analyses.
Table 2. Concentrations of inflammatory biomarkers that were above the lower limit of quantitation in individual participants of biomarkers

excluded from statistical analysis due to a majority of participants being below the detectable range.

<table>
<thead>
<tr>
<th></th>
<th>GM-CSF</th>
<th>IL-6</th>
<th>IL-7</th>
<th>IL-8</th>
<th>IL-10</th>
<th>IL-17</th>
<th>MIP-1α</th>
<th>MMP-9</th>
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<tbody>
<tr>
<td>Lower Limit of Quantitation</td>
<td>35 pg·mL⁻¹</td>
<td>4 pg·mL⁻¹</td>
<td>42 pg·mL⁻¹</td>
<td>17 pg·mL⁻¹</td>
<td>7 pg·mL⁻¹</td>
<td>1.7 pg·mL⁻¹</td>
<td>34 pg·mL⁻¹</td>
<td>38 ng·mL⁻¹</td>
</tr>
<tr>
<td>Sarcopenic</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Participant 1</td>
<td>69 pg·mL⁻¹</td>
<td></td>
<td>30 pg·mL⁻¹</td>
<td>10 pg·mL⁻¹</td>
<td>39 pg·mL⁻¹</td>
<td>77 ng·mL⁻¹</td>
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<td></td>
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<tr>
<td>Participant 2</td>
<td>30 pg·mL⁻¹</td>
<td></td>
<td>23 pg·mL⁻¹</td>
<td>8.5 pg·mL⁻¹</td>
<td>2.2 pg·mL⁻¹</td>
<td></td>
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<td>Participant 3</td>
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<td></td>
<td>47 ng·mL⁻¹</td>
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<td>Participant 4</td>
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<td>35 pg·mL⁻¹</td>
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<tr>
<td>Participant 5</td>
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<td>30 pg·mL⁻¹</td>
<td></td>
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<td></td>
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<tr>
<td>Participant 6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>14 pg·mL⁻¹</td>
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<td>Participant 7</td>
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<td></td>
<td>42 pg·mL⁻¹</td>
<td></td>
<td>54 ng·mL⁻¹</td>
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<td>Non-Sarcopenic</td>
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<td></td>
<td></td>
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<tr>
<td>Participant 8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>40 ng·mL⁻¹</td>
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<td>Participant 9</td>
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<td></td>
<td></td>
<td></td>
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<td>40 ng·mL⁻¹</td>
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Table 3. Body composition, muscle function, and muscle strength characteristics of participants that had inflammatory biomarker concentrations that were above the lower limit of quantitation of the biomarkers excluded from statistical analysis due to a majority of participants being below the detectable range.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age (years)</th>
<th>Fat Mass (kg)</th>
<th>Fat-Free Mass (kg)</th>
<th>Handgrip Strength (kg)</th>
<th>Short Physical Performance Battery</th>
<th>5RM Leg Strength (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sarcopenic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Participant 1</td>
<td>Male</td>
<td>84.0</td>
<td>7.6</td>
<td>40.9</td>
<td>28.3</td>
<td>10</td>
</tr>
<tr>
<td>Participant 2</td>
<td>Male</td>
<td>93.0</td>
<td>20.7</td>
<td>45.4</td>
<td>29.3</td>
<td>11</td>
</tr>
<tr>
<td>Participant 3</td>
<td>Male</td>
<td>83.0</td>
<td>24.2</td>
<td>51.1</td>
<td>26.7</td>
<td>12</td>
</tr>
<tr>
<td>Participant 4</td>
<td>Male</td>
<td>91.0</td>
<td>16.5</td>
<td>50.1</td>
<td>28.7</td>
<td>10</td>
</tr>
<tr>
<td>Participant 5</td>
<td>Female</td>
<td>76.0</td>
<td>28.3</td>
<td>38.2</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>Participant 6</td>
<td>Female</td>
<td>71.0</td>
<td>26.1</td>
<td>26.1</td>
<td>16</td>
<td>11</td>
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<tr>
<td>Participant 7</td>
<td>Female</td>
<td>85.0</td>
<td>32.6</td>
<td>39.2</td>
<td>13</td>
<td>9</td>
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<tr>
<td><strong>Non-Sarcopenic</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Participant 8</td>
<td>Male</td>
<td>65.0</td>
<td>12.6</td>
<td>53.5</td>
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<tr>
<td>Participant 9</td>
<td>Female</td>
<td>70.0</td>
<td>20.9</td>
<td>35.5</td>
<td>25.3</td>
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Table 4. Means ± standard deviations (SD) for baseline demographics, anthropometrics, body composition, strength, and repetitions to failure of non-sarcopenic (n=10) and sarcopenic (n=11) older adults. P-values are type I errors of the interactions and main effects.

<table>
<thead>
<tr>
<th></th>
<th>NON-SARCOPENIC</th>
<th>SARCOPENIC</th>
<th>Interaction Effect</th>
<th>Main Effect</th>
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<td></td>
<td>Males</td>
<td>Females</td>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td>Sample Size (n)</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Age (y)</td>
<td>71.8 ± 5.7</td>
<td>74.4 ± 6.9</td>
<td>87.0 ± 9.4</td>
<td>74.2 ± 7.3</td>
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<tr>
<td>Height (cm)</td>
<td>172.2 ± 6.0</td>
<td>160.7 ± 8.7</td>
<td>167.1 ± 4.3</td>
<td>157.8 ± 8.5</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>80.2 ± 9.5</td>
<td>63.4 ± 7.2</td>
<td>69.0 ± 8.9</td>
<td>71.48 ± 6.6</td>
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<tr>
<td>Body Mass Index (BMI) (kg·m⁻²)</td>
<td>27.0 ± 1.4</td>
<td>24.7 ± 2.1</td>
<td>24.7 ± 3.1</td>
<td>28.8 ± 3.5</td>
</tr>
<tr>
<td>Percent Body Fat (%)</td>
<td>22.6 ± 3.5</td>
<td>36.0 ± 2.8</td>
<td>26.8 ± 6.5</td>
<td>41.8 ± 1.6</td>
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<tr>
<td>Fat Mass (FM) (kg)</td>
<td>18.2 ± 3.6</td>
<td>22.7 ± 2.9</td>
<td>18.7 ± 6.1</td>
<td>29.1 ± 2.8</td>
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<tr>
<td>Fat-free Mass (FFM) (kg)</td>
<td>58.4 ± 6.9</td>
<td>38.4 ± 4.9</td>
<td>46.6 ± 3.7</td>
<td>38.2 ± 3.3</td>
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<tr>
<td>Relative Skeletal Muscle Index (RSMI) (%)</td>
<td>40.1 ± 2.2</td>
<td>29.6 ± 0.8</td>
<td>34.7 ± 2.4</td>
<td>25.9 ± 0.7</td>
</tr>
<tr>
<td>Handgrip Strength (kg)</td>
<td>42.1 ± 8.5</td>
<td>25.9 ± 5.4</td>
<td>27.7 ± 2.1</td>
<td>16.5 ± 2.4</td>
</tr>
<tr>
<td>Gait Speed (m·s⁻¹)</td>
<td>1.2 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>1.0 ± 0.2</td>
<td>0.9 ± 0.2</td>
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<tr>
<td>Short Physical Performance Battery (SPPB) (score)</td>
<td>12.0 ± 0.0</td>
<td>11.8 ± 0.4</td>
<td>10.5 ± 1.0</td>
<td>10.2 ± 1.6</td>
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<tr>
<td></td>
<td>Estimated VO$_2$max (ml·kg$^{-1}$·min$^{-1}$)</td>
<td>Leg Extension Strength (5RM) (kg)</td>
<td>Repetitions to Exhaustion @ 30% 1RM (reps)</td>
<td></td>
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<tr>
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<tr>
<td></td>
<td>32.1 ± 4.9</td>
<td>19.5 ± 1.3</td>
<td>26.6 ± 10.1</td>
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<td>22.4 ± 3.2</td>
<td>10.2 ± 1.9</td>
<td>14.0 ± 2.3</td>
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<td></td>
<td>26.6 ± 4.2</td>
<td>11.7 ± 3.3</td>
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<td>17.9 ± 5.4</td>
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<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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</table>
Table 5. Means ± standard deviations (SD) for inflammatory biomarkers of non-sarcopenic (n=10) and sarcopenic (n=11) older adults. P-values are type I errors between groups.

<table>
<thead>
<tr>
<th></th>
<th>NON-SARCOPENIC</th>
<th>SARCOPENIC</th>
<th>P-value</th>
<th>Sex p-value</th>
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<tbody>
<tr>
<td></td>
<td>Males (n=5)</td>
<td>Females (n=5)</td>
<td>Males (n=6)</td>
<td>Females (n=5)</td>
</tr>
<tr>
<td>Alpha-1-Antitrypsin (AAT)</td>
<td>2.22 ± 0.74</td>
<td>2.06 ± 0.09</td>
<td>2.25 ± 0.29</td>
<td>2.28 ± 0.33</td>
</tr>
<tr>
<td>Brain-Derived Neurotrophic Factor (BDNF)</td>
<td>1.51 ± 1.20</td>
<td>1.30 ± 0.78</td>
<td>2.47 ± 4.69</td>
<td>0.45 ± 0.59</td>
</tr>
<tr>
<td>Complement C3 (C3)</td>
<td>1.76 ± 0.44</td>
<td>1.76 ± 0.23</td>
<td>1.58 ± 0.37</td>
<td>1.88 ± 0.42</td>
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<tr>
<td>Eotaxin-1</td>
<td>670.80 ± 287.63</td>
<td>565.00 ± 152.94</td>
<td>499.20 ± 469.36</td>
<td>498.00 ± 282.32</td>
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<td>Factor VII</td>
<td>473.00 ± 297.78</td>
<td>566.20 ± 165.41</td>
<td>349.00 ± 169.27</td>
<td>537.60 ± 44.18</td>
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<td>Fibrinogen</td>
<td>2.30 ± 0.54</td>
<td>2.04 ± 0.50</td>
<td>2.67 ± 1.18</td>
<td>2.64 ± 0.50</td>
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<td>Haptoglobin</td>
<td>2.14 ± 2.26</td>
<td>1.52 ± 0.76</td>
<td>1.19 ± 0.73</td>
<td>2.28 ± 1.97</td>
</tr>
<tr>
<td>Immunoglobulin A (IgA)</td>
<td>4.36 ± 3.83</td>
<td>2.78 ± 1.40</td>
<td>2.83 ± 1.16</td>
<td>3.51 ± 3.06</td>
</tr>
<tr>
<td>Immunoglobulin M (IgM)</td>
<td>2.34 ± 1.20</td>
<td>1.62 ± 0.67</td>
<td>2.42 ± 1.81</td>
<td>1.90 ± 1.17</td>
</tr>
<tr>
<td>Intercellular Adhesion Molecule 1 (ICAM-1)</td>
<td>99.40 ± 25.91</td>
<td>102.00 ± 22.29</td>
<td>77.25 ± 25.08</td>
<td>119.75 ± 26.63</td>
</tr>
<tr>
<td>Interleukin-1 receptor antagonist (IL-1ra)</td>
<td>123.00 ± 19.05</td>
<td>131.25 ± 28.32</td>
<td>176.25 ± 34.87</td>
<td>192.40 ± 86.67</td>
</tr>
<tr>
<td>Interleukin-12 Subunit p40 (IL-12p40)</td>
<td>0.57 ± 0.23</td>
<td>0.50 ± 0.18</td>
<td>0.57 ± 0.19</td>
<td>0.50 ± 0.18</td>
</tr>
<tr>
<td>Interleukin-18 (IL-18)</td>
<td>296.00 ± 92.58</td>
<td>286.80 ± 68.58</td>
<td>317.17 ± 240.58</td>
<td>345.60 ± 169.30</td>
</tr>
<tr>
<td>Macrophage Inflammatory Protein-1β (MIP-1)</td>
<td>208.00 ± 79.54</td>
<td>174.60 ± 50.09</td>
<td>378.67 ± 263.89</td>
<td>339.80 ± 230.45</td>
</tr>
<tr>
<td>Protein</td>
<td>Unit</td>
<td>Mean ± SD</td>
<td>95% CI</td>
<td>p-value</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>---------------</td>
<td>------------</td>
<td>---------</td>
<td>-----------</td>
</tr>
<tr>
<td>Matrix Metalloproteinase-3 (MMP-3)</td>
<td>ng mL⁻¹</td>
<td>12.82 ± 4.51</td>
<td>5.46 ± 1.33</td>
<td>16.12 ± 17.89</td>
</tr>
<tr>
<td>Monocyte Chemotactic Protein 1 (MCP-1)</td>
<td>pg mL⁻¹</td>
<td>380.60 ± 105.00</td>
<td>432.00 ± 136.36</td>
<td>470.33 ± 377.14</td>
</tr>
<tr>
<td>Vascular Endothelial Growth Factor (VEGF)</td>
<td>pg mL⁻¹</td>
<td>77.50 ± 9.81</td>
<td>61.20 ± 11.86</td>
<td>85.60 ± 37.48</td>
</tr>
<tr>
<td>Vitamin D-Binding Protein (VDBP)</td>
<td>μg mL⁻¹</td>
<td>405.80 ± 24.75</td>
<td>227.60 ± 143.83</td>
<td>286.33 ± 22.10</td>
</tr>
</tbody>
</table>

a indicates n = 20
b indicates n = 18
c indicates n = 17
Table 6. Means ± standard deviations (SD) for inflammatory biomarkers normalized to fat mass of non-sarcopenic (n=10) and sarcopenic (n=11) older adults. P-values are type I errors between groups.

<table>
<thead>
<tr>
<th></th>
<th>NON-SARCOPENIC</th>
<th>SARCOPENIC</th>
<th>Sex p-value</th>
<th>Sarcopenic Status p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males (n=5)</td>
<td>Females (n=5)</td>
<td>Males (n=6)</td>
<td>Females (n=5)</td>
</tr>
<tr>
<td>Alpha-1-Antitrypsin (AAT)</td>
<td>0.13 ± 0.04</td>
<td>0.09 ± 0.01</td>
<td>0.14 ± 0.08</td>
<td>0.08 ± 0.02</td>
</tr>
<tr>
<td>Brain-Derived Neurotrophic Factor (BDNF)</td>
<td>0.08 ± 0.06</td>
<td>0.06 ± 0.03</td>
<td>0.13 ± 0.23</td>
<td>0.02 ± 0.02</td>
</tr>
<tr>
<td>Complement C3 (C3)</td>
<td>0.10 ± 0.01</td>
<td>0.08 ± 0.01</td>
<td>0.10 ± 0.06</td>
<td>0.07 ± 0.02</td>
</tr>
<tr>
<td>Eotaxin-1 a</td>
<td>37.99 ± 16.12</td>
<td>25.22 ± 7.48</td>
<td>43.72 ± 65.95</td>
<td>17.24 ± 10.08</td>
</tr>
<tr>
<td>Factor VII</td>
<td>26.11 ± 9.19</td>
<td>24.73 ± 5.00</td>
<td>24.03 ± 22.71</td>
<td>18.67 ± 3.00</td>
</tr>
<tr>
<td>Fibrinogen b</td>
<td>0.13 ± 0.02</td>
<td>0.09 ± 0.03</td>
<td>0.22 ± 0.20</td>
<td>0.09 ± 0.02</td>
</tr>
<tr>
<td>Haptoglobin c</td>
<td>0.12 ± 0.13</td>
<td>0.07 ± 0.03</td>
<td>0.09 ± 0.09</td>
<td>0.08 ± 0.08</td>
</tr>
<tr>
<td>Immunoglobin A (IgA)</td>
<td>0.22 ± 0.15</td>
<td>0.12 ± 0.07</td>
<td>0.17 ± 0.12</td>
<td>0.12 ± 0.11</td>
</tr>
<tr>
<td>Immunoglobin M (IgM)</td>
<td>0.13 ± 0.06</td>
<td>0.07 ± 0.03</td>
<td>0.16 ± 0.14</td>
<td>0.06 ± 0.04</td>
</tr>
<tr>
<td>Intercellular Adhesion Molecule 1 (ICAM-1) b</td>
<td>5.58 ± 1.46</td>
<td>4.51 ± 0.96</td>
<td>3.68 ± 0.92</td>
<td>4.02 ± 0.86</td>
</tr>
<tr>
<td>Interleukin-1 receptor antagonist (IL-1ra) c</td>
<td>7.41 ± 1.73</td>
<td>5.66 ± 0.61</td>
<td>12.26 ± 9.07</td>
<td>6.73 ± 3.54</td>
</tr>
<tr>
<td>Interleukin-12 Subunit p40 (IL-12p40)</td>
<td>0.03 ± 0.02</td>
<td>0.02 ± 0.01</td>
<td>0.04 ± 0.04</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>Interleukin-18 (IL-18)</td>
<td>17.53 ± 8.94</td>
<td>12.93 ± 4.03</td>
<td>20.09 ± 16.26</td>
<td>12.07 ± 6.58</td>
</tr>
<tr>
<td>Protein</td>
<td>Unit</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>--------------------------------------------------</td>
<td>------</td>
<td>-----------</td>
<td>-----------</td>
<td>-----------</td>
</tr>
<tr>
<td>Macrophage Inflammatory Protein-1β (MIP-1)</td>
<td>pg/mL·kg⁻¹</td>
<td>11.55 ± 4.08</td>
<td>7.69 ± 2.08</td>
<td>21.73 ± 16.20</td>
</tr>
<tr>
<td>Matrix Metalloproteinase-3 (MMP-3)</td>
<td>ng/mL·kg⁻¹</td>
<td>0.72 ± 0.26</td>
<td>0.24 ± 0.07</td>
<td>1.50 ± 2.62</td>
</tr>
<tr>
<td>Monocyte Chemotactic Protein 1 (MCP-1)</td>
<td>pg/mL·kg⁻¹</td>
<td>21.39 ± 5.85</td>
<td>18.76 ± 3.47</td>
<td>39.25 ± 58.33</td>
</tr>
<tr>
<td>Vascular Endothelial Growth Factor (VEGF)</td>
<td>pg/mL·kg⁻¹</td>
<td>4.54 ± 1.53</td>
<td>2.75 ± 0.74</td>
<td>5.57 ± 3.62</td>
</tr>
<tr>
<td>Vitamin D-Binding Protein (VDBP)</td>
<td>μg/mL·kg⁻¹</td>
<td>23.20 ± 5.37</td>
<td>9.97 ± 6.40</td>
<td>17.62 ± 9.02</td>
</tr>
</tbody>
</table>

* indicates n = 20

b indicates n = 18
c indicates n = 17
Table 7. Means ± standard deviations (SD) for nutritional biomarkers of non-sarcopenic (n=10) and sarcopenic (n=11) older adults. The number of individuals in each group with low values of each biomarker are reported in parentheses. P-values are type I errors between groups.

<table>
<thead>
<tr>
<th></th>
<th>NON-SARCOPENIC</th>
<th>SARCOPENIC</th>
<th></th>
<th></th>
<th></th>
<th>Sarcopenic Status p-value</th>
<th>Sex p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males (n=5)</td>
<td>Females (n=5)</td>
<td>Males (n=6)</td>
<td>Females (n=5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25(OH)D</td>
<td>50.73 ± 49.23</td>
<td>46.60 ± 31.98</td>
<td>36.82 ± 26.10</td>
<td>18.70 ± 4.70</td>
<td>0.146</td>
<td>0.441</td>
<td></td>
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<tr>
<td>Ferritin</td>
<td>170.15 ± 93.55</td>
<td>49.76 ± 30.01</td>
<td>79.71 ± 67.33</td>
<td>120.96 ± 130.19</td>
<td>0.025</td>
<td>0.393</td>
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</tr>
<tr>
<td>Hemoglobin (Hb)</td>
<td>3.49 ± 2.32</td>
<td>5.08 ± 3.48</td>
<td>6.66 ± 3.86</td>
<td>3.38 ± 0.86</td>
<td>0.524</td>
<td>0.472</td>
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<tr>
<td>Iron</td>
<td>117.47 ± 20.02</td>
<td>107.72 ± 10.43</td>
<td>117.10 ± 39.52</td>
<td>137.98 ± 24.07</td>
<td>0.246</td>
<td>0.648</td>
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</tr>
<tr>
<td>Total Iron Binding Capacity (TIBC)</td>
<td>390.60 ± 31.35</td>
<td>428.73 ± 109.24</td>
<td>387.63 ± 125.08</td>
<td>507.85 ± 117.92</td>
<td>0.512</td>
<td>0.092</td>
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</tr>
<tr>
<td>Transferrin Saturation</td>
<td>30.00 ± 3.74</td>
<td>26.03 ± 4.99</td>
<td>32.19 ± 11.25</td>
<td>27.53 ± 3.13</td>
<td>0.509</td>
<td>0.146</td>
<td></td>
</tr>
<tr>
<td>Insulin Growth Factor-1 (IGF-1)</td>
<td>155.30 ± 52.66</td>
<td>134.64 ± 40.73</td>
<td>91.26 ± 31.26</td>
<td>108.34 ± 22.37</td>
<td>0.011</td>
<td>0.024</td>
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</table>

186
Table 8. Means ± standard deviations (SD) for nutritional biomarkers normalized to fat mass of non-sarcopenic (n=10) and sarcopenic (n=11) older adults. P-values are type I errors between groups.

<table>
<thead>
<tr>
<th></th>
<th>NON-SARCOPENIC</th>
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<th>SARCPENIC</th>
<th></th>
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<td></td>
<td>Males (n=5)</td>
<td>Females (n=5)</td>
<td>Males (n=6)</td>
<td>Females (n=5)</td>
</tr>
<tr>
<td>25(OH)D</td>
<td>3.39 ± 4.21</td>
<td>2.07 ± 1.51</td>
<td>2.20 ± 1.10</td>
<td>0.65 ± 0.20</td>
</tr>
<tr>
<td>Ferritin</td>
<td>9.46 ± 4.96</td>
<td>2.18 ± 1.36</td>
<td>6.56 ± 9.88</td>
<td>4.28 ± 5.02</td>
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<tr>
<td>Hemoglobin</td>
<td>0.20 ± 0.12</td>
<td>0.23 ± 0.17</td>
<td>0.48 ± 0.48</td>
<td>0.12 ± 0.03</td>
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<tr>
<td>Iron</td>
<td>6.64 ± 1.51</td>
<td>4.80 ± 0.72</td>
<td>7.91 ± 7.07</td>
<td>4.78 ± 1.00</td>
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<tr>
<td>Total Iron Binding Capacity (TIBC)</td>
<td>22.14 ± 4.30</td>
<td>29.34 ± 6.29</td>
<td>25.16 ± 19.47</td>
<td>17.59 ± 4.65</td>
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<td>Insulin Growth Factor-1 (IGF-1)</td>
<td>8.86 ± 3.23</td>
<td>6.07 ± 2.20</td>
<td>6.54 ± 6.71</td>
<td>3.71 ± 0.62</td>
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<td>Abbreviation</td>
<td>Term</td>
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<tr>
<td>AAC</td>
<td>Area Above the Curve</td>
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<tr>
<td>AAT</td>
<td>Alpha-1-1Antitrypsin</td>
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<td>ACT</td>
<td>α1-antichymotrypsin</td>
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<td>ADL</td>
<td>Activities of Daily Living</td>
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<td>ANOVA</td>
<td>Analysis of Variance</td>
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<td>ASMI</td>
<td>Appendicular Skeletal Muscle Index</td>
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<td>ATP</td>
<td>Adenosine Tri-Phosphate</td>
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<tr>
<td>AUC</td>
<td>Area Under the Curve</td>
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<td>BMI</td>
<td>Body Mass Index</td>
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<td>BDNF</td>
<td>Brain-Derived Neurotrophic Factor</td>
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<td>C3</td>
<td>Complement C3</td>
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<td>CBC</td>
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<td>CSA</td>
<td>Cross-sectional Area</td>
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<td>DXA</td>
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<td>ELISA</td>
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<td>FFA</td>
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<td>Hb</td>
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<tr>
<td>HDL</td>
<td>High Density Lipoprotein</td>
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<tr>
<td>HHb</td>
<td>Deoxygenated Hemoglobin + Myoglobin</td>
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<tr>
<td>HOMA-IR</td>
<td>Homeostatic Model Assessment for Insulin Resistance</td>
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<tr>
<td>ICAM</td>
<td>Intracellular Adhesion Molecule</td>
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<tr>
<td>IFN-γ</td>
<td>Interferon Gamma</td>
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</table>
Ig  Immunoglobulin
IGF  Insulin-like Growth Factor
IL  Interleukin
IMAT  Intramuscular Adipose Tissue
IRB  Institutional Review Board
LDL  Low Density Lipoprotein
MAP  Multi-Analyte Profile
Mb  Myoglobin
MCP  Monocyte Chemoattractant Protein
MIP  Macrophage Inflammatory Protein
MMP  Matrix Metalloproteinase-3
MVC  Maximal Voluntary Contraction
NIRS  Near-infrared Spectroscopy
OGTT  Oral Glucose Tolerance Test
O$_2$Hb  Oxygenated Hemoglobin + Myoglobin
PCA  Principle Components Analysis
PCr  Phosphocreatine
PORH  Post-Occlusive Reactive Hyperemia
PTH  Parathyroid Hormone
RBC  Red Blood Cell
RDA  Recommended Daily Allowance
RDI  Recommended Dietary Intake
RM  Repetition Maximum
RQ  Respiratory Quotient
RSMI  Relative Skeletal Muscle Index
SPPB  Short Physical Performance Battery
sTfR  Soluble Transferrin Receptor
StO2  Tissue Oxygen Saturation
<table>
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<tr>
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<td>TGF</td>
<td>Transforming Growth Factor</td>
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<td>THb</td>
<td>Total Hemoglobin + Myoglobin</td>
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<tr>
<td>TIBC</td>
<td>Total Iron Binding Capacity</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumor Necrosis Factor</td>
</tr>
<tr>
<td>TUG</td>
<td>Timed-up-and-go</td>
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<tr>
<td>$\dot{V}CO_2$</td>
<td>Rate of Carbon Dioxide Production</td>
</tr>
<tr>
<td>$\dot{V}O_2$</td>
<td>Rate of Oxygen Consumption</td>
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<tr>
<td>VDBP</td>
<td>Vitamin D-binding Protein</td>
</tr>
<tr>
<td>VDR</td>
<td>Vitamin D Receptor</td>
</tr>
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<td>VEGF</td>
<td>Vascular Endothelial Growth Factor</td>
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<td>White Blood Cell</td>
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<td>WHO</td>
<td>World Health Organization</td>
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Relationship between Anemia, Hemoglobin Concentration and Frailty in Brazilian Older Adults.


CURRICULUM VITAE

Marni Shoemaker received her Bachelor of Arts in biology and exercise science from Augustana College in Sioux Falls, South Dakota and her Masters of Science in Exercise Physiology and Nutrition from the University of Nebraska-Lincoln (UNL). She completed her dietetic internship at UNL and was certified as a Registered Dietitian in 2017. She began her doctoral program at UNL under the mentorship of Dr. Joel Cramer in Human Sciences in 2017 and transferred with Dr. Cramer to complete her Ph.D. in Interdisciplinary Health Sciences at The University of Texas at El Paso (UTEP) in 2020. During her doctoral program, Marni has 21 publications and is lead author on seven of the publications including recent manuscripts published in *Journal of Cachexia, Sarcopenia, and Muscle*, *Nutrients*, *Clinical Nutrition*, and *Journal of the International Society of Sports Nutrition*. She has been involved in nine grant proposals to external funding agencies and was the recipient of the internal Dodson Research Grant at UTEP. Over the years, she has received scholarships and awards including the Dean’s Fellowship at UNL, Arnold E. Schaefer Memorial Scholarship from the Academy of Nutrition and Dietetics Foundation, Dr. Marie E. Knickrehm Memorial Scholarship from the Academy of Nutrition and Dietetics Foundation, SCAN Graduate Student Poster Presentation Award, and the NSCA Foundation Women’s Scholarship from the National Strength and Conditioning Association. She is certified as a Registered Dietitian, Certified Strength and Conditioning Specialist, and USA Weightlifting Level 1 Sports Performance Coach. Throughout her graduate career, Marni has been the instructor of record or the teaching assistant for a variety of courses including Healthy Living, Practicum in Exercise and Health Behavior Planning, Introduction to Sports Nutrition, Exercise, Sports, and Performance Nutrition, Human Nutrition and Metabolism, Essentials of Strength and Conditioning, Fundamentals of Nutrition, and Sports Nutrition. After graduation, Marni will begin her academic career as a tenure-track Assistant Professor of Nutrition at South Dakota State University in Brookings, South Dakota.