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Oxygenation and Activation of the Vastus Lateralis during Dynamic Constant External Resistance Leg Extension Muscle Actions in Older Women with and without Sarcopenia

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OXYGENATION AND ACTIVATION OF THE VASTUS LATERALIS DURING DYNAMIC CONSTANT EXTERNAL RESISTANCE LEG EXTENSION MUSCLE ACTIONS IN OLDER WOMEN WITH AND WITHOUT SARCOPENIA

By

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The purpose of the present study was to compare muscle strength, size, activation, and oxygenation between older women with and without sarcopenia during dynamic fatiguing leg extension bouts with high (5-repetition maximum[5-RM]) and low (30% of estimated 1-RM[30%1-RM]) loads. Eleven women (n = 6 non-sarcopenic [mean ± SE; age = 75.8 ± 2.6y] and n = 5 sarcopenic [age = 74.5 ± 3.1y]) were screened for eligibility and sarcopenic status. Descriptive assessments including demographics (age, height, and weight), body composition by dual-energy x-ray absorptiometry (fat mass[FM], fat-free mass[FFM] and percent body fat[BF%]), muscle size by ultrasonography (leg extensor muscle cross-sectional area[mCSA], vastus lateralis [VL] thickness, subcutaneous fat thickness, and echo intensity[EI]), muscle strength (leg extensor[5-RM], handgrip[HG]), muscular endurance (30%1-RM to exhaustion), and functionality (gait speed) were measured. During the 5-RM and 30%1-RM tasks, muscle activation was measured by surface electromyography (EMG), while muscle oxygenation was measured by near-infrared spectroscopy (NIRS). FM, BF%, subcutaneous fat, and EI indicated the presence of sarcopenic obesity (p ≤ 0.05). Relative skeletal mass index and HG were lower (p ≤ 0.05) in the sarcopenic group, but no other descriptive measures were different between...
groups (p > 0.05). Despite no differences (p > 0.05) in leg extensor muscle size or
strength between sarcopenic and non-sarcopenic older women, the sarcopenic women
exhibited 13 – 21% lower (p ≤ 0.05) muscle oxygenation across all repetitions of the
high- and low-load tasks. EMG amplitude (EMG_{RMS}) increased, while EMG mean power
frequency (EMG_{MPF}) decreased (p ≤ 0.05) across repetitions during both tasks, but there
were no differences between groups. These findings suggest the presence of a clinical
sarcopenic classification may not uniformly impact the size or strength of all muscles. If
greater variability is expected among muscle activation strategies of older adults, using
EMG to distinguish between sarcopenic and non-sarcopenic women without differences
in leg extensor muscle size, strength, or endurance may be difficult. However, lower
muscle oxygenation, which may reflect skeletal muscle blood flow, in clinically
sarcopenic older women may be important to consider when recommending exercise or
nutrition interventions for either oxygen or dietary nutrient delivery.
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CHAPTER 1
INTRODUCTION

In 2015, the United Nations Department of Economic and Social Affairs published a report on World Population Aging where they projected the population of adults older than 60 years to nearly double from 66.5 million in 2015 to over 108 million by 2050 in the United States alone (73). With increasing life expectancy and a dramatically increasing population of older persons, there is a growing interest to emphasize the prevention and postponing of age-related disability, specifically through nutrition and exercise-related interventions (73). The progressive loss of muscle mass with age and the accompanied decreases in strength and function, collectively known as sarcopenia, is of particular interest due to the relationship of this condition with increased risk of adverse outcomes in older adults (15). Adverse outcomes associated with sarcopenia include, but are not limited to, increased risk of falls, fractures, disability, institutionalization, and all-cause mortality (10,26,27,37). Furthermore, sarcopenia may be prevalent in more than 20% of healthy, community-dwelling adults ages 65 years and older and the prevalence increases to as much as 30% in females and 50% in males over 80 years old (36). More research is needed to understand the mechanisms of sarcopenia so that interventions can be explored that may help slow the acceleration of sarcopenia and reduce adverse outcomes in older adults (72).

Maintaining adequate blood flow, or improving blood flow with exercise, has been proposed as a mechanism to enhance the delivery of nutrients to skeletal muscles in older adults (14,71). Previous studies have demonstrated that aging muscle exhibits a degree of anabolic resistance following protein (41,61) and mixed nutrient ingestion (76),
which has led to higher recommendations of protein intake for older adults to maintain net protein balance (42,70). Also, older adults exhibit age-related insulin resistance that impairs muscle protein synthesis (21,62,74,76). It has also been shown that the attenuated effects of insulin on muscle protein synthesis may be due to an impaired endothelial response to the vasodilatory effects of insulin (16). However, exercise seems to improve endothelial-dependent dilation and the subsequent anabolic effects of nutrient consumption in healthy older adults (16,19,20,53,67,68). For example, Timmerman et al. (71) demonstrated increased blood flow, muscle perfusion, amino acid delivery, and muscle protein synthesis after mixed nutrient intake in older adults who had completed a bout of aerobic exercise the night prior. Thus, acute exercise may enhance the anabolic response to nutrient intake in older adults by improving nutrient delivery to the muscle via increased blood flow and muscle perfusion. Understanding how skeletal muscle perfusion changes with the presence of sarcopenia may be important for both exercise and nutrition recommendations in older adults.

In a study by Cramer et al. (14), individuals who were more severely sarcopenic (impaired gait speed and handgrip strength) benefited less from mixed nutrient supplementation than those who were only moderately sarcopenic (impaired gait speed, but normal grip strength). Since the moderately sarcopenic subgroup demonstrated higher baseline handgrip and leg strength, but no differences in fat-free mass, a more robust blood flow to the working muscles may have existed, which could have accounted for the better response to oral nutritional supplementation (14). Alternatively, the individuals who were more severely sarcopenic may have had a greater degree of impaired nutrient delivery, and subsequently, less favorable responses to the oral nutritional
supplementation (14). These results emphasize the need to compare strength and blood flow measurements between sarcopenic and non-sarcopenic older adults, which may help to explain the mechanisms underlying any responses (or lack thereof) to nutritional interventions.

Sex-related differences in fatigability during leg extension tasks have been well-explored (12,52,77). However, less is known about the effects of sarcopenia during skeletal muscle fatiguing (30–32). Since sex-related differences in skeletal muscle fatigability have been attributed to muscle size, muscle activation, fiber type properties, and muscle blood flow (33), it is reasonable to hypothesize that the presence of sarcopenia may impact muscle fatigue via similar mechanisms. Clark et al. (12) demonstrated differences in time to failure during a sustained submaximal leg extension task between men and women, where men fatigued more quickly than women. The authors then completed the same protocol with the addition of femoral artery occlusion, which subsequently eliminated the differences in time to failure between men and women. These findings suggest that differences in blood flow and muscle perfusion between men and women may contribute to differences in fatigability. If the presence of sarcopenia does impact the delivery of nutrients, including oxygen, to skeletal muscles, it is possible that sarcopenic versus non-sarcopenic individuals would respond similarly to the men versus women studied by Clark et al. (12). If other factors, such as muscle size, muscle activation, and fiber type properties can be held reasonably constant between sarcopenic versus non-sarcopenic groups, differences in skeletal muscle fatigability could be attributable to blood flow / perfusion.
The purpose of the present study is to compare muscle strength, size, activation, and tissue oxygenation between older women with and without sarcopenia during dynamic fatiguing leg extension bouts with high (5-RM) and low (30% of estimated 1-RM) external loads. It is hypothesized that (a) oxygenated hemoglobin will decrease while deoxygenated hemoglobin and total hemoglobin will increase during the fatiguing task and 5-RM, (b) $\text{EMG}_{\text{AMP}}$ will increase and $\text{EMG}_{\text{MPF}}$ will decrease during the fatiguing task and 5-RM, and (c) patterns of response during the fatiguing task will vary between sarcopenia status.
CHAPTER II
REVIEW OF LITERATURE

As defined by the European Working Group on Sarcopenia in Older People (EWGSOP), the parameters used to identify sarcopenia include low muscle mass, and either low muscle strength or low physical performance (15). However, the underlying causes of the decreased muscle mass, strength, and physical performance are not entirely understood. Numerous studies have shown that compared to younger adults, older adults have reduced skeletal muscle mass, decreased blood flow to the periphery, decreased oxygen demand in the skeletal muscle, reduced glucose uptake, reduced glucose transporter expression, greater insulin resistance, increased fat oxidation, increased intramyocellular fat content, and reduced mitochondrial activity (8,9,17,56). The availability and oxidation of energy substrates may be impacted by any of these differences which in turn, may contribute to the anabolic resistance associated with aging. In fact, Timmerman et al. (71), found that aging is associated with a decrease in nutritive flow. In other words, older adults have a decreased ability to deliver nutrients to skeletal muscle in part due to decreased blood flow and microvascular perfusion. Additionally, they found that impaired nutritive flow may be attenuated by increased physical activity (71). Recently the term “metabolic flexibility” has been used to describe the ability of individuals to switch between available substrates under various conditions (23). Substrate utilization has been well documented in healthy young adults at rest and during exercise and an ability to switch between substrates under various conditions is considered healthy (7,22). The effects of aging on muscle metabolic characteristics and blood flow have been extensively explored, interestingly, however, older adults with
normal muscle mass and strength/function have not yet been compared to older adults classified as sarcopenic (low muscle mass and strength/function).

Due to the multi-faceted nature of sarcopenia, the etiology is poorly understood and difficult to define (9). One factor contributing to the increased loss of muscle mass with age may be nutrient insufficiency due to an inability to utilize available nutrients, an inability to deliver necessary nutrients, or perhaps a combination of the two. The purpose of this literature review is to explore the age-related changes in skeletal muscle metabolism, oxygenation, and blood flow and how these changes may contribute to an accelerated loss of muscle mass (sarcopenia).

**Changes in skeletal muscle with aging**

*Bonadonna, Groop, Simonson, and DeFronzo, 1994*

The Randle Cycle describes the competition between free-fatty acid (FFA) and glucose for substrates under various conditions. The purpose of this study was to examine insulin sensitivity in a group of nondiabetic elderly subjects and verify whether or not the Randle cycle is present in these individuals. Seven healthy young (four men, three women, mean ± SD: age = 21 ± 1 years, height = 174 ± 1.4 cm, weight = 67.4 ± 2.6 kg) and seven old (four men, three women, 71 ± 2 years, 171 ± 3 cm, 69.6 ± 4.3 kg) volunteers gave informed consent and participated in this study. Four of the older subjects had a nondiagnostic oral glucose tolerance. Lean body mass was determined using the tritiated water dilution technique. Two different euglycemic insulin clamps were performed in random order within 5 to 7 days. Indirect calorimetry was assessed during the final 60 minutes of each clamp. Blood and breathe samples were gathered at 10-minute intervals to assess glucose, FFA, and CO₂ specific activities and plasma
insulin concentration. Two-way repeated measures ANOVA were used to assess the impact of age and insulin on all variables. Bonferroni’s t test and unpaired Student’s t tests were used for additional comparisons. Older subjects had higher fat mass (22.7± 2.9 kg) and body fat percent (32.4 ± 3.8%) than the young subjects (13.1 ± 2.2 kg, 19.4 ± 3.4%), but lean body mass was not significantly different (46.9 ± 3.7 kg [old], 54.3 ± 3.3 kg [young]). Plasma glucose concentrations were similar in the older and younger subjects during the basal states (4.8 ± 0.1mM [old], 5.0 ± 0.1mM [young]) and during both insulin clamp techniques (4.9 ± 0.2mM [old], 4.9 ± 0.2mM [young]). Plasma insulin concentrations were similar for both groups in the basal state (47.4 ± 6.6pM [old], 57.6 ± 72 [young]), however, insulin concentrations were higher in the older (1536 ± 120 pM) than younger (1332 ± 72pM) subjects at higher insulin infusion rates (p = 0.16). The older subjects had higher basal plasma FFA concentrations, and higher FFA concentrations at all five insulin infusion rates. Plasma FFA utilization, oxidation, and net lipid oxidation rate relative to LBM were significantly greater in the older than in the young in all states. However, when relative to FM, there were no significant differences.

It is unknown though, if this is purely due to the older adults having a larger source of adipose tissue, or if it is due to increased lipolytic activity. Hepatic glucose production and whole-body glucose disposal was similar between the two groups at baseline and during all insulin infusion rates. However, total glucose oxidation was lower in the older group at all time-points. The authors conclude that the combination of increased FFA oxidation and decreased glucose oxidation may be indicative of substrate competition and thus increased Randle Cycle activity.
Evidence suggests that blood flow and vascular conductance may decrease with age, however, how and why this occurs is not well understood. The purpose of this study was four-fold: 1) to compare limb blood flow at rest in younger and older subjects, and if a difference exists 2) determine if it is a function of lower systemic arterial blood flow, 3) determine if it is due to increased sympathetic vasoconstrictor activity, and 4) determine if it is related to a decreased oxygen demand and tissue mass. Sixteen young (28 ± 1 years) and 15 older (63 ± 1 years) healthy men gave informed consent and participated in this study. Body composition was assessed using DXA or hydrodensitometry. Femoral blood flow and vascular conductance were assessed using a duplex ultrasound machine. Vascular conductance was calculated as femoral blood flow/mean arterial pressure. Vascular resistance was calculated as mean arterial pressure/femoral blood flow. Echocardiography was used to assess stroke volume and calculate cardiac output. Muscle sympathetic nerve activity (MSNA) of the right peroneal nerve was assessed using a microneurographic technique. Leg oxygen consumption was estimated using whole-body resting oxygen consumption measured by indirect calorimetry (leg oxygen consumption = 7.5% of whole-body oxygen consumption). One-way ANOVAs and ANCOVAs were used to assess differences between the older and younger groups. Univariate correlation analysis was used to assess specific physiological variables. There were no significant differences in height, body mass, or leg composition between the older and younger groups (p > 0.05), however, the older men had higher body fat percent (25 ± 2%) and diastolic blood pressure (74 ± 2 mmHg) than the younger men (15 ± 2%, 65 ± 3 mmHg). Femoral artery blood flow was 26% lower in the older men (p < 0.005) due to lower
mean blood velocity, as opposed to artery diameter. Older men had 32% lower femoral vascular conductance and 45% higher femoral vascular resistance than the younger men. Heart rate, stroke volume, and cardiac output were not significantly different between the two groups. MSNA was 74% higher in the older men than the younger men. Femoral blood flow \( (r = -0.55) \), vascular conductance \( (r = -0.65) \), and vascular resistance \( (r = 0.61) \) were all significantly related to MSNA. Age-related differences in femoral blood flow, vascular conductance, and vascular resistance were reduced by 60%, 57%, and 67%, respectively, after controlling for MSNA. Older men \( (217 \pm 9 \text{ mL/min}) \) had lower whole-body oxygen consumption than younger men \( (254 \pm 8\text{ mL/min}) \). Thus, estimated leg oxygen consumption was 15% lower in the older men than younger men. Femoral blood flow was correlated to estimated leg oxygen consumption \( (r = 0.78) \). Age-related differences in femoral blood flow was reduced by 50% after correcting for leg oxygen consumption. The results of this study suggest that reduced lower limb blood flow is due to elevated sympathetic vasoconstrictor activity (decreased conductance and increased resistance) rather than decreased cardiac output. Secondly, estimated leg consumption was lower in the older men, independent of leg tissue mass. The results of this study add to our understanding of age-related differences in hemodynamics.

*Donato, Uberoi, Wray, Nishiama, Lawrenson, and Richardson, 2006.*

Numerous studies have documented decreased blood flow and increased vascular resistance in the leg in older adults, however, less data exists regarding age-related changes in forearm bloodflow. The purpose of this study was to examine leg and forearm blood flow before and during submaximal dynamic leg extensions and forearm flexions in older and younger adults. Eight young \( (20 – 29 \text{ years old}) \) and six older \( (65 – 80 \text{ years old}) \)
old) healthy males gave informed consent and participated in this study. The subjects performed absolute submaximal workloads and relative workloads of single leg dynamic extensions and rhythmic handgrip exercises. Subjects exercised for 3 minutes before blood flow measurements were taken to ensure steady-state had been achieved. Doppler ultrasound was used to measure blood flow at the common femoral artery and brachial artery. Leg blood flow was taken in a seated position, which is different from many other studies who examine blood flow in a supine position. Indirect calorimetry was used to calculate leg VO$_2$ during the leg extension exercises by subtracting resting VO$_2$. Forearm and thigh muscle volume were estimated using circumference and skinfold measurements. Two-factor mixed model ANOVAs were used to assess the impact of age and exercise intensities. Wilcoxon’s and Freidman nonparametric tests were also used. The older group had a significantly higher BMI (27 ± 2 kg/m$^2$), systolic and diastolic blood pressure (129 ± 7/ 82 ± 10), and heart rate (70 ± 7 bpm) than the younger group (24 ± 3 kg/m$^2$, 119 ± 11/ 70 ± 8, 62 ± 8 bpm). The older group had significantly lower quadriceps muscle mass (1.6 ± 0.1 kg) than the younger group (2.1 ± 0.2 kg), but forearm muscle mass was similar between the two groups (0.84 ± 0.2 vs. 0.97 ± 0.1 kg). Additionally, the older group had significant lower maximal handgrip strength (28 ± 6 kg) and knee extensor strength (31 ± 17 W) than the younger group (51 ± 5 kg, 61 ± 11 W). Seated resting leg blood flow was 26% lower in the older adults but was not different when expressed per kilogram of quadriceps muscle mass. Resting arm blood flow was not different between the two groups in relative or absolute terms. At all absolute submaximal leg extension intensities, relative leg blood flow was similar between young and older subjects. However, for relative workloads older adults had decreased relative
blood flow compared to younger adults. Relative leg VO$_2$ was similar between the two groups under both absolute and relative workloads. Absolute and relative forearm blood flow was similar between young and old subjects at absolute workloads. The authors concluded that their leg blood flow results seemed to be much lower than previous studies findings, which indicates the importance of position while taking blood flow measurements. Additionally, this paper helps aid in our understanding peripheral limb blood flow and the differences that may exist between the upper and lower body.

*Nilwik, Snijders, Leenders, Groen, Kranenburg, Verdiijk, et al., 2013*

There has been some debate on whether loss of muscle mass with aging is due to muscle fiber loss or muscle fiber atrophy. The purpose of this study was to determine if differences in muscle cross-sectional area (CSA) between young and older men are mainly due to differences in muscle fiber size. Furthermore, a secondary purpose was to determine if increases in muscle mass in older adults after a resistance training program were due to muscle fiber hypertrophy. Twenty-five healthy young (23 ± 1 year) and 26 healthy old (71 ± 1 year) participated in this cross-sectional study, the old group also participated in 6-month resistance training program. Whole-body DXA scans, thigh CT scans, and vastus lateralis muscle biopsies were performed at baseline for both groups and additional after the training program for the old group. Muscle biopsies were used to determine the relative percentage of type I and type II muscle fibers, and these percentages were used to determine the CSA occupied by each fiber type. Exercises included leg press, leg extension, chest press, horizontal row, lateral pull down, and biceps curl. One-repetition strength tests for leg press and leg extension were used at baseline and repeated every 4 weeks until the end of the training program. Independent
samples t-tests were used to determine difference between young and old men for all variables. Paired samples t-tests were used to determine differences in strength and CSA in the old men from pre- to post-training. A repeated measures ANOVA was used to determine changes in fiber size in the old men from pre- to post-training with fiber type as a within-subjects factor. Correlation coefficients (r) were used to determine if muscle fiber size was associated with greater muscle strength in the older men at baseline and post-training. Whole body lean mass was not different between young (62.7 ± 1.4 kg) and older men (62.2 ± 1.1 kg) however, leg lean mass was lower in older men (9.9 ± 0.2 kg) than young (11.0 ± 0.3 kg). Additionally, quadriceps CSA was 14% smaller in older (68 ± 2 cm²) than young men (80 ± 2 cm²). Average muscle fiber size was 20% smaller in the older than younger men. Type II muscle fiber size was much smaller in the older (5050 ± 198µm²) versus the young men (7136 ± 309µm²), however type I muscle fibers exhibited no difference in size between the two groups (p > 0.05). Consequently, absolute CSA occupied by type II muscle fibers was smaller in the older (29 ± 2 cm²) than younger men (46 ± 2 cm²). The number of muscle fibers was not different between older (119 ± 4 • 10⁴) and younger men (127 ± 3 • 10⁴). After the training program, older men exhibited a 1.7 ± 0.4% increase in whole body lean mass and a 3.2 ± 0.7% increase in leg lean mass (p < 0.05) and CSA significantly increased from 68±2 to 74±2 cm²). Additionally, leg press and leg extension strength significantly increased by 25 ± 2 and 41± 3%, respectively. No changes in muscle fiber type distribution occurred. Type II muscle fiber size significantly increased 24 ± 8% to 6096 ± 338 µm² (p ≤ 0.05) whereas there was no significant increase in type I muscle fiber size after the training intervention (p > 0.05). Additionally, type II muscle fibers were significantly smaller than type I in the older men
at baseline, however, this difference did not remain at post-training. There were no changes in the number of muscle fibers from pre- to post-training. Type II muscle fiber hypertrophy explained 100±3% of the increase in quadriceps CSA from pre- to post-training. Baseline correlations indicated that type II muscle fiber size was significantly correlated with leg press (r = 0.62) and leg extension (r = 0.38). However, this relationship did not exist after training. The authors concluded that type II muscle fiber size alone can explain the differences in leg muscle CSA between young and old men. Additionally, type II muscle fiber hypertrophy after 6 months of resistance training explains the increase in thigh CSA in older men. The results of this study contribute to our understanding of the etiology of age-related loss of muscle mass.

**Defining the population: Sarcopenic vs. nonsarcopenic**

Currently, there is no established clinical definition of sarcopenia, which makes recognition and appropriate treatment a difficult feat (75). It is debated whether sarcopenia should refer only to age-related loss of muscle mass, or whether the definition should include a decrease in strength and function as well (9). The prevalence of sarcopenia and risk of disability may vary greatly depending on how the condition is identified and classified (36). The EWSOP has identified sarcopenic stages which identify the severity of the condition. Pre-sarcopenia is characterized by low muscle mass without decreased strength or physical function. Sarcopenia is characterized by low muscle mass with either low strength or low physical function. Severe sarcopenia is characterized by low muscle mass with both low strength and low physical function (15). The purpose of this section is to gain an understanding on the relationships between
physical function/strength and sarcopenia and how the severity of the condition affects incidence of disability and responsiveness to nutritional intervention.

*Iannuzzi-Suchich, Prestwood, and Kenney, 2002*

The purpose of this study was to determine the prevalence of sarcopenia in a cohort of community-dwelling older adults using the definition established by Baumgartner and colleagues. One-hundred ninety-five healthy women (75.0 ± 4.7 years, BMI 26.5 ± 5.1 kg/m²) and 142 healthy men (73.8 ± 5.3 years, BMI 26.5 ± 3.9 kg/m²) gave informed consent and participated in this study. Body composition was measured using whole body dual x-ray absorptiometry (DXA) scans. Appendicular (ASM) and total skeletal muscle mass (TSM) were adjusted for height. Sarcopenia was defined as skeletal muscle mass greater than two standard deviations below the sex-specific normal mean. Physical activity levels were estimated using the Physical Activity Scale for the Elderly. 1RM leg press strength and power were measured in the men only. The Short Physical Performance Test (SPPB), Physical Performance Test, and SF-36 health survey were also completed to measure physical function and quality of life. Fasting blood and urine samples were collected to measure 25-hydroxy vitamin D (25OHD), estrone, estradiol, bioavailable testosterone (men only), sex hormone-binding globulin (SHBG). Pearson correlation coefficients were used to examine the relationship between skeletal muscle mass and hormone levels, physical activity, physical function, strength, and power. Multiple linear regression was used to determine which variables were the best predictors of the variance in skeletal muscle mass. Sarcopenia was prevalent in 22.6% (44) of the women and 26.8% (38) of the men using the definition proposed by Baumgartner and colleagues. A subgroup of 80+year olds were analyzed and the prevalence increased to
31.0% (9 out of n = 29) in women and 52.9% (10 out of n = 19) in men. BMI was significantly correlated with ASM in women (r = 0.695) and men (r = 0.682). Estrone (r = 0.244), estradiol (r = 0.357), and 25OHD (r = -0.161) were significantly correlated with ASM in women. Leg press strength (r = 0.504) and power (r = 0.409) and bioavailable testosterone (r = 0.357) were significantly correlated with ASM in men. BMI accounted for 47.9% of the variability in ASM in women (p < 0.001). BMI (50.1%), leg press strength (10.3%), leg press power (4.1%), and bioavailable testosterone (2.6%) accounted for 67.1% of the variability in ASM in men (p < 0.001). The results of this study helped confirm incident rates of sarcopenia based on the cut points of less than 2 standard deviations below the sex-specific healthy young adult average for skeletal muscle mass.

Janssen, 2006

Many studies examining sarcopenia are cross-sectional and therefore it is unknown whether sarcopenia precedes physical disability or vice versa. The purpose of this study was to determine if the onset of physical disability can be predicted by the presence of sarcopenia. Five-thousand thirty-six older men and women from the Cardiovascular Health Study were analyzed in this study. At baseline subjects were categorized by muscle mass (normal, moderate sarcopenia, or severe sarcopenia) and disability status (yes or no). Follow-up examinations were used to understand the relationships between sarcopenia and disability in the 3,694 subjects who were not disabled at baseline. Whole body muscle mass was estimated using bioelectrical impedance analysis (BIA) and was normalized for height (skeletal muscle index, SMI). Age, race, socioeconomic status, smoking status, adiposity status, cognitive function, and prevalence of cardiovascular and non-cardiovascular disease were used as covariates or
as potential effect modifiers in subgroup analyses. Disability scores were assessed according to an individual’s ability to complete heavy housework, light housework, shopping, preparing meals, paying bills, and using the telephone. Logistic regression was used to determine cross-sectional odds ratios at baseline for the relationship between muscle mass and disability prevalence. Cox proportional hazards regression was used to determine the risk of developing disability according to muscle mass. At baseline, 70.7% of the men and 41.9% of the women had moderate sarcopenia and 17.1% of the men and 10.7% of the women had severe sarcopenia. Disability likelihood was significantly greater in the severe sarcopenia group than the normal muscle mass group (p < 0.001), however, the likelihood was not greater in moderate sarcopenia than normal muscle mass (p = 0.38). The risk of developing disabilities was 27% greater in the severe sarcopenia group than the normal muscle mass group (p < 0.05). There was no significant difference in increase in risk of disability between the normal muscle mass group and moderate sarcopenia group. The authors conclude that the relationship between sarcopenia and disability is bidirectional.

_Cramer, Cruz-Jentoft, Landi, Hickson, Zomboni, Pereira, et al., 2016._

Nutritional interventions may improve muscle-related outcomes in individuals with sarcopenia due to the close relationship with malnutrition. The purpose of this study was to examine the effects of 6-month supplementation of 2 different oral nutrition supplements (ONS) in older adults with malnutrition and sarcopenia. Subjects were instructed to consume 2 servings daily between meals and continue their regular diet and exercise. Subjects visited the laboratory every 6 weeks to assess compliance, medication changes, and adverse events. Height, weight, body composition, leg strength, grip
strength, gait speed, and fasting blood draws were measured at baseline, 12 and 24 -
weeks. A total of 330 subjects enrolled in this study in 8 countries across Europe and
North America. Subjects were randomized to either control (n = 165, 625 women, mean
[25th, 75th interquartile range]; age 77 [71, 81], weight 70 [60, 78] kg) or experimental
ONS (n = 165, 62% women, age 77 [71, 81], weight 68 [58, 78] kg). The control ONS
consisted of the following: 14 g protein, 11 g fat, 44 g carbohydrate, 147 IU vitamin D₃,
and additional vitamins and minerals. The experimental ONS consisted of 20 g protein,
11 g fat, 36 g carbohydrate, 1.5g CaHMB, 499 IU vitamin D₃, and additional vitamins and
minerals. Leg extension strength (peak torque) was measured on an isokinetic
dynamometer set at 60 degrees/second. Grip strength was measured with a calibrated
dynamometer adjusted to grip width. Body composition was measured using dual x-ray
absorptiometry (DXA). Muscle quality was assessed by calculating peak torque relative
to total lean muscle mass (nm/kg). Subjects were classified as either severe sarcopenia or
mild-moderate sarcopenia. Mild-moderate sarcopenia group was further classified as
sarcopenia with normal gait speed or sarcopenia with normal grip strength. Wilcoxon
rank sum test and Wilcoxon signed rank tests were used to examine between group and
within group differences, respectively. The primary finding from this study was that
sarcopenia staging (severe vs mild-moderate) differentially affected peak torque in
response to ONS supplementation. In the mild-moderate sarcopenic group, greater
increases in peak torque at 12 weeks were seen in the experimental ONS group than the
control ONS group. In the severe sarcopenic group, increases in peak torque were seen at
24 weeks but not 12 weeks. The authors concluded that there may be a delayed time
course in skeletal muscle adaptations in severely sarcopenic individuals. The authors also
concluded that this delay may be due to physical differences between the groups at baseline as the severe sarcopenic group had lower lean muscle mass, peak torque, muscle quality, grip strength, and gait speed, and higher fat mass than the mild-moderate group. Furthermore, the authors hypothesized that the delayed response in the severely sarcopenic individuals may be due to decreased delivery of nutrients to the muscle (termed “nutritive flow”) due to impaired blood flow.

**Age-related reductions in metabolism related to sarcopenia**

The term “metabolic flexibility” has been coined to describe the healthy fluctuations of substrate utilization from fasted to fed states as well as from rest to exercise. An inability to switch between carbohydrate and fat oxidation under various conditions may, in part, be due to decreased availability of nutrients to skeletal muscle via decreased blood flow or impaired glucose uptake (72). The purpose of this section of the literature review is to gain an understanding of normal and abnormal patterns of substrate utilization under various conditions.

*Kelley, Goodpaster, Wing, and Simoneau, 1999*

There is significant evidence of impaired glucose uptake in response to insulin in obese individuals, however, less is known about substrate utilization during fasting conditions in obese individuals. The purpose of this study was to examine the relationship between fat oxidation and skeletal muscle fat infiltration and to explore the relationship between fatty acid and glucose utilization during fasting conditions and obesity-related insulin resistance. Lean (n = 16, mean ± SD: weight = 71.1 ± 2.3 kg, 18% body fat [male], 63.1 ± 2.7 kg, 30% body fat [female]) and obese (n = 40, weight = 108.1 ± 3.2 kg, 31% body fat [male], 92.5 ± 3.8 kg, 42% body fat [female]) adults between the ages of 25
and 40 who were sedentary, glucose tolerant, and had normal blood lipid profiles participated in this study. Blood samples were obtained from the radial artery and femoral vein during a fasting period, after FFA infusion, and during a continuous insulin plus 20% dextrose infusion. Resting blood flow of the leg was measured using venous occlusion strain-gauge plethysmography and a muscle biopsy of the vastus laterals was taken. Computed tomography (CT) was used to obtain muscle and fat cross-sectional area of the thigh and dual-energy X-ray absorptiometry (DXA) was used to determine fat mass (FM) and fat-free mass (FFM). A modified incremental Bruce protocol on a cycle ergometer was used to determine VO$_2$ max. Two-way analysis of variance (ANOVA) was used to compare obese vs. lean and men vs. women on substrate metabolism, body composition, and insulin sensitivity. To examine the correlations among fat mass, VO$_2$ max, and substrate metabolism linear regression and step-wise multiple regression analyses were used. The main findings from this study were that obese had significantly higher fasting RQ values (0.90 ± 0.02) than lean (0.83 ± 0.002), but also had significantly lower insulin-stimulated RQ values (0.91± 0.02) than lean (0.99 ± 0.03). Additionally, obese fasting RQ was not significantly different than obese insulin-stimulated RQ. Because of this, the authors concluded that obese individuals have a decreased ability to adjust between fat and glucose utilization under various conditions. Fasting FFA uptake was similar for lean and obese subjects, however, fasting FFA oxidation was significantly greater in lean than obese. Thus, net FFA storage was significantly greater in obese. Conversely, insulin-stimulated FFA uptake was similar for lean and obese, however, insulin-stimulated FFA oxidation was higher for obese than lean. CT scans of the thigh indicated increased lipid content within the skeletal muscle of obese individuals.
Additionally, fasting RQ values were significantly and negatively correlated with insulin sensitivity ($r = -0.57$). Finally, fasting RQ and cross-sectional area of low-density muscle together predicted 48% of the variability in insulin-sensitivity. This was one of the original articles to coin the term “metabolic flexibility” and adds to our understanding of the term with regards to healthy, lean individuals and obese individuals.

*Bergman and Brooks, 1999*

The purpose of this paper was to compare respiratory exchange ratios (RER) during various intensities of cycling in trained versus untrained men in both fed and fasted conditions. Seven untrained (mean ± SD: age; 22.1 ± 1.3 years, weight; 75.6 ± 6.2 kg, % body fat; 180.0 ± 2.9) and seven trained (age; 25.1 ± 1.8 years, weight 73.7 ± 3.5, % body fat; 9.6 ± 1.8) healthy men gave informed consent and participated in this study. Progressive maximal exercise tests were completed on two different occasions to familiarize the subjects and determine maximal (peak) VO$_2$ (VO$_{2\text{peak}}$). Blood samples were taken after each workload stage to determine lactate concentrations and calculate lactate threshold (trained men only). Body composition was assessed using the seven-site skinfold method. Once VO$_{2\text{peak}}$ was determined, subjects were randomized to exercise intensity (20% [2 hours], 40% [2 hours], 60% [1.5 hours], and 80% [trained: 45 min, untrained: 30 min] VO$_2$) and nutrition state (fed vs. fasted). Therefore, each subject completed 8 separate exercise trials. Meals were controlled the night before and morning of each exercise trial. RER values were determined using open-circuit calorimetry and total workload (kcal/minute) and relative carbohydrate and lipid oxidation rates were calculated. Independent sample t-tests were used to compare characteristics of trained and untrained men. Factorial ANOVAs were used to determine differences between absolute
oxidation rates of carbohydrates and lipids in trained versus untrained. Mixed model ANOVAs were used to determine differences in mean RER over time between groups (trained vs. untrained) and nutrition status (fed vs. fasted). There were no significant differences in age or weight between trained and untrained subjects (p > 0.05). The trained men had lower % body fat and higher VO$_{2\text{peak}}$ (58.04 ± 1.58 ml/kg/min) than untrained men (38.55 ± 2.39 ml/kg/min). Fasting RER values were significantly lower than fed values at 22, 40, and 59% VO$_{2\text{peak}}$ in trained subjects and 40 and 59% VO$_{2\text{peak}}$ in untrained subjects. RER values were lower in trained subjects than untrained subjects at absolute workloads in both fasted and fed states. There was an exponential relationship between RER and power output in both fasted and fed states. Since trained subjects only had decreased RER at intensities of less than 40% VO$_{2\text{peak}}$, the authors concluded that training may increase relative lipid oxidation during lower intensity exercise only.

Additionally, ingesting carbohydrates prior to exercise increases carbohydrate oxidation for intensities less than 60% VO$_{2\text{peak}}$. The results of this study help us understand the patterns of substrate utilization and oxidation in healthy young individuals at rest and up to 80% VO$_{2\text{peak}}$.

*Rizzo, Barbieri, Ragno, Grella, Provenzano, Villa, et al., 2005*

It is evident that changes in resting metabolic rate (RMR) and body composition occur with aging. However, less in known about the relationship between human longevity and energy expenditure. The purpose of this cross-sectional study was to compare RMR and respiratory quotients (RQ) in adults (< 65 years old), aged-adults (66 – 94 years old), and long-lived adults (> 94 years old). A sample of 81 healthy females (adults, n = 26; aged-adults, n = 27; long-lived, n = 28) participated in this study.
Following IRB approval and informed consent all subjects underwent a 2-week period of a standard diet (50% CHO, 27% FAT, 23% PRO, 1500 kcal). Waist-to-hip ratio (WHR), skinfold measurements, plasma blood samples, Mini Mental State Examination, and indirect calorimetry was assessed and recorded for all subjects. Analysis of variance (ANOVA) was used to determine differences among the three study groups. Pearson product moment correlations, partial correlations, and multivariate linear regression was used to examine the relationships among variables and the strongest predictors of RMR and RQ. The authors reported that the subjects were not obese or malnourished, had plasma glucose and lipid parameters within normal range, and had normal cognitive function. In the whole sample, RMR was significantly correlated with age (r = -0.561), WHR (r = -0.365), fat mass (FM, r = -0.387), and % body fat (%BF, r = -0.240). RQ was significantly correlated with age (r = -0.240), WHR (r = -0.503), FM (r = -0.302), %BF (r = -0.483), and glucose plasma levels (r = -0.291). Together, WHR, fat-free mass (FFM), and total volume of expired air (TV) predicted 40% of the variance in RMR. Age, WHR, FFM, and plasma glucose levels predicted 46% of the variance in RQ. VO\textsubscript{2}, VCO\textsubscript{2}, and fasting RQ in long-lived was higher than aged-adults, but lower than adults. TV and RMR in long-lived adults were greater than aged-adults, but not different from adults. The authors analyzed the long-lived adults separately and found RQ was negatively correlated with %BF (r = -0.420), fasting plasma glucose (r = -0.596), free-fatty acids (FFA, r = -0.389), and WHR (r = -0.582) whereas RMR was only significantly negatively correlated with WHR (r = -0.603). The authors concluded that even though there is a significant age-related decline in RMR and RQ, long-lived females had greater RMR and RQ than aged-females. Thus, long-lived females may have a
protective mechanism against metabolic age-related decline. However, this may be explained by differences in anthropometric measurements since the long-lived females had a lower BMI, FFM, and WHR than the aged-females. The results of this study contribute to the understanding of resting metabolism, age-related changes in metabolism, and body composition in older females.

Prior, Ryan, Stevenson, and Goldberg, 2014

The abnormal fat and carbohydrate oxidation seen in overweight older adults with impaired glucose tolerance may contribute to impairments in the ability to switch fuel substrate selection while transitioning from rest to exercise. The purpose of this study was to examine differences fuel substrate selection during submaximal exercise in overweight older adults with and without impaired glucose tolerance. Twenty-three sedentary overweight men and women with impaired glucose tolerance (IGT, n = 10, age 62 ± 3 years) and without impaired glucose tolerance (NGT, n = 13, age 63 ± 2 years) gave informed consent and participated in this study. Body composition was assessed via dual energy x-ray absorptiometry (DXA) and intra-abdominal and subcutaneous abdominal fat was measured using computed tomography (CT). An oral glucose tolerance test (75-glucose ingestion) was administered to all subjects and plasma glucose and insulin levels were measured over a 2-hour period. An incremental treadmill protocol was used to measure VO2max via indirect calorimetry. Indirect calorimetry was also used to measure substrate utilization during rest, after insulin-stimulation, and during submaximal exercise (10 minutes of steady-state treadmill exercise at 50 – 60% VO2max). Repeated measures ANCOVA were used to identify differences between IGT and NGT groups, using sex as a covariate. Correlations and multivariate regression were used to
determine relationships among metabolic variables. Baseline analyses indicated there were no differences in age, weight, or body composition between the IGT and NGT groups. VO$_2$max was lower in IGT (45.0 ± 1.3 ml/kg/min) than in NGT (49.0 ± 1.2 ml/mg/min) only when expressed relative to lean body mass. There was no significant difference in RER between IGT and NGT at rest, however, during submaximal exercise, RER was significantly greater in NGT than IGT. Indicating that the NGT group was better able to switch from primarily fat oxidation to carbohydrate oxidation during exercise. The authors conclude that older overweight individuals with glucose intolerance have an inability to efficiently switch between fuel substrates from rest to exercise may limit the ability to provide sufficient nutrients to the working muscles.

**Near-infrared spectroscopy (NIRS) as a measurement of blood flow and skeletal muscle metabolism with sarcopenia**

NIRS has been used as tool to measure microvascular perfusion under numerous conditions. Soares et al. (2018) recently demonstrated the sensitivity of NIRS with its ability to detect differences in vascular responsiveness between obese and healthy lean individuals (69). Additionally, numerous studies have used NIRS devices to examine oxygen recovery kinetics following varying modes of exercise in patients with cardiovascular, pulmonary, or neurological diseases (43,49,55,58). The purpose of the following section of the literature review is to gain an understanding of NIRS as a tool of measuring muscle microvascular perfusion.

*Timmerman, Dhanani, Glyn, Fy, Drummond, Jennings et al. 2012.*

The purpose of this study was to determine if increases in physical activity improves muscle protein anabolism after essential amino acid (EAA) and sucrose intake
in older adults. Six healthy, sedentary older adults (age 70 ± 4 years) gave informed consent and participated in this study. Each subject completed two identical EAA+sucrose ingestions separated by 4–6 weeks. The night before one of the ingestions, subjects completed a 45-minute bout of aerobic exercise on a treadmill at 60–70% of their heart rate reserve, the night before the other ingestion, no exercise was completed. During the test visit, over a three-hour period, 30 mL of EAA+sucrose was ingested every 10 minutes. Leg blood flow was assessed using Doppler ultrasound. Contrast enhanced ultrasound imaging was used to measure blood perfusion into the vastus lateralis. Blood samples from the femoral vein and muscle biopsies of the vastus lateralis were also taken. Paired t-tests were used to compare basal values between conditions (exercise vs. no exercise), to compare basal values to treatment values (infused EAA+sucrose) in each condition, and to compare change scores (EAA+sucrose – baseline) between the two conditions. There were no differences any baseline measurements between the control and exercise conditions. Blood flow was increased significantly more from baseline after EAA+sucrose ingestion in the exercise condition than control (p < 0.05). Microvascular perfusion increased significantly from baseline to EAA+sucrose infusion in the exercise group only. There were no baseline differences in insulin and glucose kinetics between the control and exercise conditions. Insulin and glucose concentrations and leg glucose uptake increased significantly during EAA+sucrose consumption under both conditions. Endogenous glucose production decreased after nutrient consumption in both conditions. Phenylalanine concentrations significantly increased after nutrient consumption under both conditions. Phenylalanine delivery to the leg, output from the leg, transport into the muscle, transport out of the
muscle, and intracellular availability increased after nutrient consumption in both conditions. Additionally, phenylalanine rate of appearance, rate of disappearance, and utilization for protein synthesis significantly increased after nutrient consumption only in the exercise condition. The change scores for phenylalanine delivery to the leg, rate of disappearance, and utilization for protein synthesis were greater in the exercise group than control group. The authors concluded that an acute bout of moderate aerobic physical activity enhances the anabolic response to the consumption of amino acids and carbohydrates in older adults, due to an increase in blood flow, microvascular perfusion, and amino acid delivery to the working muscles. In the control condition, consumption of amino acids and carbohydrates alone was unable to increase muscle protein synthesis, blood flow, and microvascular perfusion. The results of this study add to our understanding of the importance of blood flow for nutrient delivery in healthy older adults.

*Martin, Mumford, Haun, Luera, Muddle, Colquhoun, et al., 2017*

The purpose of this study was to determine the effects of a post-workout supplement on the hyperemic response following leg extension exercise at 80% and 30% 1-repetition maximum. Thirty healthy trained males (mean ± SD: age = 22.1 ± 3.5 years, weight = 86.0 ± 9.5 kg) gave informed consent and participated in this study which included three separate visits to the laboratory. On visit one, subjects were assigned to placebo (PLA, maltodextrin and glycine) or pre-workout supplementation (PWS) and instructed to supplement for 7 days leading up to visit two. During visit two, blood samples, heart rate, blood pressure, and femoral artery blood flow were measured at rest, 45-minutes post-supplement consumption, and 5 minutes post-exercise. The subjects then
consumed their supplement, rested for 45 minutes and then completed the leg extension protocol (40 sets of dynamic bilateral leg extension to failure at 30% or 80% 1RM). During the exercise, a NIRS device was placed on the left vastus lateralis to measure minimum and maximum oxygenated hemoglobin ($O_2$Hb), deoxygenated hemoglobin (HHb), difference between $O_2$Hb and HHb (HbDiff), and total hemoglobin (tHb). Visit three was also preceded by 7 days of supplementation and was identical to visit two expect subjects completed the opposite training load (30% or 80%). Independent t-tests were used to examine baseline differences between groups. Three-way (time x group x load) mixed model ANOVAs were used to examine all dependent variables. There were no significant differences in baseline characteristics between PLA and PWS ($p > 0.05$). There was no difference between final volume load (sum of all four sets) between 80% and 30% loads. Significantly lower max tHb and min $O_2$Hb in the 80% load condition than the 30% load condition were observed during the first set of leg extension exercises. The authors concluded that this suggested oxygen consumption and metabolic demand was greater than blood perfusion to the working muscle. The results of this study aid in our understanding of the effects of leg extension exercise on variables measured with NIRS.

de Oliveira, Morgado, Conte-Junior, and Alvares, 2017

The purpose of this study was to determine if beetroot-based nutritional supplement promotes muscle oxygenation, blood volume, and muscle strength in the forearm after fatiguing handgrip exercise. Twelve healthy older adults ($n = 3$ male, $n = 9$ female; age = 68.8 ± 3.5 years) provided informed consent and completed this randomized, double-blind, crossover design study. Each subject visited the laboratory 3
times for a familiarization trial, then two experimental trials (placebo and beet-root), with a one-week wash out period between all visits. All subjects consumed 100g of a beetroot based nutritional gel (BG) or a nitrate-depleted placebo gel (PLA). Urine samples were obtained at baseline, and 90, 150, and 180 minutes after supplementation intervention to measure urinary nitrite and nitrate. Approximately 150 minutes after supplementation intervention, a near-infrared spectroscopy (NIRS) device was placed on subjects anterior forearm and they began the handgrip exercise protocol. The exercise protocol consisted of one minute of rhythmic contractions at 30% MVC. Tissue saturation index (TSI%) and blood volume (tHb) were measured with the NIRS at baseline, during forearm exercise, and during exercise recovery. Minimum TSI%, half-desaturation time, desaturation rate, resaturation rate, and blood volume amplitude during exercise ($\Delta tHb_{Ex}$) and exercise recovery ($\Delta tHb_{Rec}$) were calculated. A paired t-test was used to determine differences in desaturation rate, resaturation rate, $\Delta tHb_{Ex}$, and $\Delta tHb_{Rec}$ between BG and PLA. A two-way repeated measures ANOVA was used to determine differences in TSI%, tHb, muscle strength, nitrite, and nitrate between BG and PLA. Urinary concentrations of nitrite and nitrate were greater at T150 and T180 in BG than PLA. Resaturation rate and $\Delta tHb_{Rec}$ were significantly greater in BG ($1.43 \pm 0.77 \% \cdot s^{-1}, 10.25 \pm 5.47 \mu M$) than PLA ($1.02 \pm 0.48 \% \cdot s^{-1}, 6.72 \pm 4.55 \mu M$). Minimum TSI% was lower in BG (-13.68 ± 6.77 %) than PLA (-10.70 ± 5.02%). There were no differences in decrease in MVC ($\Delta MVC$) immediately following the fatiguing exercise between PLA (-0.77 ± 0.45 N) and BG (-0.71 ± 0.40 N). However, after 30 minutes of recovery, $\Delta MVC$ was lower in BG (-0.24 ± 0.18 N) than PLA (-0.39 ± 0.20 N). The authors conclude that BG supplementation increased muscle oxygen extraction, muscle resaturation rate following exercise, and
force recovery in the forearm muscles following a fatiguing exercise in older adults. This suggests that the supplementation was able to increase nitric oxide bioconversion in the muscle, thus improving the vasodilatory effect and improving blood and oxygen delivery to the active muscle. The results of this study aid in our understanding of blood oxygenation and blood volume measurements in older adults as measured with the NIRS device.

Niemeijer, Jansen, Van Dijk, Spee, Meijer, Kemps et al., 2017

NIRS devices that utilize spatially-resolved spectroscopy offer an effective tool to monitor muscle microvascular oxygen function, however, the effects of adipose tissue thickness (ATT) are often underestimated. The purpose of this study was to explore the extent to which subcutaneous adipose tissue effects NIRS derived measurements in vivo and in vitro using a blood phantom device to isolate the effects of adipose tissue thickness. Fifty-six subjects with chronic heart failure (CHF, n = 49 male, n = 7 female, age 64 ± 9 years, weight 86 ± 15 kg) and 20 healthy active individuals (n = 16 male, n = 14 female, age 63 ± 6 years, weight 85 ± 14 kg) gave informed consent and completed this study. All subjects completed a maximal ramp exercise protocol on a cycle ergometer to calculate peak work rate, peak VO\textsubscript{2}, peak respiratory exchange ratio (RER), and the gas-exchange threshold (GET). Submaximal exercise tests consisted of a 6-minute bout at 80% of the work rate corresponding to the GET, or 50% of the peak work rate. A NIRS device was placed on the vastus lateralis during the cycling exercise to measure tissue oxygen saturation (StO\textsubscript{2}), tissue saturation index (TSI%), and deoxygenated hemoglobin (HHb). Adipose tissue thickness was measured using a skinfold caliper. To measure the effects of ATT in vitro, the NIRS device was placed on a blood mixture with known
concentration without no layer of porcine fat, then with 8 consecutive measurements of increasing porcine fat thickness (2 mm up to 14 mm). Independent t-tests and Mann-Whitney U tests were used to compare CHF and healthy groups. Pearson correlation coefficients were used to examine the relationships between the NIRS measurements and potential confounding factors (age, gender, ATT, etc.). Multivariate linear regression was used to determine the best predictors of the NIRS variables. ATT had the strongest correlation with with all NIRS variables ($r^2 = 0.098 – 0.393$) than any other variables (gender, age, CHF). Additionally, in vitro measurements indicated that adding adipose tissue up to 14mm resulted in a increase of TSI to 73.5% for 100% SO$_2$, and a TSI of 68% for 0% SO$_2$. The average absolute deviate was approximately 5%. Thus, it appears that adipose tissue thickness underestimates oxygenation, and overestimates deoxygenation. The results of this study emphasis the importance of correcting for adipose tissue thickness when comparing NIRS parameters between subjects.

Soares, Reimer, Alenezi, Doyle-Baker, and Murias, 2018

The purpose of this study was to determine if near-infrared spectroscopy (NIRS) is able to detect differences in vascular responsiveness following an oral-glucose tolerance test between obese and lean individuals. Fifteen lean individuals (n = 9 males, n = 6 females, age: 25.2 ± 1.2 years, BMI: 21.3 ± 1.7 kg/m$^2$) and 13 obese individuals (n = 10 males, n = 3 females, age: 23.4 ± 1.1 years, BMI: 34.4 ± 2.0 kg/m$^2$) gave informed consent and participated in this study. Vascular responsiveness and blood glucose measurements were taken at baseline, and 30, 60, 90, and 120 minutes after ingesting 75 g of glucose. Vascular responsiveness was measured with NIRS during vascular occlusion tests (VOT). NIRS device was placed on the tibialis anterior and a pneumatic
cuff attached to a rapid inflation system was placed just below the knee. Five minutes of baseline was collected, followed by 5 minutes of occlusion at 250 mmHg, and finally 20 minutes of recovery. Baseline oxygen saturation (StO₂, %), StO₂ resaturation rate (%·s⁻¹), and StO₂ area under the curve (StO₂AUC) were reported. One-way repeated measures ANOVAs were used to examine differences between lean and obese. Blood glucose levels were not different between lean and obese individuals at baseline. At 120 minutes after ingesting glucose, glucose levels returned to baseline levels in the lean group, but not the obese group. Glucose levels were significantly greater in the obese than lean at 90 and 120 minutes post-glucose ingestion. Resaturation was greater in lean than obese at 90 minutes post-glucose ingestion. However, at 120 minutes post glucose ingestion, resaturation rate returned to baseline values in the lean but remained elevated in the obese compared to baseline values. The authors concluded that the NIRS device was sensitive enough to detect differences in vascular responsiveness between obese and lean individuals. The results of this study support the use of NIRS as a tool to measure vascular responsiveness via muscle oxygen saturation and resaturation rate after occlusion.

Overall, the age-related declines in muscle mass, strength, and physical function are apparent. However, due to the complexity of the aging process, the mechanisms underlying the anabolic resistance that is associated with aging are less clear. The use of near-infrared spectroscopy as a measure of microvascular perfusion may provide insight into the issue of nutritive flow impairments in older adults. Furthermore, comparing otherwise healthy older adults with normal muscle mass to those with low muscle mass
and function may provide insight into the mechanisms that contribute to the accelerated loss of muscle mass with aging.
CHAPTER III

METHODOLOGY

Subjects and Research Design

This project is a part of a larger study funded by Abbott Nutrition entitled, “A pilot study to explore muscle energy metabolism and metabolic flexibility in older men and women.” Five sarcopenic and six non-sarcopenic females aged 65 and older were recruited from the Lincoln community with the use of flyers and verbal recruitment for this cross-sectional study.

To be eligible for participation, subjects did not have any of the following active/treated (under physician care) diseases: metabolic, hepatic, cardiovascular, gastrointestinal, renal, respiratory, neuromuscular diseases, or any active malignancies as self-reported on a health history questionnaire. They did not have any current infections, any chronic, contagious, infectious diseases (i.e., HIV, hepatitis A), any recent unexplained weight loss (i.e., 10 lbs. over the past 6 months), or any history of any significant neurological or psychiatric disorders (i.e., eating disorders, substance abuse, dementia). Subjects were not currently participating in any regular resistance training exercises. Subjects were able to walk without assistance (excluding use of walker or cane) and were classified as low- or moderate-risk as assessed by the American College of Sports Medicine (ACSM) Health/Fitness Facility Pre-Participation Screening Questionnaire. All subjects had a body mass index (BMI) of greater than 20.0, but less than 39.0 kg/m², were not current smokers (within the past 10 years) and were not taking any medications or dietary supplements that could profoundly modulate metabolism (i.e.,
progestational agents, steroids, growth hormone). Multi-vitamins and minerals and topical steroids were exceptions.

All subjects visited the laboratory twice to complete a screening visit and a test visit separated by 4 to 10 days. During the screening visit, all testing procedures were explained to potentially eligible subjects, and they signed an approved informed consent document. Anthropometrics (height [cm], weight [kg]) were measured, exercise risk stratification was determined using ACSM’s pre-participation screening questionnaire, and a self-reported medical history (including list of current medications) was obtained. Body composition, handgrip strength, gait speed, a test for post-occlusive resting hyperemia (PORH), and a five-repetition maximum (5-RM) test for leg extension strength was also measured or performed during the screening visit. During the testing visit, qualified subjects completed one set of submaximal, unilateral leg extension exercises (30% of estimated 1-RM) to volitional exhaustion with the right leg. During the 5-RM and fatiguing leg extension exercises, surface electromyography (EMG) and near-infrared spectroscopy (NIRS) measurements were recorded from the surface of the skin over the right vastus lateralis muscle.

This study was a non-randomized, cross-sectional pilot study designed to compare muscle size, strength, activation, and tissue oxygenation during a 5-RM bout and a fatiguing bout of dynamic constant external resistance leg extension muscle actions in sarcopenic versus non-sarcopenic women. The independent variable in this study is sarcopenia status (sarcopenic vs. non-sarcopenic). Descriptive dependent variables used to compare sarcopenic vs. non-sarcopenic mean values included demographics, body composition, muscle size, muscle strength, muscular endurance, and functionality. The
descriptive outcome variables assessing demographics are age, height, and weight. Body composition assessments included percent body fat, fat mass, fat-free mass, and relative skeletal mass index, while muscle size assessments included muscle cross-sectional area, muscle thickness, adipose tissue thickness, and echo intensity. Muscle strength and endurance assessments are absolute leg extension strength, repetitions to failure during the leg extension fatiguing task, and handgrip strength, while functionality was assessed with gait speed. The primary dependent variables in this study assessed muscle activation and muscle tissue oxygenation from the vastus lateralis muscle during the 5-RM bout and fatiguing bout of leg extension muscle actions. The specific dependent variables assessing muscle activation are EMG amplitude ($EMG_{RMS}$) and EMG center frequency ($EMG_{MPF}$), while the variables assessing muscle tissue oxygenation are tissue saturation index ($TSI\%$), total hemoglobin ($THb$), oxygenated hemoglobin ($O_2Hb$), deoxygenated hemoglobin ($HHb$), and the difference between oxygenated and deoxygenated hemoglobin ($HBDiff$).

**Descriptive Assessments**

**Demographics.** Age was calculated from each participant’s self-reported birthdate. Height and weight were measured on a calibrated digital scale with attached stadiometer (Seca gmbh & co., Hamburg, Germany, Model: 769 1321994).

**Body Composition.** Total body skeletal muscle (TBSM) and appendicular skeletal muscle (ASM) was assessed using dual-energy x-ray absorptiometry (DXA) (Lunar iDXA, GE Healthcare, Madison, WI). Before all scans, the device was calibrated daily using a quality assurance phantom that consists of varying bone mineral density and percent fat standards (Lunar iDXA User Manual, GE Healthcare, Madison, WI). Scans
were performed after at least eight hours of fasting. Before each scan, the subjects’
height, weight, sex, birthdate, and race was entered into the enCORE software (GE
Healthcare, Version 14) by a licensed technician (JTC). Subjects’ bodies were free from
all external metal objects, while internal metal devices (except pacemakers) were noted.
Subjects laid supine on the padded scanner table with their hands pronated and near their
body, but without touching the hip. A Velcro strap was wrapped just proximal to the
ankles to keep the subject’s legs adducted. The appropriate thickness (thin, standard, or
thick) based on chest depth was selected prior to completing each total-body scan (Lunar

To classify sarcopenic status, the results from the DXA scan were divided into
axial and appendicular regions of interest to determine TBSM, ASM, and total fat mass
(15). The right and left arms were separated from the axial skeleton through the necks of
the humeri, while the legs were separated from the axial skeleton through the necks of the
femurs. ASM was calculated as the sum of the lean masses calculated by the DXA
software from the left and right arms and legs. TBSM was predicted using the equation
developed ($R^2 = 0.96$, SEE = 1.58 kg) by Kim et al. (44), where females = 0:

$$\text{TBSM} = (1.13*\text{ASM}) - (0.02*\text{Age}) + (0.61*\text{sex}) + 0.97$$  \hspace{1cm} (Eq. 1)

Relative skeletal muscle index (RSMI, %), absolute skeletal muscle mass index
(SMI, kg·m⁻²), and an adjusted lean mass (ALM, kg) were also calculated for sarcopenia
classification. RSMI was calculated using the following equation established by Janssen
et al. (37):

$$\text{RSMI} = (\text{TBSM ÷ body mass in kg}) \times 100$$  \hspace{1cm} (Eq. 2)
SMI was calculated using the following equation established by Baumgartner et al. (5):

\[ SMI = \frac{ALST}{(\text{height}^2 \text{ in m})} \quad \text{(Eq. 3)} \]

ALM was calculated using the following sex-specific equations established by Newman et al. (54), where FM = fat mass:

\[ ALM \text{ (women)} = -13.19 + (14.75 \times \text{height in m}) + (0.23 \times \text{FM in kg}) \quad \text{(Eq. 4)} \]

Using the above equations, subjects were defined as sarcopenic at screening if their values fell below any 1 of 4 cut-off models: (a) RSMI ≤ 28% (37), (b) SMI < 5.45 kg·m⁻² (5), (c) SMI < 5.67 kg·m⁻² (54), or (d) ALM < -1.73 kg (54).

Using the same total-body DXA scan to classify sarcopenic status with the variables in Equations 1 – 4, fat mass (FM), fat-free mass (TBSM), and percent body fat were calculated by the enCORE software, recorded, and used in all subsequent statistical analyses.

**Muscle Size.** A portable, brightness mode (B-mode) ultrasound imaging device (GE Logiq e, USA) with a multi-frequency linear array probe (12L-RS; 5–13 MHz; 38.4 mm field-of-view) was used to determine muscle cross-sectional area, muscle thickness, and echo intensity of the leg extensor muscles. Subcutaneous adipose tissue thickness was also determined from the muscle thickness ultrasound assessment (1,39). During the screening visit, subjects laid supine on a padded plinth with legs extended, relaxed, and feet secured with a Velcro strap. A generous amount of water-soluble transmission gel was applied to the skin to enhance acoustic coupling and reduce near field artifacts. Minimal pressure necessary was applied to the probe to limit compression of the adipose tissue and skeletal muscle. For leg extensor muscle cross-sectional area, panoramic
ultrasound images were taken at 50% of the distance from the ASIS to the medial, superior border of the patella (beginning laterally, working medially) using GE Logiq e LogiqView software™ (46). For vastus lateralis muscle thickness, subcutaneous adipose tissue thickness, and echo intensity transverse images were taken at 66% of the distance between the ASIS and the lateral border of the patella (location of the EMG electrode) and immediately proximal to the EMG electrode at the location where the NIRS device was placed. All images were taken using the musculoskeletal mode using a gain of 58 dB and a frequency of 10 MHz. These settings were held constant between subjects; however, the depth was adjusted according to each subject’s leg size, but still held constant within each subject. Images were taken until three panoramic and three transverse scans with acceptable quality were obtained as determined by the tester (BDM). All images were taken prior to any exercise.

All images were analyzed in Image-J Software (National Institutes of Health, USA, Version 1.51s). Each image was scaled from pixels to cm using the straight-line function in Image-J prior to analyses. To determine leg extensor muscle cross-sectional area, the region of interest was selected using the polygon function to include as much of the muscle as possible, while avoiding surrounding fascia and adipose tissue. Muscle thickness of the leg extensor muscles and overlying subcutaneous fat thickness was determined using the straight-line function. Echo intensity (EI) of the leg extensor muscles was determined using the polygon function and a computer-aided gray-scale analysis. The mean EI was reported as a value between 0 (black) and 255 (white) arbitrary units (a.u.).
**Muscle Strength, Endurance, and Functionality.** Subjects completed a 5-RM unilateral, dynamic constant external resistance leg extension test on a commercially-available, plate-loaded leg extension device (Hammer Strength Plate-Loaded, Iso-Lateral Leg Extension Machine; LifeFitness, Rosemont, IL, USA). Subjects were seated with restraining straps over the pelvis, trunk, and contralateral thigh. They were asked to sit with their back against the chair and hands firmly grasped on the handles near their hips. The lateral epicondyle of the right femur was aligned with the axis of rotation of the leg extension device. Subjects performed a brief warm-up set of 10 repetitions with 4.5 kg external resistance to familiarize with the movement. Based on the performance during the first set and after a 2-minute rest period, an appropriate weight was added to elicit failure after the fifth repetition. The 5-RM was considered successful if the subject was able to complete five repetitions throughout the full range of motion, but not a sixth repetition. A trial-and-error process was used with external resistance added or subtracted to reach the 5-RM trial. A minimum of two minutes and maximum of 5 minutes rest was allowed between attempts. Upon determination of the 5-RM, the external resistance was recorded and used to estimate the one-repetition maximum (1-RM) using the following equation \( r = 0.994 \) (63):

\[
1-\text{RM} = 1.0970 \times (5-\text{RM loaded resistance in kg}) + 14.2546 \quad (\text{Eq. 5})
\]

During the test visit, the same leg extension device was loaded with an external resistance equal to 30% of the estimated 1-RM (rounded to the nearest 1.2 kg) to complete the submaximal, fatiguing leg extension exercise task. Subjects completed dynamic, unilateral leg extension exercises until volitional failure (66). A metronome was set to 20 beats per minute, so that one repetition was completed approximately every 3
seconds. The number of repetitions completed was recorded as an assessment of muscle
e endurance. For surface EMG and NIRS data analysis, five individual repetitions were
selected for analysis that corresponded with approximately 25% completion of the total
fatiguing task. Specifically, the first and last repetitions, plus the closest repetitions
responding to 25%, 50%, and 75% of task failure, were selected. For example, if 20
repetitions were completed, then repetitions 1, 5, 10, 15, and 20 were selected for analysis.

For all leg extension muscle actions, linear force (N) was recorded from a load
cell (Omegadyne, model LCHD-500, 0–500 lbs; Stamford, CT, USA) custom fitted
between the shin pad and lever arm of the leg extension device. During testing and
exercise, the subjects were seated on the leg extension machine with a strap securing their
pelvis. The axis of rotation of the lever arm was aligned with the lateral epicondyle of the
femur of the dominant leg. The lever arm length (m) from the fulcrum to the shin pad was
measured and used to calculate torque (Nm). The force signal was sampled at 1 kHz with
a Biopac data acquisition system (MP150WSW, Biopac Systems, Inc., Santa Barbara,
CA, USA), recorded on a personal computer, and processed off-line with custom written
software (Labview v. 12.0, National Instruments, Austin, TX, USA). Torque values were
calculated from the signal epochs corresponding to the 60° range of motion occurring
between 100° and 160° of leg extension (180° = full extension) during the concentric
portion of each repetition based on the position of the lever arm monitored by a triaxial
accelerometer (TSD109C1, Biopac Systems, Inc., Santa Barbara, CA) attached at the
location of external load placement.

Maximal isometric handgrip strength of the dominant hand was measured with a
standard handgrip dynamometer with adjustable grip width (Hydraulic Hand
Dynamometer, Jamar Technologies, Inc, Hatfield, PA). Subjects were instructed to perform the handgrip strength test in a standing position facing the investigator. Subjects were asked to keep the arm adducted, forearm flexed to 90°, and neutral (semi-pronated) grip position (2). Subjects were given three attempts to perform a maximal handgrip contraction, and the average force (kg) of the two highest attempts was used as a representation of handgrip strength and sarcopenic classification. A score of < 20 kg was required for classification as sarcopenic (48).

A handheld stop watch was used to time the subjects during the gait speed assessment. Subjects were asked to walk at their normal walking speed on a 4-meter, straight, level course. Subjects were allowed to use walking aids (i.e., canes, walkers); however, no assistance was provided by the tester (BDM). The fastest of two trials was recorded. A gait speed of ≤ 0.8 m·s⁻¹ on the 4-meter course was considered low-gait speed for sarcopenic classification.

**Surface Electromyography**

A pre-amplified, bipolar active electrode pair (TSD150B, Biopac Systems, Inc., Santa Barbara, CA) was placed over the vastus lateralis muscle of the right thigh. A single-differentiated, difference EMG signal was recorded from the surface of the skin during all leg extension exercises. The center of the electrode pair was placed at 66% of the distance between the anterior superior iliac spine and the superior, lateral border of the patella (25). The longitudinal axis of the electrode pair was angled at 20° relative to the longitudinal axis of femur to mimic the pennation angle of the muscle fibers (40). The electrode has a center-to-center inter-electrode distance of 20 mm, gain of 330 (nominal), input impedance of 100 MΩ, common mode rejection ratio of 95 dB (nominal), and a
bandwidth of 12 – 500 Hz. A single pre-gelled electrode (EL503, Biopac Systems Inc., Santa Barbara, CA) was placed on the tibial tuberosity to serve as the reference electrode. Prior to placing the electrodes, the local skin areas were prepared by light dry shaving, light abrasion with a cotton towel, and cleaning with isopropyl alcohol to reduce inter-electrode impedance and improve signal to noise ratio (29).

EMG (μV) signals were sampled at 1 kHz with a Biopac data acquisition system (MP150, Biopac Systems, Inc., Santa Barbara, CA). All signals were stored on a personal computer and processed offline with custom written software (LabVIEW v. 17.0, National instruments, Austin, TX). EMG amplitude (EMG_{RMS}) was expressed as the root mean square value, and EMG mean power frequency (EMG_{MPF}) was expressed in Hz. EMG_{RMS} and EMG_{MPF} were calculated for the surface EMG signal epochs corresponding to the 60° range of motion occurring between 100° and 160° of leg extension (180° = full extension) during the concentric portion of each leg extension repetition. Position of the lever arm was monitored by a triaxial accelerometer (TSD109C1, Biopac Systems, Inc., Santa Barbara, CA) attached at the location of external load placement. To quantify EMG_{MPF} each signal epoch was processed with a Hamming window and a discrete Fourier transformation (DFT) based on the recommendations of Diemont et al. (78) and calculated as described by Kwatny et al. (47). The absolute values of EMG_{RMS} and EMG_{MPF} during the fatiguing leg extension exercises were normalized as a percentage of the first repetition of the 5-RM trial. Consequently, EMG_{RMS} and EMG_{MPF} are expressed as a %5-RM during the fatiguing leg extension exercises, with the first repetition of the 5-RM equaling 100% for every subject.
Near-infrared Spectroscopy (NIRS)

A portable, continuous wavelength NIRS device (PortaMon MKII, Artinis, Einsteinweg 17, Netherlands) was positioned over the right vastus lateralis muscle immediately proximal to the EMG electrode pair (45). Prior to placement, the local skin area was prepared by light dry shaving, light abrasion with a cotton towel, and cleansed with isopropyl alcohol. The device was secured to the skin with dark-colored, opaque self-adherent Coban™ wrap (3M™, Maplewood, MN, USA), which limited extraneous light interfering with the sensor. The NIRS device measures relative changes from baseline (rest) in oxygenated hemoglobin (O$_2$Hb) concentration, deoxygenated hemoglobin (HHb) concentration, total hemoglobin (tHb = O$_2$Hb + HHb), and tissue saturation index (TSI% = [O$_2$Hb/tHb]*100) within a 2.0-cm tissue depth from the surface of the skin (45). Spatially-resolved spectroscopy with a sampling rate of 10 Hz is used by the NIRS device based on light absorbance at 760 and 850 nm using the modified Lambert-Beer Law (65). All NIRS variables were calculated as the average during the signal epochs corresponding to the 60° range of motion occurring between 100° and 160° of leg extension (180° = full extension) during the concentric portion of each leg extension repetition.

During the screening visit, a post-occlusion reactive hyperemia (PORH) procedure was used as a physiological calibration to account for inter-individual variations in NIRS measurements between subjects. The PORH procedure was performed while the subject was at rest, laying supine on a plinth with the NIRS device secured over the vastus lateralis muscle (described above). A blood pressure cuff was placed at the most proximal portion of the thigh and inflated to 200-220 mmHg to cause arterial occlusion. This cuff pressure was maintained for five minutes. The pressure in the cuff was then released and the cuff was
removed. The NIRS device recorded data for five minutes prior to cuff inflation, during the five minutes while the cuff was inflated, and for five minutes after cuff deflation. All subsequent NIRS data during the leg extension exercises were expressed as a percentage of the difference between the minimal tissue oxygenation during ischemia and maximal oxygenation following recovery from ischemia that occurs during the PORH procedure (24).

**Statistical Analyses**

Means ± standard errors for descriptive assessments are reported in Table 1. Independent sample’s t-tests were used to compare the means of the all descriptive assessments between sarcopenic and non-sarcopenic groups. Three separate two-way mixed factorial analyses of variance (ANOVA) with a 2 × 2 model and repeated measures on the Site factor (site [EMG vs. NIRS] × group [sarcopenic vs. non-sarcopenic]) were used to compare the means for vastus lateralis muscle thickness, subcutaneous fat thickness, and echo intensity. Nine separate three-way mixed factorial analyses of variance (ANOVAs) with a 5 × 2 × 2 model and repeated measures on the Repetitions and Task factors (Repetitions [1 vs. 2 vs. 3 vs. 4 vs. 5] × task [5-RM vs. fatigue] × group [sarcopenic vs. non-sarcopenic]) were used to compare the means for absolute EMG<sub>RMS</sub> (μV), normalized EMG<sub>RMS</sub> (%MAX), absolute EMG<sub>MPF</sub> (Hz), normalized EMG<sub>MPF</sub> (%MAX), HHb (%PORH), O<sub>2</sub>Hb (%PORH), THb (%PORH), HBDiff (%PORH), and TSI (%). Significant interactions, main effects of the marginal means, or simple main effects were followed up with lower-order ANOVAs, paired t-tests for repeated measures factors, and independent t-tests for between-subjects factors and Fisher’s Least Significant Difference adjustments for multiple comparisons. Partial $\eta^2$ effect sizes and Cohen’s d effect sizes ($d$) were calculated and reported for all F- and t-ratios, respectively (13). All statistical analyses were performed using
IBM SPSS for Macintosh, Version 24 (IBM Corp., Chicago, IL, USA). An alpha of $p \leq 0.05$ was considered statistically significant for all comparisons.
CHAPTER IV
RESULTS

Descriptive Assessments

**Demographics.** There were no significant differences (p > 0.05) in age (d = 0.18), height (d = 0.30), or weight (d = 1.35) between the means of the sarcopenic and non-sarcopenic groups (Table 1). However, BMI was lower in the non-sarcopenic group (p = 0.03).

**Body Composition.** There were no significant differences (p > 0.05) in fat-free mass (d = 0.42) between the means of the sarcopenic and non-sarcopenic groups (Table 1). The sarcopenic group exhibited greater mean fat mass (p = 0.003, d = 2.41) and percent body fat (p = 0.007, d = 2.16) than the non-sarcopenic group, while mean RSMI was significantly greater (p = 0.001, d = 4.259) in the non-sarcopenic than the sarcopenic group.

**Muscle Size.** There were no site × group interactions for vastus lateralis muscle thickness (p = 0.313, η² = 0.113), subcutaneous adipose tissue thickness (p = 0.652, η² = 0.024), or echo intensity (p = 0.485, η² = 0.056). There were no main effects for site (p = 0.472, η² = 0.059) or group (p = 0.626, η² = 0.027) for vastus lateralis muscle thickness. However, there were main effects for *site* for subcutaneous fat thickness (p = 0.002, η² = 0.687) and echo intensity (p = 0.007, η² = 0.577) and main effects for *group* for subcutaneous fat thickness (p = 0.047, η² = 0.369) and echo intensity (p = 0.039, η² = 0.392). Subcutaneous adipose tissue thickness was greater and echo intensity was lower (p = 0.015, d = 1.21; p = 0.004, d = 1.64) at the EMG site than the NIRS site for the non-sarcopenic group only. Subcutaneous adipose tissue thickness (p = 0.026, d = 1.56) and echo
intensity \((p = 0.027, d = 1.63)\) were greater in the sarcopenic group than the non-sarcopenic group at the NIRS site only.

**Muscle Strength, Endurance, and Functionality.** There were no significant differences \((p > 0.05)\) in mean absolute 5-RM leg extension strength \((d = 0.88)\) or mean gait speed \((d = 1.00)\) between the sarcopenic and non-sarcopenic groups (Table 1). However, the sarcopenic group exhibited significantly lower \((p = 0.008, d = 2.13)\) mean handgrip strength than the non-sarcopenic group (Table 1).

**Surface Electromyography**

There were no repetition \(\times\) task \(\times\) group interactions for absolute EMG\(_{RMS}\) \((p = 0.142, \eta^2 = 0.170)\), normalized EMG\(_{RMS}\) \((p = 0.091, \eta^2 = 0.195)\), absolute EMG\(_{MPF}\) \((p = 0.845, \eta^2 = 0.037)\), or normalized EMG\(_{MPF}\) \((p = 0.611, \eta^2 = 0.070)\). There were no repetition \(\times\) task interactions for absolute EMG\(_{RMS}\) \((p = 0.726, \eta^2 = 0.054)\), normalized EMG\(_{RMS}\) \((p = 0.482, \eta^2 = 0.085)\), absolute EMG\(_{MPF}\) \((p = 0.374, \eta^2 = 0.108)\), or normalized EMG\(_{MPF}\) \((p = 0.171, \eta^2 = 0.168)\). There were no repetition \(\times\) group interactions for absolute EMG\(_{RMS}\) \((p = 0.997, \eta^2 = 0.004)\), normalized EMG\(_{RMS}\) \((p = 0.482, \eta^2 = 0.085)\), absolute EMG\(_{MPF}\) \((p = 0.675, \eta^2 = 0.061)\), or normalized EMG\(_{MPF}\) \((p = 0.171, \eta^2 = 0.168)\). There were no task \(\times\) group interactions for absolute EMG\(_{RMS}\) \((p = 0.781, \eta^2 = 0.009)\) or normalized EMG\(_{RMS}\) \((p = 0.657, \eta^2 = 0.023)\); however, there task \(\times\) group interactions for absolute EMG\(_{MPF}\) \((p = 0.008, \eta^2 = 0.560)\) and normalized EMG\(_{MPF}\) \((p = 0.040, \eta^2 = 0.390)\). The marginal means for absolute EMG\(_{MPF}\) \((p = 0.017, d = 0.973)\) and normalized EMG\(_{MPF}\) \((p = 0.039, d = 1.98)\) (collapsed across repetition) were greater during the 5-RM than the fatiguing task for the non-sarcopenic group (sarcopenic group; \(p = 0.229, d = 0.895\) [normalized EMG\(_{MPF}\)], \(p = 0.167, d = 0.648\) [raw EMG\(_{MPF}\)])(Figure 2).
marginal means for both absolute and normalized EMG\textsubscript{MPF} (collapsed across repetition) were not different between groups during the 5-RM (p = 0.075 – 0.638, \(d = 0.224 - 1.17\)) or fatiguing task (p = 0.530 – 0.937, \(d = 0.047 - 0.402\)).

There were significant main effects for repetition for absolute EMG\textsubscript{RMS} (p = 0.001, \(\eta^2 = 0.534\)), normalized EMG\textsubscript{RMS} (p = 0.001, \(\eta^2 = 0.600\)), absolute EMG\textsubscript{MPF} (p = 0.001, \(\eta^2 = 0.434\)), and normalized EMG\textsubscript{MPF} (p = 0.001, \(\eta^2 = 0.478\)). Post-hoc comparisons indicated that the marginal means (collapsed across task and group) for absolute EMG\textsubscript{RMS} during repetitions 3 (p = 0.002, \(d = 0.673\)), 4 (p = 0.001, \(d = 0.688\)), and 5 (p = 0.001, \(d = 0.773\)) were greater than repetition 1, but there were no other differences among repetitions (p = 0.060 – 0.701, \(d = 0.029 - 0.420\)) (Figure 4). The marginal means (collapsed across task and group) for normalized EMG\textsubscript{RMS} during repetitions 2 (p = 0.025, \(d = 0.884\)), 3 (p = 0.001, \(d = 1.373\)), 4 (p = 0.001, \(d = 1.409\)), and 5 (p = 0.001, \(d = 1.754\)) were greater than repetition 1, and repetition 5 was greater than repetition 2 (p = 0.039, \(d = 0.581\)); however, no other repetitions were different (p = 0.059 – 0.877, \(d = 0.025 – 0.403\)) (Figure 4). The marginal means (collapsed across task and group) for absolute EMG\textsubscript{MPF} during repetitions 4 (p = 0.009 – 0.041, \(d = 0.287 – 0.579\)) and 5 (p = 0.004 – 0.014, \(d = 0.309 – 0.590\)) were lower than repetition 1, 2, and 3, but there were no other differences among repetitions (p = 0.093 – 0.721, \(d = 0.037 – 0.299\)) (Figure 5). The marginal means (collapsed across task and group) for normalized EMG\textsubscript{MPF} during repetitions 4 (p = 0.004 – 0.010, \(d = 0.358 - 0.471\)) and 5 (p = 0.007 – 0.009, \(d = 0.434 – 0.555\)) were lower than repetitions 1 and 2, repetition 3 was also lower than repetition 5 (p = 0.013, \(d = 0.295\)), but there were no other differences among repetitions (p = 0.063 – 0.720, \(d = 0.067 – 0.236\)) (Figure 5). There were no main effects
for task for absolute EMG\textsubscript{RMS} (p = 0.778, $\eta^2 = 0.009$) or normalized EMG\textsubscript{RMS} (p = 0.847, $\eta^2 = 0.004$), and there were no main effects for group for absolute EMG\textsubscript{RMS} (p = 0.331, $\eta^2 = 0.105$) or normalized EMG\textsubscript{RMS} (p = 0.704, $\eta^2 = 0.017$).

**Near-infrared Spectroscopy**

There were no repetition $\times$ task $\times$ group interactions for HHb (p = 0.765, $\eta^2 = 0.016$), O$_2$Hb (p = 0.485, $\eta^2 = 0.073$), THb (p = 0.194, $\eta^2 = 0.163$), HBDiff (p = 0.780, $\eta^2 = 0.019$), or TSI (p = 0.735, $\eta^2 = 0.022$). There were no repetition $\times$ task interactions for HHb (p = 0.634, $\eta^2 = 0.034$), O$_2$Hb (p = 0.431, $\eta^2 = 0.086$), THb (p = 0.489, $\eta^2 = 0.80$), HBDiff (p = 0.412, $\eta^2 = 0.088$), or TSI (p = 0.466, $\eta^2 = 0.071$). There were no task $\times$ group interactions for HHb (p = 0.282, $\eta^2 = 0.127$), O$_2$Hb (p = 0.091, $\eta^2 = 0.285$), THb (p = 0.085, $\eta^2 = 0.294$), HBDiff (p = 0.991, $\eta^2 = 0.001$), or TSI (p = 0.550, $\eta^2 = 0.041$). There were no repetition $\times$ group interactions for THb (p = 0.467, $\eta^2 = 0.063$) or HBDiff (p = 0.321, $\eta^2 = 0.117$); however, there were repetition $\times$ group interactions for O$_2$Hb (p = 0.046, $\eta^2 = 0.230$), HHb (p = 0.020, $\eta^2 = 0.270$) and TSI (p = 0.045, $\eta^2 = 0.231$).

For the marginal means in the non-sarcopenic group (collapsed across task) there were main effects for repetition for HHb (p = 0.001, $\eta^2 = 0.769$) and TSI (0.001, $\eta^2 = 0.754$), but not for O$_2$Hb (p = 0.167, $\eta^2 = 0.265$). Post-hoc comparisons indicated that the marginal means for HHb during repetitions 2 (p = 0.007, $d = 0.687$), 3 (p = 0.010, $d = 1.285$), 4 (p = 0.006, $d = 1.637$), and 5 (p = 0.006, $d = 1.598$) were greater than repetition 1. HHb during repetitions 3 (p = 0.041, $d = 0.596$), 4 (p = 0.017, $d = 0.905$), and 5 (p = 0.019, $d = 0.886$) were greater than repetition 2. HHb during repetitions 4 (p = 0.007, $d = 0.277$) and 5 (p = 0.004, $d = 0.275$) were greater than repetition 3; however, HHb during
repetition 5 (p = 0.928, d = 0.004) was not greater than repetition 4. TSI during repetitions 2 (p = 0.043, d = 0.615), 3 (p = 0.017, d = 1.180), 4 (p = 0.009, d = 1.394) and 5 (p = 0.008, d = 1.383) were less than repetition 1. TSI during repetitions 3 (p = 0.012, d = 0.629), 4 (p = 0.003, d = 0.857), and 5 (p = 0.006, d = 0.853) were less than repetition 2. TSI during repetition 4 (p = 0.001, d = 0.212) was less than repetition 3, but TSI during repetition 3 (p = 0.076, d = 0.193) and 4 (p = 0.803, d = 0.021) were not different from repetition 5 (Figure 6).

For the marginal means in the sarcopenic group (collapsed across task) there were main effects for repetition for HHb (p = 0.008, $\eta^2 = 0.559$), O$_2$Hb (p = 0.021, $\eta^2 = 0.496$), and TSI (p = 0.002, $\eta^2 = 0.629$). However, post-hoc comparisons indicated no differences in HHb (p = 0.066 – 0.273, d = 0.121 – 0.836) or O$_2$Hb (p = 0.077 – 0.758, d = 0.037 – 0.530) among any repetitions. TSI during repetitions 3 (p = 0.049, d = 0.348) and 5 (p = 0.047, d = 0.582) were different than repetition 1, TSI during repetitions 4 (p = 0.018, d = 0.438) and 5 (p = 0.023, d = 0.534) were different than repetition 2, but no other repetitions were different (p = 0.070 – 0.488, d = 0.074 – 0.492) (Figure 6).

For all 5 repetitions, the marginal mean for TSI (collapsed across task) was lower in the sarcopenic group than the non-sarcopenic group (p = 0.005 – 0.014, d = 1.861 – 2.258). There were no differences between groups for HHb (p = 0.207 – 0.718, d = 0.231 – 1.017) or O$_2$Hb (p = 0.300 – 0.847, d = 0.118 – 0.406) for any repetitions (Figure 6).

There were no main effects for task for HHb (p = 0.288, $\eta^2 = 0.124$), O$_2$Hb (p = 0.307, $\eta^2 = 0.115$), THb (p = 0.577, $\eta^2 = 0.036$), HBDiff (p = 0.240, $\eta^2 = 0.150$), or TSI (p = 0.508, $\eta^2 = 0.050$). There were no main effects for group for THb (p = 0.449, $\eta^2 = 0.065$) or HBDiff (p = 0.357, $\eta^2 = 0.095$). There was a significant main effect for
repetition for HBDiff ($p = 0.001, \eta^2 = 0.670$), but not for THb ($p = 0.066, \eta^2 = 0.212$).

Post-hoc comparisons indicated that the marginal means for HBDiff (collapsed across task and group) during repetitions 2 ($p = 0.015, d = 0.394$), 3 ($p = 0.001, d = 0.958$), 4 ($p = 0.001, d = 0.93$) and 5 ($p = 0.001, d = 1.135$) were less than repetition 1. Additionally, HBDiff during repetitions 3 ($p = 0.003, d = 0.509$), 4 ($p = 0.002, d = 0.620$), and 5 ($p = 0.004, d = 0.702$) were less than repetition 2. There were no other differences among repetitions ($p = 0.059 – 0.150, d = 0.079 – 0.219$) (Figure 7).
CHAPTER V

DISCUSSION

This was the first study to compare muscle strength, size, activation, and tissue oxygenation between older women with and without sarcopenia during dynamic fatiguing tasks. Despite no differences in leg extensor muscle size or strength between sarcopenic versus non-sarcopenic older women, the sarcopenic older women exhibited 13–21% lower TSI scores across all repetitions of the 5-RM and 30% 1-RM fatiguing tasks. Although EMG_{RMS} increased, while EMG_{MPF} decreased from repetitions 1 to 5 during both the 5-RM and 30% 1-RM loads, there were no differences between the sarcopenic versus non-sarcopenic groups.

Based on handgrip strength and RSMI cutoffs, the sarcopenic group met the existing qualifications (15). Despite meeting the sarcopenic qualifications, the sarcopenic and non-sarcopenic groups did not differ in age, height, weight, gait speed, fat-free mass, leg extensor muscle cross sectional area, or vastus lateralis muscle thickness, nor were there differences in leg extension strength or endurance. There were, however, greater body fat indicators in the sarcopenic group by fat mass, percent body fat, subcutaneous fat thickness, and echo intensity (Table 1). These findings suggested that the sarcopenic group was also more obese than the non-sarcopenic group. Sarcopenic obesity has been studied and may adversely impact the health and function of older adults (4,6,28). The results of the present study indicate that body fat was present at a greater degree in nearly all measured locations, such as overall fat mass, percent body fat, site-specific subcutaneous fat thickness, and possibly infiltrated within the muscle via echo intensity. Even sample body mass index (BMI) calculations showed that only 2 out of 6 non-
sarcopenic older women were greater than 25 kg·m⁻¹, while the remaining 4 were 22–24 kg·m⁻¹. In contrast, 4 out of 5 sarcopenic women were greater than 25 kg·m⁻¹, with 1 greater than 30 kg·m⁻¹. Interestingly, a previous study, Ohara et al. (57) found that pulse wave velocity, which is a reflection of arterial stiffness, was higher in older individuals with sarcopenic-obesity than individuals classified as only sarcopenic and only obese. This suggests that blood flow may be impaired to a greater degree in sarcopenic-obese individuals than sarcopenia or obesity alone.

Another interesting finding in the present study was the lack of differences in leg extensor strength or muscle size between the sarcopenic and non-sarcopenic women. The fact that upper-body handgrip strength was lower in the sarcopenic group, while lower-body leg extension strength was not different, suggests that upper-body, non-weight bearing muscles may deteriorate in strength quicker than lower-body, weight-bearing muscles in older women. It is well accepted that age-related declines in muscular performance are muscle-specific and depend on the amount and intensity of physical activity performed across a life span (39). Since the subjects recruited for this study self-reported no involvement in resistance training, it may be reasonable to assume the daily physical activity these individuals perform may be predominately using weight-bearing muscles. Therefore, the presence of sarcopenia may be most evident in non-weight-bearing muscles, such as the finger flexors during handgrip strength, but less evident in weight-bearing muscles, such as the leg extensors during leg extension strength.

A consequence of the research design of the present study was a distinguished clinical classification of sarcopenic based on handgrip strength and RSMI. However, subjects ended up being matched for leg extension strength, endurance, and muscle size.
Therefore, when comparing the fatiguing leg extension tasks at 5-RM and 30% of estimated 1-RM, the only measured descriptive physiological difference between sarcopenia and non-sarcopenia groups was subcutaneous fat and echo intensity. Previous studies have hypothesized that subcutaneous fat can dampen the EMG signal (59,60) and the NIRS signal (55). However, it should be noted that subcutaneous fat and echo intensity were not different between sarcopenic and non-sarcopenic at the EMG electrode site. Subcutaneous fat and echo intensity were greater in the sarcopenic group at the NIRS site only (Table 1). Thus, it may be reasonable to consider body fat as a confounding factor for NIRS, but not for EMG time or frequency domain variables. The range of adipose tissue thickness was 0.61 – 1.064 cm for the non-sarcopenic group and 0.83 – 1.754 cm for the sarcopenic group. The Portamon NIRS device has a range of 2cm, therefore, we can reasonably assume that the NIRS device was able to detect the muscle underlying the subcutaneous adipose tissue for all of our subjects. Even yet, adipose tissue reflects light to a greater degree than muscle tissue. Therefore, the presence of subcutaneous and intramuscular adipose tissue may increase the scattering of light, which artificially decreases the amount of light detected at the light source, thus decreasing or dampening the signal. This highlights the need of normalization methods such as the PORH protocol when comparing group mean values of the NIRS variables. By expressing the NIRS values to each individual’s range, it may be possible to account for the heterogeneity in the underlying tissue, whether it was varying thicknesses of subcutaneous adipose tissue or varying concentrations of infiltrated intramuscular adipose tissue (3,45).
Previous studies have shown greater variability in muscle activation in older versus younger adults (64). Previous studies have also shown that older adults tend to demonstrate less muscle fatiguable than younger adults (34). Collectively, these previous findings may explain why neither EMG amplitude nor EMG frequency were different between sarcopenic and non-sarcopenic groups for either the 5-RM or 30% 1-RM fatiguing tasks. If greater variability is expected among muscle activation strategies of older adults in general, finding differences in EMG between sarcopenic and non-sarcopenic women without differences in leg extension strength, endurance, or muscle size may be difficult.

The patterns of responses in EMG amplitude and frequency in the present study followed the same characteristics of high- versus low-load fatigue in younger adults (38). Jenkins et al (38) showed during high load and low load leg extension exercises to failure in college-aged men and women, EMG\textsubscript{AMP} increased and EMG\textsubscript{MPF} will decreased across repetitions. Thus, the present study supported the conclusions of previous authors (35) that neuromuscular control strategies during fatigue are similar between young versus older adults.

Another interesting finding of the present study was that despite no differences in muscle size, strength, or endurance, significant differences in TSI during the 5-RM and 30% 1-RM fatiguing tasks between the sarcopenic and non-sarcopenic did still exist. Therefore, oxygen supply was not able to keep up with oxygen utilization as much in the sarcopenic group than the non-sarcopenic group. This is similar to findings of a previous studies showing impaired oxygen kinetics in mobility-impaired individuals (49) and adults with chronic obstructive pulmonary disease (58) compared to healthy individuals.
Thus, the present study supports previous conclusions suggesting that one mechanism contributing to sarcopenia may involve the nutritive flow hypothesis or the inability to deliver nutrients and oxygen to working skeletal muscles (14).

The patterns of responses in NIRS variables in the present study followed the same characteristics of previous studies examining dynamic fatiguing exercises (11,51). As the oxygen demand increases for the working muscles, $O_2$HB and HHB will exhibit an inverse relationship where $O_2$Hb decreases and HHB increases during the fatiguing task as oxygen is consumed by the muscle. Furthermore, tissue saturation index (TSI) represents the balance between oxygen supply to the muscle and oxygen utilization in the muscle and is expected to decrease during fatiguing tasks as oxygen utilization exceeds oxygen supply. Changes in total hemoglobin (tHB) represents changes in blood volume and is expected to increase with fatiguing exercise due to the increase in oxygen demand (50). HBDiff is not commonly reported in previous studies, however, recent recommendations (3) have emphasized the need to report all available NIRS variables. HBDiff represents the difference between $O_2$Hb and HHb and may serve as additional variable describing the balance between oxygen supply and utilization.

It is hypothesized that (a) oxygenated hemoglobin will decrease while deoxygenated hemoglobin and total hemoglobin will increase during the fatiguing task and 5-RM, (b) EMG$\text{AMP}$ will increase and EMG$\text{MPF}$ will decrease during the fatiguing task and 5-RM, and (c) patterns of response during the fatiguing task will vary between sarcopenia status.

This cross-sectional, exploratory pilot study identified differences in RSMI, handgrip strength, and vastus lateralis muscle tissue oxygenation, despite no differences
in leg extensor size, strength, or endurance. Even though clinical classifications exist for sarcopenia, age-related losses in muscle mass, strength, and function may not impact all muscles uniformly. The findings of our previous study support the hypothesis that \( \text{EMG}_{\text{AMP}} \) increased and \( \text{EMG}_{\text{MPF}} \) decreased during the fatiguing task. However, if greater variability is expected among muscle activation strategies of older adults (64), using EMG to monitor differences between sarcopenic and non-sarcopenic women without differences in leg extensor size, strength, and endurance may be difficult. Future studies are needed to further explore the findings in the present study that muscle tissue oxygenation, and possibly skeletal muscle blood flow, is lower in sarcopenic than non-sarcopenic older women. This study supported the hypothesis that deoxygenated hemoglobin will increase during the 5-RM and fatiguing tasks in the non-sarcopenic group only. Interestingly, deoxygenated hemoglobin did not increase in the sarcopenic group. Additionally, oxygenated hemoglobin did not decrease during the 5-RM and fatiguing tasks for either group. However, there were differences in tissue saturation index during the 5-RM and fatiguing tasks. NIRS measurements may be important to consider when recommending exercise or nutrition interventions for either oxygen or dietary nutrient delivery to working skeletal muscles. (18)
**Table 1. Means (± standard error) for descriptive outcome measures in non-sarcopenic and sarcopenic women.**

<table>
<thead>
<tr>
<th></th>
<th>Non-sarcopenic (n = 6)</th>
<th>Sarcopenic (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>75.8 ± 2.6</td>
<td>74.5 ± 3.1</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>160.2 ± 2.9</td>
<td>157.8 ± 3.8</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>62.2 ± 2.9</td>
<td>71.5 ± 3.0</td>
</tr>
<tr>
<td>Fat Mass (kg)</td>
<td>22.7 ± 1.1*</td>
<td>29.1 ± 1.2</td>
</tr>
<tr>
<td>Lean Mass (kg)</td>
<td>41.0 ± 3.7</td>
<td>38.2 ± 1.5</td>
</tr>
<tr>
<td>Percent Body Fat (%)</td>
<td>36.7 ± 1.2*</td>
<td>41.8 ± 0.7</td>
</tr>
<tr>
<td>Relative Skeletal Muscle Index (%)</td>
<td>29.3 ± 0.9*</td>
<td>25.9 ± 0.7</td>
</tr>
<tr>
<td>Leg Extensor Muscle Cross-sectional Area (cm²)</td>
<td>30.5 ± 1.6</td>
<td>31.6 ± 1.1</td>
</tr>
<tr>
<td>Vastus Lateralis Muscle Thickness&lt;sub&gt;NIRS&lt;/sub&gt;(cm)</td>
<td>1.90 ± 0.08</td>
<td>2.03 ± 0.11</td>
</tr>
<tr>
<td>Vastus Lateralis Subcutaneous Fat Thickness&lt;sub&gt;NIRS&lt;/sub&gt; (cm)</td>
<td>0.89 ± 0.08*‡</td>
<td>1.34 ± 0.16</td>
</tr>
<tr>
<td>Vastus Lateralis Echo Intensity&lt;sub&gt;NIRS&lt;/sub&gt; (a.u.)</td>
<td>105.8 ± 3.2*‡</td>
<td>116.5 ± 2.2</td>
</tr>
<tr>
<td>Vastus Lateralis Muscle Thickness&lt;sub&gt;EMG&lt;/sub&gt;(cm)</td>
<td>1.93 ± 0.07</td>
<td>1.87 ± 0.05</td>
</tr>
<tr>
<td>Vastus Lateralis Subcutaneous Fat Thickness&lt;sub&gt;EMG&lt;/sub&gt; (cm)</td>
<td>1.25 ± 0.15</td>
<td>1.63 ± 0.16</td>
</tr>
<tr>
<td>Vastus Lateralis Echo Intensity&lt;sub&gt;EMG&lt;/sub&gt; (a.u.)</td>
<td>89.2 ± 4.9</td>
<td>105.6 ± 7.7</td>
</tr>
<tr>
<td>Absolute leg extension strength (kg)†</td>
<td>9.5 ± 1.1</td>
<td>7.4 ± 0.9</td>
</tr>
<tr>
<td>Repetitions Completed at 30% Est. 1-RM</td>
<td>13.2 ± 1.2</td>
<td>13.6 ± 3.0</td>
</tr>
<tr>
<td>Handgrip Strength (kg)</td>
<td>25.1 ± 2.1*</td>
<td>16.5 ± 1.1</td>
</tr>
<tr>
<td>Gait Speed (m·s⁻¹)</td>
<td>1.10 ± 0.06</td>
<td>0.93 ± 0.07</td>
</tr>
</tbody>
</table>

* Indicates significant difference between non-sarcopenic and sarcopenic subjects (p ≤ 0.05)
‡ Indicates significant difference from EMG site (p ≤ 0.05)
†Mass lifted during the 5RM task (kg)

**Table 2. Means (± standard error) for EMG time and frequency domains for the first repetition of the 5RM.**

<table>
<thead>
<tr>
<th></th>
<th>Non-sarcopenic (n = 6)</th>
<th>Sarcopenic (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EMG Amplitude (μV&lt;sub&gt;RMS&lt;/sub&gt;)</td>
<td>92.5 ± 13.5</td>
<td>71.5 ± 9.1</td>
</tr>
<tr>
<td>EMG Frequency (Hz&lt;sub&gt;MPF&lt;/sub&gt;)</td>
<td>72.1 ± 4.7</td>
<td>52.1 ± 8.5</td>
</tr>
</tbody>
</table>
Figure (1). Torque (Nm) across the 5RM (left) and fatiguing (right) leg extension repetitions for all participants. Gray lines indicate sarcopenic and black lines indicate non-sarcopenic.
Figure (2). Absolute and normalized EMG variables across the 5RM (left) and fatiguing leg extensions (right). Black lines indicate non-sarcopenic and gray lines indicate sarcopenic.

*Indicates EMG MPF collapsed across repetition was greater during the 5RM than fatiguing task for the non-sarcopenic group only (p ≤ 0.05).
Figure (3). NIRS variables across the 5RM and fatiguing leg extensions. Black lines indicate non-sarcopenic and gray lines indicate sarcopenic.
Figure (4). Absolute EMG<sub>RMS</sub> (top) and normalized EMG<sub>RMS</sub> (Bottom) collapsed across group and task.
* Indicates significantly greater than repetition 1.
† Indicates significantly greater than repetition 2.
Figure (5). Absolute $\text{EMG}_{\text{MPF}}$ (Top) and normalized $\text{EMG}_{\text{MPF}}$ (bottom) collapsed across group and task.

* Indicates significantly less than repetition 1.
† Indicates significantly less than repetition 2.
‡ Indicates significantly less than repetition 3.
Figure (6). HHb (Top), O$_2$Hb (Middle), and TSI (Bottom) collapsed across task in the non-sarcopenic (black) and sarcopenic (gray).

* Indicates significantly different than repetition 1.
† Indicates significantly different than repetition 2.
‡ Indicates significantly different than repetition 3.
§ Indicates significant difference between groups.
Figure (7). HBDiff collapsed across group and task. *Indicates significantly different than repetition 1. †Indicates significantly different than repetition 2.
REFERENCES


22. Goedette, JH, Gibson, ASC, Grobler, L, Collins, M, Noakes, TD, and Lambert, EV. Determinants of the variability in respiratory exchange ratio at rest and during


APPENDIX A

Department of Nutrition and Health Sciences
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University of Nebraska-Lincoln
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IRB #: 18543

Formal Study Title: A pilot study to explore muscle energy metabolism and metabolic flexibility in older men and women

Authorized Study Personnel

Principal Investigator: Joel T. Cramer, Ph.D. Phone: (402) 472 - 7533
Study Coordinator: Brianna D. McKay Phone: (308) 380 - 7861
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Additional Personnel: Zachary Gillen, M.S. Phone: (402) 472 - 7738
Marni Shoemaker, M.S. Phone: (402) 472 - 7738

Key Information:
If you agree to participate in this study, the project will involve:

- Males and females 65 years and older
- Procedures will include:
  - questionnaires to assess your health status and eligibility
  - body composition assessments including dual-energy x-ray absorptiometry (DXA) and ultrasonography
  - one occlusion test using a blood pressure cuff on your thigh for about 5 minutes
  - ten venous blood draws while you rest for 3 hours
  - exercise tests (grip strength, walking speed, balance and chair stand tests, walking on a treadmill, and leg extension exercises)
  - surface electromyography and near-infrared spectroscopy
- Two visits to our laboratory are required
- These visits will take approximately 9 hours total (Visit 1: 2 hours, Visit 2: 7 hours)
- The risks of DXA include being exposed to small amounts of radiation that is no greater than the amount of natural background radiation received in two days
- The risks of the occlusion test include leg discomfort while the blood pressure cuff is inflated
- The risk of surface electromyography and near-infrared spectroscopy include mild discomfort to local skin areas due to light shaving and cleansing with alcohol wipes
- The risks of exercise tests include cramping, fatigue, sweating, or becoming short of breath. You may also experience soreness in the days immediately following these tests.
- The risks of the venous blood samples include potential pain, bruising, or bleeding.
- You will be paid up to $100 for your participation ($25 for screening, $25 for completing Visit 1, $50 for completing Visit 2)
- You will be provided a copy of this consent form
- Your participation is voluntary, and you may decide not to participate at any time

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Initials
Invitation

You are invited to take part in this research study. The information in this form is meant to help you decide whether or not to participate. If you have any questions, please ask.

Why are you being asked to be in this research study?

You are being asked to be in this study because you are a healthy adult age 65 or older.

What is the reason for doing this research study?

As we age, we tend to lose muscle mass which can lead to a decrease in strength and function. Maintaining muscle mass is important for healthy aging. This research is designed to explore how your muscles use energy during rest, after eating a meal, and during exercises.

This study is being done at the University of Nebraska-Lincoln (UNL). A total of about 20 people will participate in this study

What will be done during this research study?

You will be asked to complete 2 study visits, a Screening Visit and a Test Visit, between 4 – 10 days. The following will be completed during the Screening Visit:

Questionnaires: We will ask you to complete 2 questionnaires. The first questionnaire will ask for contact information, demographic information, and a few health history questions to determine your eligibility for this study. The second questionnaire will ask about your health history to determine if you are able to exercise safely.

Height, Weight, and Waist Circumference: We will ask you to step on a scale to obtain your body weight and height, we will use these measurements to calculate your body mass index (BMI). We will also use a tape to measure your waist circumference. This procedure will take approximately 5 minutes.

Strength and Performance Tests: We will ask you to squeeze a handle as hard as you can to obtain your grip strength. We will ask you to complete a series of balance tests with your 1) feet side-by-side, 2) heel of one foot against the big toe of the other foot, and 3) feet aligned heel to toe. We will also ask you to walk at your normal walking speed on a 4-meter track. Finally, we will ask you to complete a chair stand test where we will ask you to raise from the chair 5 times with your hands placed across your chest. We will time you as you complete all balance, walking, and chair stand tests.

The above items will allow us to determine if you are eligible. If you are eligible for the study, you will then complete the following during the screening visit:

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**Dual X-ray Absorptiometry (DXA):** We will measure your body composition (how much fat and muscle your body is made of) with a dual energy x-ray absorptiometry (DXA) scanner at the first visit. You will be asked to remove jewelry, body piercings, clothing with zippers or metal buttons, or any clothing containing metal and to put on unrestrictive clothing (provided in the laboratory or brought by you) for the scan. You will lie still on a padded table and breathe normally for the duration of the scan which is approximately 5 minutes for a whole-body scan.

**Ultrasonography:** We will also measure composition of your right thigh with a non-invasive ultrasound device. Using the ultrasound device, we will take a cross-sectional picture across your thigh to measure the cross-sectional area of your quadriceps muscle. We will also take a few ultrasound pictures of your thigh at the site of the sensors (see below: Surface Electromyography and Near-Infrared Spectroscopy), to measure muscle thickness and adipose tissue thickness.

**Post-occlusion Reactive Hyperemia Test:** We will place a standard blood pressure cuff around the upper portion of your right thigh and inflate the cuff to at least 200mmHg and no higher than 280mmHg. This is very similar to when you have blood pressure taken on your arm. However, we will keep the cuff inflated for at least 5 minutes to maintain arterial occlusion. We will then release the blood pressure cuff and ask you to remain laying down with your legs relaxed for approximately 10 minutes. During this test, we will have a small device placed on your right leg that will monitor oxygenation in your thigh muscles (see Near-Infrared Spectroscopy below).

**VO_{2}max Estimation and Leg Extension Strength:** We will calculate your maximal aerobic capacity (VO_{2}max) using an equation that considers your age, sex, physical activity status, and waist circumference. This will tell us how fast we will ask you to walk on the treadmill at your second visit. We will also ask you to complete a 5-repetition maximum leg extension test. We want to see how much weight you can successfully lift 5 times in a row. This will allow us to estimate the maximum amount of weight you would be able to lift one time. This will help us determine how much weight we will ask you to lift on your second visit.

If you meet our eligibility criteria, we will also schedule you for the Test Visit. The following will be completed leading up to and during the Test Visit:

**Food Record Forms and Test Visit Instructions:** At the end of your Screening Visit, we will provide you a food record form that we will ask you to bring back to the Test Visit. We will ask you to record everything you eat and drink for the 3 days preceding your scheduled Test Visit. We will also provide instructions for your Test Visit. We will ask you to 1) consume a minimum of 150 grams of carbohydrates per day for the 3 days preceding your Test Visit 2) not consume alcohol or participate in strenuous exercise the day before your Test Visit 3) fast (no food or drink except medications and water) for 8 – 16 hours the night before your Test Visit. Finally, we will ask you to wear appropriate clothes and shoes to the test visit so that you can exercise safely and comfortably. We will ask you if there were any changes to your health since your Screening Visit and we will verify that you adhered to the pre-Test Visit procedures.
Blood Draws and Meal Tolerance Tests: We will collect ten 12 mL blood samples (less than 1 Tbsp. each) from a vein in your forearm during the Test Visit (maximum of 120 mL total). The blood draws will be done by a trained phlebotomist who will leave the catheter in your arm so we only poke you once. We will ask you to rest in our laboratory for 30 minutes after you arrive after which we will complete a fasting blood draw. We will then ask you to eat a meal (1/2 English muffin with 1 Tbsp. peanut butter and 20 oz. Gatorade) within 15 minutes, and then continue resting for up to three hours. During this time, we will collect blood samples every 15 minutes for the first 90 minutes, and then every 30 minutes up to 180 minutes. During the entire resting period, we will ask you to wear a mask that measures how much oxygen you inhale and carbon dioxide you exhale. You will also be allowed to watch TV, play games on your phone or electronic tablet or read, and have access to toilets. Your blood samples will be analyzed to determine serum concentrations of glucose, c-peptide, insulin, amino acids, free-fatty acids, gastric inhibitory polypeptide (GIP), and glucagon-like peptide-1 (GLP-1). Additionally, your blood samples will be archived for subsequent analysis of unique proteins and other metabolites that are intended to help identify additional biomarkers related muscle function. These biomarkers may include markers of inflammation e.g., c-reactive protein, interleukin 6, IGF-1, TNF-alpha, and/or muscle tissue injury e.g., 3-methylhistidine. No genetic analysis or disease risk assessments will be performed. Your blood samples will be stored in a freezer in a locked room in Ruth Leverton Hall with limited access. Only the investigators of this study will have access to these samples. All samples will be managed and maintained by Dr. Joel Cramer and with the help of his graduate students. When the samples are analyzed at a later date, the results will be shared with Abbott Nutrition. By signing this document, you agree to allow your biospecimen stored for future analysis and therefore cannot withdraw your blood sample at any time.

Lunch Break: We will give you a 30-minute lunch break where we will provide you a standard meal (1 slice of white bread, 1 oz. American Cheese, and 20 oz. Gatorade).

Aerobic and Anaerobic Exercise Tests: We will ask you to walk on a treadmill for 15 minutes at low-moderate intensity so we can assess your aerobic metabolism. We will also ask you to complete submaximal leg extension exercises until you are unable to complete a repetition throughout your full range of motion to assess your anaerobic metabolism. During these two tests, we will ask you to wear a mask that measures how much oxygen you inhale and carbon dioxide you exhale. During the leg extension test we will also attach sensors to your thigh muscles.

Surface Electromyography: During the leg extension tests at the screening visit and test visit, we will put non-invasive sensors on your right thigh to measure the activity of your muscle during the exercise. We will need to prep your skin before applying the sensors which will consist of light shaving and cleansing with alcohol wipes.

Near-Infrared Spectroscopy: We will place a small non-invasive device on your right thigh during the post-occlusion reactive hyperemia test and all exercise tests. This device measures oxygenation in your muscle. Before securing this device to your thigh with Coban™ wrap, we will need to prepare your skin by light shaving and cleansing with alcohol wipes.
How will my data be used?

Your data will be sent to researchers outside of the University of Nebraska-Lincoln for the purpose of sharing results with the sponsoring company. The data that are sent to these researchers will not contain identifiable information, only the participant ID that is linked to your personal information. No personal information will be sent to Abbott Nutrition, only coded data will be sent.

Your blood samples will not be used for commercial profit and therefore, you will not share in any commercial profit from the use of your blood samples.

Your blood samples that are collected for this research study will not include whole genomic, germline, somatic, and/or exome sequencing. This means that the researchers have no plans to look at or try to “read,” the genetic information from your sample.

Will I be notified if my blood samples or DXA scan result in an unexpected finding?

When blood samples and DXA results are collected and analyzed, there is the chance of finding something unexpected. There may be benefits to learning such results (such as early detection and treatment of a medical condition), but there are risks as well (such as feeling worried about a finding for which no treatment is required or appropriate).

The results from the blood samples we collect in this research study are not the same quality as what you would receive as part of your health care. The blood sample results will not be reviewed by a physician who normally reads such results. Due to this, you will not be informed of any unexpected findings. The results of your blood samples will not be placed in your medical record with your primary care physician or otherwise. If you believe you are having symptoms that may require care, you should contact your primary care physician.

In this study, you will be informed of any unexpected findings of possible clinical significance that may be discovered during review of results from your DXA scan. The results of your DXA scan will not be placed in your medical record with your primary care physician or otherwise.

The results from the DXA scan we collect in this research study are the same quality as what you would receive as part of your health care. The results will be reviewed by a physician who normally reads such results and they will inform us if there are any unexpected findings and we will provide you with this information so that you may discuss it with your primary care physician. However, if you believe you are having symptoms that may require care prior to receiving any information from this study, you should contact your primary care physician. If you would rather not receive incidental findings, please indicate so at the end of this form.
What are the possible risks of being in this research study?

This research presents a slight risk of loss of confidentiality since your data will be able to be identified and shared with researchers from outside UNL. However, only coded data will be shared with researchers from outside UNL.

There is a possibility that you will feel hungry and uncomfortable during the fasting the night before each visit, and it may be inconvenient for you to not eat anything.

The risks associated with the exercise tests include cramping, fatiguing, sweating, or becoming short of breath. You may also experience soreness in the days immediately following the tests. However, this soreness is normal and is naturally alleviated by the body. Stretching exercises will be demonstrated for you to help relieved cramping, if necessary. Additionally, you will be asked repeatedly during the tests how you feel in relation to your ability to continue the test. Throughout all tests you will be monitored by study personnel trained in Cardiopulmonary Resuscitation (CPR) and use of an Automated External Defibrillator (AED).

The risks associated with blood draw include discomfort at the site of the blood draw, feeling dizzy and nauseated, and bruises and blot spots under the skin. Clotting of the blood, blocking of arteries, and infections are very rare but are also potential risks. These risks will be reduced or eliminated by having only a trained phlebotomist draw blood. Additionally, a trained assistant will closely monitor you while you lay down to have your blood collected. Blood will be drawn in the laboratory in a sterile (clean) environment.

The risks associated with the post-occlusion reactive hyperemia test include discomfort while the blood pressure cuff is inflated on your thigh. You will be asked repeatedly during this test how you feel in relation to continue the test.

There are no known risks of surface electromyography or near-infrared spectroscopy. However, prepping the skin prior to placement of the sensors includes light shaving and cleaning with alcohol wipes. This may cause mild discomfort to the local skin areas.

The amount of radiation exposure received as a result of a whole-body DXA scan is 5 μSv per scan (μSv is an abbreviation for micro-Sievert which is a measure of radiation dose). The average person living in the United States receives approximately 3000 μSv of radiation dose each year from natural sources. The amount of radiation received from a single whole-body DXA scan is less than the amount of natural background radiation received in two days, or, in other terms, less than 5 % of the radiation received from a standard medical chest X-ray. This level of radiation dose is well below known minimum amounts that would result in a direct harmful effect (for example, skin redness or rash). However, a possible indirect effect to radiation exposure is an additional risk of cancer. The normal risk of developing cancer in the general public is approximately 40 %. Based on the most current scientific understanding, the additional cancer risk from this procedure is less than 0.001 %. For these reasons, it is thought that the risks associated with radiation exposure from this study will be very small.

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It is possible that other rare side effects could occur which are not described in this consent form. It is also possible that you could have a side effect that has not occurred before.

What are the possible benefits to you?
You are not expected to get any benefit from being in this study.

What are the possible benefits to other people?
This information will progress our understanding of metabolism in older adults, thus the knowledge gained from this study may benefit older adults in the future.

What are the alternatives to being in this research study?
Instead of being in this research study you can choose not to participate.

What will being in this research study cost you?
Cost for travel and transport to our laboratory on UNL East Campus will be your responsibility. We will provide temporary visitor parking passes for you to park in East Campus parking lots for the duration of your Screening Visit and Test Visit.

Will you be compensated for being in this research study?
You will be paid in two separate payments after each visit. You will receive $25.00 cash for the screening visit. If you are eligible for the study, you will receive an additional $25.00 cash for finishing the testing during the screening visit. If you complete the test visit, you will receive $50.00 cash. Therefore, if you complete both visits you will receive a total of $100.00 cash. We will ask you to sign a receipt upon receiving your payment after each visit. This signed receipt will be securely stored for a minimum of 7 years.

Who is paying for this research?
The sponsor of the research is Abbott Nutrition. The University of Nebraska-Lincoln receives money from the sponsor to conduct this study.

What should you do if you have a problem during this research study?
Your welfare is the major concern of every member of the research team. If you have a problem or experience harm as a direct result of being in this study, you should immediately contact one of the people listed at the beginning of this consent form. If needed, seek immediate emergency care for this problem. Please note, it is the policy of UNL not to pay for any required care. Agreeing to this does not mean you have given up any of your legal rights.

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In the unlikely event that you should suffer an injury as a direct consequence of the research procedures described above, Abbott agrees to pay reasonable medical expenses necessary to treat the injury, provided that you follow the directions of the investigator and to the extent that you are not otherwise reimbursed by medical insurance. Emergency care is available at local community health providers. In the case that you seek medical treatment following an injury, you will be asked to sign an Authorization to release your medical records to the IRB and applicable associates for review. The study physician, Dr. Heather Eberspacher, will review any adverse events that might occur. If the adverse event is considered serious, Dr. Eberspacher will need to have access to your medical records for review.

How will information about you be protected?

Reasonable steps will be taken to protect your privacy and the confidentiality of your study data. The data will be stored in a locked cabinet in the investigator’s office and will only be seen by the research team during the study and will be stored for a minimum of 15 years after the study is complete. Your data will receive an identifying number and only the investigators will be able to identify you from your data. Furthermore, when your data is sent to Abbott Nutrition, only the identifying number that was assigned to you for this study will be used for identification. The data will also be stored electronically through a secure server and will only be seen by the research team during the study and for a minimum of 15 years after the study is complete.

The only persons who will have access to your research records are the study personnel, the Institutional Review Board (IRB), and any other person, agency, or sponsor as required by law. Information from this study may be published in scientific journals or presented at scientific meetings but the data will be reported as group or summarized data and your identity will be kept strictly confidential.

What are your rights as a research subject?

You may ask any questions concerning this research and have those questions answered before agreeing to participate in or during the study.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

For study related questions, please contact the investigator(s) listed at the beginning of this form.

For questions concerning your rights or complaints about the research contact the Institutional Review Board (IRB):

- Phone: 1 (402) 472 - 6965
- Email: irb@unl.edu

Initial

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What will happen if you decide not to be in this research study or decide to stop participating once you start?

You can decide not to be in this research study, or you can stop being in this research study ("withdraw") at any time before, during, or after the research begins for any reason. Deciding not to be in this research study or deciding to withdraw will not affect your relationship with the investigator or with the University of Nebraska-Lincoln.

You will not lose any benefits to which you are entitled.

If the research team gets any new information during this research study that may affect whether you would want to continue being in the study you will be informed promptly.

The researchers may also make the decision to take you out of the study, even if you want to continue, if:

- Your health changes and the study is no longer in your best interest.
- You do not follow the study rules or no longer meet the requirements to be in the study.
- The study is stopped by the sponsor, IRB, or researchers.

Documentation of informed consent

You are voluntarily making a decision whether or not to be in this research study. Signing this form means that (1) you have read and understood this consent form, (2) you have had the consent form explained to you, (3) you have had your questions answered and (4) you have agreed to participate in the research study. You will be given a copy of this consent form to keep.

Participant Feedback Survey

The University of Nebraska-Lincoln wants to know about your research experience. This 14-question, multiple-choice survey is anonymous. This survey should be completed after your participation in this research. Please complete this optional online survey at: http://bit.ly/UNLresearchfeedback.
Please indicate if you would or would not like to receive unexpected findings regarding your DXA scan. The answer to this question will not affect your participation in this study.

- I would like to receive unexpected findings
- I would NOT like to receive unexpected findings

Participant Name:

(Name of Participant: Please print)

Participant Signature:

Signature of Research Participant                      Date

Investigator certification:

Signature of Person Obtaining Consent                      Date

Please initial to indicate that you have read and understand that you cannot withdraw your blood specimens after the project ends because your blood specimens will be de-identified.

Initial
APPENDIX B

AHA/ACSM Health/Fitness Facility Preparticipation Screening Questionnaire

Assess your health needs by marking all true statements.

History
You have had:
___ A heart attack
___ Heart surgery
___ Cardiac catheterization
___ Coronary angioplasty (PTCA)
___ Pacemaker/implantable cardiac defibrillator/rhythm disturbance
___ Heart valve disease
___ Heart failure
___ Heart transplantation
___ Congenital heart disease

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

Cardiovascular risk factors
___ You are a man older than 45 years.
___ You are a woman older than 55 years, you have had a hysterectomy, or you are postmenopausal.
___ You smoke, or quite within the previous 6 mo.
___ Your BP is greater than 140/90.
___ You don't know your BP.
___ You take BP medication.
___ Your blood cholesterol level is >200 mg/dL.
___ You don't know your cholesterol level.
___ You have a close blood relative who had a heart attack before age 55 (father or brother) or age 65 (mother or sister).
___ You are physically inactive (i.e., you get less than 30 min. of physical activity on at least 3 days per week).
___ You are more than 20 pounds overweight.

___ None of the above is true.

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

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

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

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


www.acsm-msse.org/ptjpt-template-journal/msse/media/0698c.htm
APPENDIX C

Pre-Participation Demographic/Screening Questionnaire

Contact Information:
Preferred Phone Number: ____________________
E-mail Address: ____________________________________________
Emergency Contact Name: ____________________________________
Emergency Contact Phone: ____________________________________
Personal Physician: ____________________ Physician’s Phone: ____________________

Demographics:
Your Birthdate: ____________ Age: ______ Sex: M F
Are you: ( ) Hispanic or Latino ( ) Not Hispanic or Latino ( ) Prefer not to answer
Which of the following best describes you?
( ) American Indian or Alaska Native
( ) Asian
( ) Black or African American
( ) Native Hawaiian or Other Pacific Islander
( ) White
( ) Other, please specify: ____________________

Are you currently participating in any other research studies? Yes No

Health Status Questions:
1. Are you able to walk without assistance? Yes No N/A
2. Are you post-menopausal? Yes No
3. Are you currently participating in a resistance training program? Yes No
4. Have you had a poor appetite with any unexplained weight loss over the past 6 months? Yes No
5. Do you have a current infection (requiring medication or which might be expected to require hospitalization)? Yes No
6. Have you had inpatient surgery, or corticosteroid treatment (excluding topical creams) in the last 3 months, or antibiotics in the last 3 weeks? Yes No
7. Do you have any chronic, infectious diseases such as active Tuberculosis, Hepatitis A, B, or C or HIV? Yes No

8. Please check (√) if you previously had or currently have any of the following conditions:
( ) gastrointestinal disease (i.e., Crohn’s, colitis)
( ) gastrointestinal surgeries 
( ) gastroparesis 
( ) active malignancy 
( ) liver disease 
( ) kidney disease 
( ) eating disorder 
( ) severe dementia or delirium 
( ) neurological or psychiatric disorder 
( ) alcoholism 
( ) substance abuse 

9. Are you allergic or intolerant to any foods? Yes No 
If so, please list: ____________________________________________________________

10. Please list all medications you are currently taking: __________________________
__________________________________________________________________________
__________________________________________________________________________
__________________________________________________________________________

11. Please list all vitamins or dietary supplements you are currently taking: __________
__________________________________________________________________________
__________________________________________________________________________
__________________________________________________________________________
__________________________________________________________________________

__________________________  ______________________________
Participant Signature       Date