DOES DIETARY PROTEIN INTAKE, GREATER THAN THE RDA, DELAY THE CONSEQUENCES OF SARCOPENIA IN OLDER ADULTS?

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ABSTRACT

Introduction

Sarcopenia is a multifactorial age-related condition that has been referred to as the most drastic biological aspect of the aging process. Significant declines in muscle mass, efficiency, and strength are the most well-known pathophysiological changes that occur as a result of this aging phenomenon. However, the underlying causes remain unclear so the current definition and research criteria, to properly diagnosis individuals, has yet to reach a universal consensus. As a result, investigators have made few strides in determining an effective intervention in delaying sarcopenia. So far emerging nutritional research has indicated dietary protein may be potentially beneficial in deterring the development of sarcopenia, but no clinical practice guidelines or evidence-based dietetic recommendations have been established. The Evidence Analysis Library, from the Academy of Nutrition and Dietetics (AND), does not have an evidence analysis question or any information regarding this topic. Therefore, with the aging population expected to exponentially expand, the need for adequate older adult nutritional care will be essential.

Methods

The AND's evidence analysis process was utilized to answer the question, "Does dietary protein intake, greater than the RDA delay, the consequences of sarcopenia in older adults?" Based on set criteria, relevant articles were critically analyzed using Evidence Analysis Worksheets and Quality Criteria Checklists. Each study was rated based on the quality of evidence provided. From these findings and the quality of research available, a graded conclusion statement was formulated.

Results

Based on inclusionary criteria, nine primary research articles were found pertinent to the research question. Three articles received a positive quality rating, one of which was a randomized control trial; six articles were given a neutral rating. Eight studies found a higher protein intake was associated with some degree of muscle mass improvements. Three out of four studies discovered greater protein intake was positively correlated with muscle strength. Two studies, mainly focused on physical performance, found dietary protein was helpful in preserving muscle efficiency. Due to the wide study variability and complexity of sarcopenia, it was a challenge to comparatively analyze these articles.

Conclusion

The collaborative evidence suggested dietary protein may play a protective role against preserving muscle mass and possibly delaying and/or preventing sarcopenia. Since more studies focused on muscle mass, a stronger consensus was formulated compared to muscle strength and physical performance. However, a Grade II (fair) was given due to the evidence presenting many limitations of generalizability, potential biases, and study design flaws.

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CHAPTER 1: INTRODUCTION

By 2029, older adults will represent at least 20% of the United States population and this age demographic is expected to surpass those aged 18 years and younger (Colby & Ortman, 2014). With exponential in the older adult population expected to occur and individuals continuing to have longer life expectancies, the prevalence of chronic diseases and age-related conditions will undoubtedly ascend as well. A major consequence of aging is sarcopenia, defined as a natural age-associated loss of skeletal muscle mass, efficiency, and strength (Chernoff, 2014; WebMD, 2014; Phillips, 2012). With this current definition under national debate, it is difficult to determine exactly how many older adults are affected by sarcopenia on a national level (Batsis et al, 2014). The most recent estimation was in 2010, where 18 million Americans experienced this degenerative loss of muscle composition. Consequences of sarcopenia include extreme financial and social burdens, not only for the individual, but also for families and communities that provide services and assistance for age-related conditions (Robinson et al., 2012; Aging in Motion, 2011).

The overall pathophysiology of sarcopenia is multifaceted since it affects both cognitive and muscle-dependent mechanisms (Palmio & Udd, 2014; Lynch, 2011). In addition to natural loss, sarcopenia can also accelerate during inactive periods resulting from illness or surgical recovery, which indicates a possible connection between physical activity and sarcopenic advancements (Phillips, 2012). It is estimated that an individual can experience about one-percent muscle loss per year, which equates to approximately 50% between the ages of 20 and 80 years (Palmio & Udd, 2014; Phillips, 2012). As a result, some of the major health consequences that may develop from sarcopenia include declines in physical activity, quantity of

motor neurons and function, protein synthesis, altered hormonal status, and decreases in protein and caloric intakes (Chernoff, 2014; Palmio & Udd, 2014). Therefore, the underlying preventative measures are still unclear in order to make solid recommendations, but it is apparent various lifestyle behaviors seem to play a preventative role as well (Phillips, 2012; Lynch, 2011).

The two most well-known interventions shown to delay sarcopenic effects in older adults are through diet, usually protein supplementation, and muscle resistance training (Phillips, 2012). There appears to be less research on dietary interventions emphasizing food intake (e.g. dietary protein sources) and the impact on sarcopenia advancements. Evidence from the National Health and Nutrition Examination Survey (NHANES), *What We Eat in America*, illustrates that older adults above the age of 50 years consume the least amount of protein than that of any other adult age group. Older males and females, between the ages of 50 and 70 years old, show significant intake declines averaging 10 grams less of protein per day (USDA, 2012).

Currently, the recommended daily allowance (RDA) for protein consumption is 0.8 g/kg to help maintain adequate muscle stores (Bauer et al., 2013). However, the RDA does not take into account age-related metabolic changes and sex so this recommendation is universally prescribed to all healthy adults (Bauer et al., 2013). Emerging research is now suggesting that the RDA for older adults should be increased to 1.0 to 1.2 g/kg to meet increased sarcopenic needs and any other catabolic changes, like inflammation that can occur with chronic diseases (Bauer et al., 2013). Other research suggests that this amount of protein will better preserve and regain muscle mass losses for the older adult population (Bauer et al., 2013). Therefore, promoting protein-rich foods instead of supplements should become a higher priority when implementing nutrition interventions for older patients. If a more accurate and evidence-based RDA is established, then older adults could receive better nutritional care suitable to meet their aged

metabolic and pathophysiological requirements (Chernoff, 2014; Bauer et al., 2013). Hence an Evidence Analysis Library (EAL) project is essential to determine if there is a correlation between dietary protein intake and the progression of sarcopenia.

The evidence-based conclusions drawn from this EAL project could help dietitians and other health professionals establish more accurate nutrition recommendations to determine the next steps in providing age appropriate nutritional care for this population. If the final consensus validates dietary protein intake is associated with the progression of sarcopenia, then this may create a better understanding of how to protect middle aged individuals against sarcopenia in the future. Evaluating the current literature, will help dietitians gain more background knowledge on sarcopenia and its association with dietary protein. As a result, more empirical evidence can be formulated on whether this is an effective nutrition intervention for older adults. In general there is a need for stronger evidence on sarcopenia, especially on what the most effective preventive strategies are to ensure the highest quality of care is prescribed. The nutrition intervention domains of food and/or nutrient delivery, nutrition education, and coordination of care could all be impacted by this EAL conclusion as well as dietetic practice.

Research Question

The original research question used for this EAL project was, "Does dietary protein improve age-related lean muscle mass losses in older adults to prevent sarcopenic affects?" The question was later modified to encompass all physiological changes that encompass sarcopenia (muscle composition, strength, and functionality) to better fit within the scope of evidence-based practice. The newly refined question is, "Does dietary protein intake, greater than the RDA, delay the consequences of sarcopenia in older adults?"

Subproblems

The secondary outcome will be to help establish a more predominant consensus on whether increased dietary protein can be an appropriate nutrition intervention to offset the consequences of sarcopenia. Gathering valid and reliable scientific evidence to answer this question has the potential to fill in the current research gaps that predominantly surround this complex condition.

Limitations

The main limitation for this EAL project may be the ability to generalize the results considering this is restricted by study populations within the relevant studies chosen for analysis. After reviewing the primary articles, there may still not be enough supportive evidence to encourage the dietetic community to modify the RDA for protein. This could be due to the lack studies that depict a cause-and-effect relationship between protein intake and the characteristics of sarcopenia. Despite the fact sarcopenia starts to develop around the age of 30 years old, the primary focus will be on dietary protein intakes of older adults. Therefore, it may be difficult to establish if and when sarcopenia can be delayed or prevented since all older adult probably already have moderate to severe muscle losses. In regards to study design, if the studies are well-controlled and free of bias, this will help develop a stronger overall conclusion statement.

Delimitations

Inclusionary delimitations have been set to increase the likelihood of formulating a stronger and more relevant project. In order for a study to be included for critical analysis, it must be published after 2008, peer-reviewed, include subjects $\geq$ 50 years old at any health status, involve minimum measurements of lean body mass and protein intake (observational or as an

intervention), and in English. Some exclusionary delimitations consist of studies that only evaluate frailty and weight status without considering body composition measurements and involve any form of protein supplementation (observationally or as an intervention). Also studies that incorporate a meta-analysis or review study design and are animal-based will be further excluded. As a whole, this topic may interest other researchers to conduct more studies emphasizing protein-rich food interventions and how this affects the overall well-being of elders. The understanding of sarcopenia on a pathophysiological level may not be any clearer, but this may help to develop more effective interventions to delay this aging process. Since older adults are more susceptible to sarcopenia and current interventions are generally limited, it is essential this EAL project primarily focuses on prolonging sarcopenia for this target population before working on preventative interventions for younger adults. Overall, this will allow dietitians to better understand the impact natural food sources can have on the aging body and how important protein is to an older adult's livelihood.

Assumptions

This proposed project assumes that the analyzed findings are accurate and truthful in order to reach a valid conclusion. Additionally, it is implied that all study methodologies are measured consistently amongst all study participants and self-reported data is honestly obtained to maintain study reliability.

Definitions:

BIA (bioelectrical impedance analysis): method for determining body fat, fat-free mass, and total body water by measuring flow resistance from a small electrical current passed through the body.

BMI (body mass index): an anthropometric screening tool to determine how much body fat a person had by using measurements of height and weight (formula: weight (kg) / height (m^2)).

CRP (C-reactive protein): a protein released as a response to inflammation.

DXA (dual-energy x-ray absorptiometry): a tool used to measure mineral mass, mineral-free mass, and fat mass by conducting a body scan with radiation photons at two different energy levels.

EAR (estimated allowance required): the median usual intake value that is estimated to meet the requirements of 50% of the population, specific to age group and gender.

FFQ (food frequency questionnaire): an assessment method used to determine usual intakes of different food items and portion sizes from each food group an individual has consumed over the past 6 months to 1 year.

IL-6 (Interleukin-6): an inflammatory marker

NHANES (National Health and Nutrition Examination): a national survey used to obtain health and nutrition information to collect various anthropometric measurements while providing standardized procedures for practitioners and researchers to apply as they use these techniques in health and disease assessment annually.

RDA (recommended daily allowance): is the daily intake level of a nutrient that is considered to be sufficient for a majority (97-98%) of the population, specific to age group and gender.

RDI (recommended daily intake): another term for RDA

TNF-α (Tumor Necrosis Factor-α): a group of cytokines that regulate the immune system and protects cells from viruses.

Abbreviations:

aLM: appendicular skeletal lean mass

BMC: bone mineral content

FM: fat mass

IBW: ideal body weight

CHAPTER 2: REVIEW OF LITERATURE

The age structure of the United States population changed dramatically when the "baby boomer" generation was born from 1946 to 1964; these 78 million children are currently reaching older adulthood (King, Matheson, Svetlana, Shankar, & Broman-Fulks, 2013). With older adults representing the majority of the nation's population and the rate of sarcopenia mounting, the elderly population may experience greater declines health outcomes, reduced functional abilities, and overall poor nutritional statuses that the nation will have to compensate for (Chen, Nelson, Zhao, Cui, & Johnston, 2013; Robinson, Cooper & Sayer, 2012). Hereby, assisting the elderly with nutritional strategies to manage and modify current life-style choices may result in healthier vigorous lives and should be of greater dietetic importance.

Although nutritional supplementation is commonly prescribed to prevent muscle loss in older adults, the most feasible and nutritionally beneficial way may be through diet and natural protein sources. Dietary protein also appears to be the one stimulus continuously overlooked by researchers and yet, there is no final consensus on how much is required to rebuild age-related muscle losses. The purpose of this literature review is to understand the current evidence on whether there is a correlation between dietary protein intake and improved body composition, strength, and/or physical performance to diminish the effects of sarcopenia in elderly individuals. First, background on sarcopenia will be discussed with additional details on the history of sarcopenia, the debated sarcopenia definition for evidence-base practice, the pathophysiology, the underlying consequences of this condition, and possible preventative strategies.

Background

Discovery of Sarcopenia

The first published research study indicating the existence of sarcopenia was in the 1970's by a researcher named Nathan Shock. Shock provided evidence, from decades of published research articles, that older adults experienced age-related muscle changes in physiologic function (Rosenberg, 1997). It was not until 1989, when a researcher named Irwin H. Rosenberg coined the term sarcopenia, which is from the Greek word *sarx* meaning flesh and *peni* for loss that other researchers started to become interested in this aging phenomenon. Rosenberg strongly believed research on sarcopenia should become a necessity since this condition was the most significant and drastic aspect of the aging process discovered at that time, as indicated in Figure 1. Therefore, he hoped this newly defined term would allow future investigators to more appropriately classify patients while having a better understanding of the underlying pathogenic mechanisms involved (Rosenberg, 1997). The original description and definition of sarcopenia was stated in 1993 as, an age-related loss of muscle mass, by researchers Evans and Campbell (Fielding et al., 2011; IWGS, 2009). However, while the phenomenon of age-related skeletal muscle loss is currently well-known, preventative research still remains minimal and unclear for evidence-based practice (Morris & Jacques, 2012).

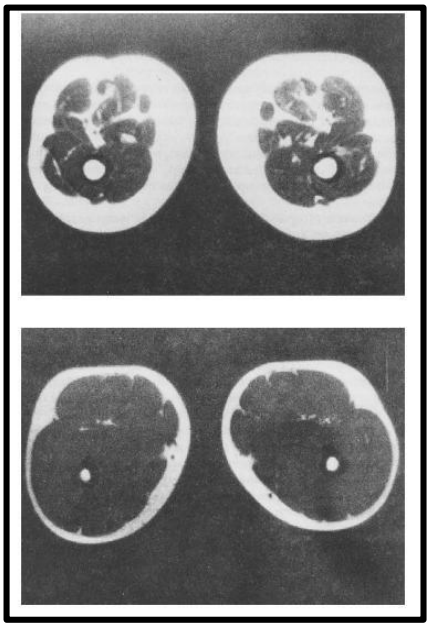

Figure 1: Sarcopenic Thigh Picture. A picture of lean body mass versus fat mass in the thighs of a 20 year old female athlete (upper panel) versus a 64 year old sedentary female (lower panel). The black color within the thigh indicates fat mass while the white color shows lean body mass (Rosenberg, 1997).

Defining Sarcopenia to Establish Research Criteria

Sarcopenia has been commonly associated with functional status, one of the most recognizable clinical indicators of health outcomes for aged individuals, and yet a universal definition to establish reliable diagnostic criteria for research and clinical practice remains elusive. Since sarcopenia was originally defined, it has been broadly defined as an age-related decrease in muscle mass and performance, which allows room for interpretation. As a consequence, a majority of research studies have relied on gender-specific cut-off points based on their own reference population. This has further caused investigators to develop study specific criteria depending on each sample population being evaluated (Beasley, Shikany, & Thomson, 2013).

Between 2009 and 2010, the European Working Group on Sarcopenia in Older People (EWGSOP) defined sarcopenia as, "a syndrome characterized by progressive and generalized loss of skeletal muscle mass and strength with a risk of adverse outcomes such as physical disability, poor quality of life and death" (Cruz-Jentoft et al., 2014). The EWGSOP used low muscle mass and function, indicative of low strength and/or physical performance, as criteria for a sarcopenia diagnosis. The measurement approaches used to clinically diagnose sarcopenia, according to EWGSOP, are illustrated in Table 1. While similarly around the same time, the International Working Group on Sarcopenia (IWGS) decided on a simpler definition of, "age-associated loss of skeletal muscle mass and function" (Cruz-Jentoft et al., 2014). This diagnostic criteria was based on low whole-body or appendicular fat-free mass and poor physical functioning (Cruz-Jentoft et al., 2014).

Comparatively, another review article evaluated multiple research studies that each set gender specific cut-off points and methodologies for assessing muscle mass, strength, and physical performance; three consensus definitions were established. Similar to the definitions developed by EWGSOP and IWGS, two of the studies used definitions that relied on using dual-energy x-ray absorptiometry (DXA) while the third used bioelectrical impedance analysis (BIA), in combination with handgrip strength, as a criteria measure for sarcopenia. However, Beasley et al. (2013) pointed out that DXA may not be suitable for clinical practice since it is expensive, impractical, and involves radiation exposure. On the other hand, BIA plus handgrip strength is more acceptable and easily available, but less precise (Beasley et al., 2013).

Table 1: *Clinical Diagnostic Approaches for Sarcopenia.*

Construct	Measurement Approach	Cut-points for Men	Cut-points for Women	Study Population
Muscle Mass	Bioelectrical Impedance Analysis	**Normal muscle:** > 10.76 kg/m^2	**Normal muscle:** > 6.76 kg/m^2	NHANES III[a]

		Moderate sarcopenia: 8.51-10.75 kg/m^2 **Severe sarcopenia:** < 8.50 kg/m^2	**Moderate sarcopenia:** 5.76-6.75 kg/m^2 **Severe sarcopenia:** < 5.75 kg/m^2	
Muscle Strength	Handgrip Strength	< 30 kg*	< 20 kg*	InCHIANTI[b]
Physical Performance	Gait Speed	< 1m/s 6-m course		Health ABC Study[c]

** For individuals that walk slower than 0.8 m/s (main data source: Beasley et al., 2013)*
Secondary sources: [a] Janssen et al. (2004), [b] Lauretani et al. (2003), and [c] Cesari, Kritchevsky, & Penninx (2005)

Overall, it is evident the definition of sarcopenia remains under national debate and there is no "gold standard" for quantifying muscle mass in clinical trials. For these reasons, the EWGSOP and IWGS have been collaborating to develop standardized methods, terminology, and possible interventions by formulating the International Sarcopenic Initiative (ISI). The overall expectation is, as technology advancements continue and professional work groups collaborate on this specialized area, a definitive definition and criteria will surface.

Prevalence

Some researchers have estimated the prevalence of sarcopenia among older adults will reach 5-13% by year 2050; this is greater than 200 million individuals (Norton & Jakeman, 2013). However determining the prevalence of sarcopenia in the aging population can be extremely difficult due to inconsistencies with current definitions and diagnostic criteria. For example, a study found the prevalence of sarcopenia ranged from 0-45.2% in men and 0-25.8% in women because they had to combine seven definitions of sarcopenia in order to prove how large the variance was (Beasley et al., 2013). Dawson, Taylor, and Favaloro (2013) found sarcopenia will increase in prevalence from 13% to 24% in older adults less than 70 years old and over 50% of those greater than 80 years old after reviewing research on this topic. So far

evidence has shown that those who are older in age, malnourished, and inactive are at greater risk for developing sarcopenia. Sarcopenia also seems to be more prevalent in older adults greater than 70 years old with no major differences between genders. Approximately 50% of older men and 60% of older women have developed this condition (Beasley et al., 2013; Wolfe, 2012).

Pathophysiology

Sarcopenia is one of many pathophysiological changes that biologically occur as a person ages. The aging process is very complex, but some of these metabolic changes that are affiliated with sarcopenia are reductions in sex hormones, mitochondrial dysfunction, and increased inflammation. Some older individuals may additionally develop a neurodegenerative disease that can negatively affect muscle signaling and function, which also can put somebody at greater risk (Beasley et al., 2013). Other researchers believe age-related muscle losses develop from a lower basal metabolic rate of protein synthesis and increased rate of protein breakdown (Phillips, 2012). Endocrine function along with greater muscle disuse, inflammation, nutrient deficiencies, and insulin resistance are also possible aging factors (Phillips, 2012; Lynch, 2011). Inflammation in particular may have detrimental effects on amino acid utilization and/or insulin signaling pathways involved in muscle synthesis after food is consumed (Beasley et al., 2013). Pertaining to insulin resistance, in a study conducted by Aleman-Mateo et al. in 2012, fasting insulin levels were significantly decreased by 10.1% in men after they added ricotta cheese to their habitual diet, compared to a 5.1% increase in insulin levels in men who just consumed their habitual diet (Aleman-Mateo et al., 2012).

Muscle composition also changes with age by decreasing the number and size of muscle fibers required for strength and power, but how much each specific type of muscle fiber diminishes is unknown (Palmio & Udd, 2014; Fielding et al., 2011). Aging muscle is expected to begin around the age of 30 years and continue throughout the life span from exposure to various environmental and genetic components (Palmio & Udd, 2014; WebMD, 2014). Subsequently as an older adult experiences declines in muscle mass, body fatness inadvertently increases, a process termed myosteatosis (Fielding et al., 2011). This initial loss of muscle mass is usually physically displayed in the intrinsic hands and feet muscles (Fielding et al., 2011). It is estimated that the average adult looses about 500 g (equivalent to about 33 pounds) of fat while losing about 250 g (equivalent to about 16 pounds) of muscle mass annually between the ages of 30 and 60 years old. By the age of 70 years old, 27% of total body weight is accounted for by skeletal muscle mass, which reflects a higher portion of body fat (Dawson, Taylor, & Favaloro, 2008). Although sarcopenia may not necessarily be a fact of aging, the underlying pathophysiology has yet to be fully understood. Nonetheless, the fact that not all elderly individuals become sarcopenic is an indicator that this condition may be preventable (Morris & Jacques, 2013).

Consequences

Similar to the predictive risk of bone fractures from osteopenia, sarcopenia can also be a predictor of the frailty development that can cause further immobilization in aged individuals. As a consequence of the drastic muscle depletion and decrease functional capacity, sarcopenia can eventually increase an individual's risk for late-life disability (Fielding et al., 2011). When an elder becomes functionally limited, this can lead to a proliferation in their overall medical expenses resulting in severe financial strains (Fielding et al., 2011). Using previous NHANES

data from 2004, it was calculated that the American Health Care System spends approximately $18.4 billion on sarcopenia due to its strong association with physical disabilities (Morley, 2008). Considering this statistic was formulated over 11 years ago, researchers can only imagine how much money is spent on sarcopenic individuals today. This burdensome lack of mobility also impacts an elder's future health outcomes since these individuals are at greater risk for falls, injuries, inability to perform daily activities, and loss of independence, all which could lead to death (Palmio & Udd, 2014).

Beyond the physical and financial complications of sarcopenia, this can also increase an older adult's risk for dehydration, decreased metabolic rates and lower energy needs, which may result in increased adiposity tissue and glucose intolerance. Considering skeletal muscle is responsible for approximately 80% of glucose uptake, when muscle mass is preserved, the burden of diabetes is lessened. Therefore, it comes to no surprise that all these negative implications of sarcopenia, can ultimately inhibit the immune system from properly defending against the development of chronic diseases or other life-threatening illnesses; the body does not have cellular strength to fight back (Dawson et al., 2008).

Preventative Strategies

Although sarcopenia cannot be completely avoided, many of these pathophysiological changes that can increase an older individual's risk for sarcopenia, particularly inflammation and insulin resistance, may be reduced by modifiable factors of exercise and diet (Beasley et al., 2013). Physical activity and diet, the two most commonly linked risk factors of sarcopenia, have been evaluated in intervention trials to analyze whether these may help delay and/or prevent the progression of sarcopenia. However, as of right now, there is no data available indicating

whether or not sarcopenia can be reversed since the current theory may depend more on the phenotype of the individual. Nonetheless, prolonging the severity of sarcopenia through diet and physical activity shows some promise and may lead investigators towards evaluating the role between diet, particularly increased dietary protein, and minimizing functional immobility through exercise (Beasley et al., 2013). While beyond the scope of this review, there is scientific evidence supporting the effectiveness of implementing physical activity, especially resistance training, as a therapeutic technique for sarcopenia in the elderly population (Beasley et al., 2013).

Protein intake is of particular interest because adequate dietary protein has been shown to prevent and delay the progression of lean muscle mass losses, which may account for approximately $\geq$ 25% of weight loss in older adults (Gray-McDonald et al., 2014). Low protein intake has been shown to have many negative health consequences such as compromising daily functioning and impaired immune system, like those affiliated with sarcopenia (Dawson et al., 2008). However, the amount of daily protein required to offset the various pathophysiological changes associated with aging was not considered when the current RDA for protein was established. The current RDA for protein of 0.8 g/kg/day was estimated in 1985 based on nitrogen balance studies involving healthy younger males (Beasley et al., 2013; Rand, Pellett, & Young, 2004). The issue with this RDA recommendation is recently nitrogen balance has been considered an insensitive tool for defining protein requirements since there is no definitive clinical endpoint (Beasley et al., 2013). Additionally, estimating a generalized protein requirement based on younger males may not be appropriate for women and especially older adults.

Now emerging evidence suggests the daily protein requirement for older adults may be more accurate when increased to approximately 1-1.5 g/kg/day of protein, almost double that of

the current RDA (Paddon-Jones & Leidy, 2014; Cermak, Res, CPGM de Groot, Saris, & Loon, 2012; Dawson et al. 2008; Morley, 2008; Wolfe, Miller, & Miller, 2008). More researchers are supporting this new RDA for protein since this amount is believed to provide older adults with optimal health and possibly protect again sarcopenia muscle losses. On the other hand, this still has not received enough universal support for it to be implemented into medical practice (Beasley et al., 2013; Padden-Jones & Rasmussen, 2009). Nevertheless, even though more researchers are expressing the need to increase the current RDA, it can be a major challenge for older adults to get in enough protein on a daily basis. Some of most recognized reasons are lack of choice, poor appetite, early satiety, poor dexterity, poor dentition, and lack of awareness for higher quality diet (Dawson et al., 2008). Therefore, it is pertinent to examine what the current scientific literature is inferring about this association between dietary protein and sarcopenia advancements. This collaborative information will help justify whether or not this newly proposed RDA for protein should be considered and can be easily implemented for evidence-based practice.

Protein Supplementation

Animal and plant-based proteins are high-quality sources of essential amino acids, but it can be difficult for elders to consume due to limited dentition, high cost, and reduced mobility to go shopping or cooking. In some instances, protein supplementation offers the most readily available form of nutrients to help improve an elder's nutritional status. Therefore, protein supplementation has been proposed as a routinely effective dietary strategy to combat skeletal muscle mass losses and enhance physical performance (Casperson, Sheffield-Moore, Hewlings, & Padden-Jones, 2012; Tieland & van de Rest et al., 2012). There are many different types of

protein supplements that older adults generally consume with the most common types being soy protein- and milk-based formulas. Shahar et al. in 2013 conducted a quasi-experimental, community-based intervention study with elderly sarcopenic Malayan individuals to test if a soy protein-based formula, combined with exercise, was effective in positively intervening with body composition, functional fitness, and oxidative stress. Sixty-five participants were placed into a control group, exercise group, protein supplementation group, or an exercise with protein supplementation group. After a 12-week intervention, the protein supplement group had the greatest impact in body weight reduction, fat-free mass increases, and better upper body strength. However, muscle strength and body composition improvements were more prevalent in the exercise group (Shahar et al., 2013). In contrast, Dawson, Taylor, and Favaloro (2008) reported soy protein resulted in lower nitrogen retention compared to milk protein, which may inhibit protein synthesis required for building muscle. Therefore, soy-based protein formulas may have potential flaws, but may be the most beneficial when this supplementation is paired with exercise.

Milk-based formulas like casein and whey, are considered the highest quality proteins. Whey protein in particular may support muscle preservation better than soy-based protein in the older adult population (Phillips, Tang, & Moore, 2009). Whey protein is considered an excellent protein source for many older adults since its amino acid profile is similar to human milk and stimulates greater muscle protein synthesis than essential amino acids alone (Dawson et al., 2008). However, casein has been shown to cause clots in the digestive system and may result in a slower amino acid release (Dawson et al., 2008). Chale et al. (2013) objectively measured whole-body lean mass, mid-thigh muscle cross-sectional area, muscle strength, and stair-climbing performance in 80 disabled older adults. The purpose was to measure whether 6 months of

resistance training would produce greater overall effects when combined with a whey protein concentrate supplement instead of an isocaloric controlled diet. Although the whey protein concentration supplement group had similar outcomes in muscle strength, compared to the isocaloric diet group, this group produced greater total lean body mass increases and in the mid-thigh muscle cross sectional area. Nevertheless, there were no significant differences between the mobility-limited older adults who consumed 40 g/day of a whey protein concentrate supplement and those who consumed an isocaloric diet. From the provided evidence, milk-based formula may have negative health consequences in older adults. However, it appears while whey-based formulas may have promise in helping to increase lean muscle mass, there may not be any greater benefits when compared against a high protein diet.

In comparison, a study in the Netherlands found no differences in skeletal muscle mass or muscle fibers between the milk-based protein supplement group (equivalent to 15 g of protein per day) and the placebo group after 24-weeks. Muscle strength and physical performance though were significantly different between the protein supplement group versus the placebo group in frail elderly individuals (Tieland & van de Rest et al., 2012). Thus, whey protein and other milk-based protein supplements may provide some additional benefits in helping sarcopenia outcomes, but not all muscle characteristics may improve (Chale et al., 2013).

In addition, the amino acid leucine has become of interest for researchers because of its anabolic effects on muscle mass (Dawson et al., 2008). Casperson et al. (2012) found after two weeks of supplementing three meals with 4 g of leucine each day, participants had significant improvements in muscle protein synthesis. Hereby leucine allowed meal-derived proteins to be more metabolically available to help the aging body with muscle tissue growth and repair. However, the long term effects of whether this intervention improves outcomes of muscle mass

and strength is uncertain. Although it is evident that protein supplementation has many positive outcomes in protecting against an older adult's risk for developing sarcopenia, older adults often report increased satiety, poor palatability, low compliance, and greater financial burdens (Casperson et al., 2012). Therefore taking the more natural dietary protein route to increase an elder's daily protein consumption may produce positive outcomes to counteract sarcopenia, without these negative perceptions relevant to supplements.

Overall, every type of protein supplement discussed appeared to have its flaws and mixed results depending on the study. Evidently more research is required to ensure no adverse health effects become present so each can be deemed as safe for the elderly population. It is important additional research figures out the appropriate quantities and what pathophysiological effects occur within the body to determine which ones are most effective in delaying sarcopenia. As more empirical evidence exists, stronger assumptions can be made on the impact protein supplementation can have on improved health outcomes relative to this syndrome, whether it be soy-based, casein-based, whey-based, or a specific amino acid like leucine.

Dietary Protein Intake

Several studies have evaluated the association between dietary protein and sarcopenia to determine if an increased protein intake is indicative of better health outcomes for older adults by assessing muscle mass, strength, and/or functional capabilities. However, prior to comparing protein intake and prevalence of sarcopenic risk, an informative cross-sectional study conducted by Tieland, Borgonjen, & Van den Berg (2012), evaluated protein intake characteristics amongst a diverse group of older adults. Daily protein intake, protein intake distribution, and the specific types of protein sources consumed were specifically investigated by these researchers. The three

main goals were: 1) determine where dietary protein inadequacies existed, 2) define a more effective dietary intervention to postpone or prevent sarcopenia, and 3) identify if community-dwelling, frail, or institutionalized older adults were at greatest risk for protein insufficiency (Tieland, Borgonjen, & Van den Berg, 2012).

The study population comprised 707 community-dwelling, 194 frail independent, and 276 institutionalized older adults from four previous studies; the institutionalized individuals were a combination of two prior studies. Energy and protein intakes (including total protein intake (g/day), protein intake per kilogram of body weight (g/kg-bw/day), and percentage of energy from protein were calculated from dietary recalls. The cut-point method for inadequate protein consumption was considered below the estimated average requirement (EAR) for protein of 0.7g/kg-bw/day. The lowest average protein intakes were amongst the institutionalized elders (56 $\pm$ 17 g/day for men and 55 $\pm$ 15 g/day for women), whereas community-dwelling older men consumed the most protein with a mean intake of 85.9 $\pm$ 23.9 g/day. The amount of protein, expressed as g/kg-bw/day, was representative of 0.8 $\pm$ 0.3 g/kg-bw/day in the institutionalized elders, 1.0 $\pm$ 0.3 g/kg-bw/day in the independently frail individuals, and 1.1 $\pm$ 0.3 g/kg-bw/day in the community-dwelling older adults. Overall, the institutionalized elders consumed significantly less protein ($p < 0.0001$) compared to the community-dwelling elders with a majority of the total sample population consuming above the EAR. On average for protein distribution (reported at breakfast, lunch, dinner, and in between mealtime snacks), the institutionalized individuals consumed the most protein at breakfast and dinner compared to the frail and community-dwelling older adults. Community-dwelling elders consumed the most during lunch time. A majority of the protein sources consumed came from animal sources (65%), such as dairy and meat products, with no difference in source preferences between genders (Tieland et al., 2012).

The researchers concluded that overall the community-dwelling and frail elders consumed above the RDA of 0.8 g/kg-bw/day for protein, whereas institutionalized elders consumed significantly below this recommended amount. Therefore, institutionalized older adults were considered at highest risk for inadequate protein intake and should be targeted for dietary interventions to possibly prevent or prolong sarcopenia. Also dietary protein was lowest at breakfast so this may be the mealtime to focus on, along with equal protein distribution, throughout the day when trying to increase an elder's daily total protein intake. However, a weakness of this current study was it comprised of four previous studies that each included different dietary recall methods and food composition tables (Tieland et al., 2012). This study also illustrated dietary protein intake of older adults at one time period so it may not be representative of usual intakes. The major limitation was the dietary assessments were not compared against body composition measures to determine if protein intake was actually associated with sarcopenia risk.

However while components of body composition (i.e. muscle mass, strength, and/or functional status) were not compared against protein intake characteristics in this study design, the results provided detailed information on overall protein consumption within a diverse aging population. Ultimately, in order to figure out an appropriate dietary intervention for sarcopenia, it is important to also understand how a variety of elderly population groups differentiate in their dietary protein intakes (Tieland et al., 2012).

Another study also evaluated protein distribution and intake, but focused more specifically on frail old adults. Taking into account the strong pathophysiological overlap between frailty and sarcopenia, investigating how protein intake affects frailty could create a more in-depth perspective into whether protein could be a modifiable risk factor for these

geriatric health concerns. Therefore, to preserve muscle mass and limit an older adult's risk for disability, Bollwein et al. (2013) conducted a cross-sectional study to evaluate if there was a correlation between protein intake distribution and the total amount consumed in frail elders. Frailty was assessed using five criteria of weight loss, exhaustion, low grip strength, low walking speed, and low physical activity. Based on these criteria, subjects were categorized into three groups as followed: frail ($\geq$ 3 positive criteria), pre-frail (1 to 2 positive criteria), and non-frail (no positive criteria). Subjects also underwent a nutrition assessment using a food frequency questionnaire (FFQ) to identify main protein sources, portion sizes, amount of protein consumed, frequency of protein consumption, and usual times (morning, noon, evening) protein was ingested. After analysis, subjects were separated into four protein quartile intake groups based on g/kg BW with the first quartile representing the lowest intake category (Bollwein et al., 2013).

Out of 194 subjects (68 male and 127 female), 40.5% were categorized as pre-frail while 15.4% were considered frail. There were no significant differences between total energy intakes between the frail, pre-frail, and non-frail elders. The average daily protein intake for the total sample population was 77.5 g, 1.07 g/kg-BW, and 15.9 % of total energy intake. There was no trend between protein intake and higher frailty status. However after a multinomial logistic regression model was calculated, the higher protein intake quartiles had a significant p-trend for low physical activity ($p < 0.021$). Protein was mostly consumed at noon (60.2%), followed by about 25% consumed in the evening, and 15.3% in the morning. As frailty levels increased, the percentage of protein consumed in the morning decreased significantly, but ended up increasing at noon. The mean coefficient of variances (CV) of protein intake differed significantly between the frail (0.77%), pre-frail (0.74%), and non-frail elders (0.68%); the more uneven the protein distribution was, the higher the individual CV was for protein intake. Subjects with a low

walking speed and exhaustion had significantly higher CVs ($p < 0.05$) compared to those without these disabilities (Bollwein et al., 2013).

In conclusion, the researchers found no differences between the amount of protein intake and the three frailty groups with most consuming above the RDA for protein (0.8 g/kg-BW/day). It was apparent frail subjects had more uneven daily protein distribution than the pre-frail and non-frail elders. However while the risk of frailty was not reduced with greater protein consumption, there was an association between protein intake and walking speed and hand grip strength (Bollwein et al., 2013).

There were many study limitations such as possible underreporting from the dietary assessment, protein intake distribution data was only from the main meals (breakfast, lunch, and dinner), and no direct cause-and-effect relationship could be established from within this study design. Furthermore, protein quality was not evaluated to determine if this influenced frailty and the only measurement tool used in relation to sarcopenia was hand grip strength; this data was not even shown. Although the statistical power was limited from a smaller sample size of frail elders, significant associations were still disclosed. In contrast, this study was the first to examine whether there was a link between protein distribution and incidence of frailty (Bollwein et al., 2013). Regardless of these study results, the effect of protein intake on functional and clinical outcomes is still limited so more studies are required to determine the true impact between protein intake and aging (Bollwein et al., 2013).

Since the primary cause of frailty could be sarcopenia, and low protein intake has been associated with a loss of muscle mass, there could be an inverse relationship between protein consumption and frailty status. Research has also shown that a specific mixture of essential amino acids may enhance muscle protein synthesis and improve physical function so these could

be influential to frailty as well (Fukagawa, 2013). Therefore, to help fill in some of the research gaps from the previous study, another recent cross-sectional multicenter study examined the association of protein and amino acid intakes among frail elderly Japanese women. Participants were grandmother's (n = 2108) of freshman dietetic students from 85 universities, colleges, and technical schools (Kobayashi et al., 2013).

The grandmothers were assessed on their dietary habits for over a month using a self-administered questionnaire. Animal protein sources were considered as fish, shellfish, meat, eggs, and dairy products. Plant protein sources included protein from cereals, pulses (a plant from the legume family), potatoes, confectionaries, fruits, vegetables, alcoholic beverages, and non-alcoholic beverages. Intakes of leucine, isoleucine, valine, methionine, cysteine, branched chain amino acids (sum of leucine, valine, methionine, cysteine, and valine), and sulfur amino acids (sum of methionine and cysteine) were also analyzed. Although participants were asked about protein supplementation on the questionnaire, this data was not analyzed due to high composition variability. Slowness and weakness, exhaustion, low physical activity, and unintentional weight loss were the four components used to evaluate frailty status; if a participant scored more than or equal to 3, they were considered frail. Furthermore participants self-reported their physical activity by recording the duration of four activities (walking, bicycling, standing, running, and high-intensity activities) along with sleeping and sitting hours. This information was then used to calculate the average metabolic equivalent-hours (MET) for study analysis purposes. Within the diet history questionnaire, participants were also asked to record their current weight status so this could be compared to their weight from the previous year to determine weight loss. Once data was gathered, subjects were divided into quintiles according to how much of each nutrient was consumed (Kobayashi et al., 2013).

The results inferred that 481 (22.8%) women were classified as frail with a mean age of 74.7 $\pm$ 5 years and BMI of 22.7 $\pm$ 3.2 kg/m^2. The mean total protein consumed was 74 g/day, 43.5 g/day for animal protein, and 30.5 g/day for plant protein. Frail participants tended to consume fewer dietary supplements and significantly less total protein and amino acids compared to those in the non-frail group. Therefore, total protein intake was inversely associated with frailty. In addition, this same association was found for animal and plant-based proteins even after a multivariate adjustment ($p \leq 0.002$). Total protein intake comprised mainly of fish and shellfish (30%), cereals (18%), and meat (14%) sources. Similarly, higher consumption of all the amino acids were also correlated to a lower prevalence of frailty in the elderly Japanese women (p trend of ≤ 0.006) (Kobayashi et al., 2013).

In conclusion, Kobayashi et al. (2013) stated this was the first study that showed the connection between protein and amino acid intake with prevalence of frailty in a large cohort of older women. Protein intake was deemed as the strongest predictor of frailty prevalence, surpassing the association discovered with any of the individual amino acids. A strength was the researchers tried to minimize the effect of reverse causality by excluding older women with diseases that had a protein intake restriction and any disabilities like Parkinson's disease. Overall, this study provided an unique perspective into the inverse relationship between protein intake and frailty development. Additionally, different types of protein sources and amino acids were specifically evaluated to determine which ones may be associated with frailty status (Kobayashi et al., 2013).

A major weakness was not being able to determine the appropriate amount of protein required to prevent frailty, but it was suggested that the required levels for protein may be higher than the current recommendations. Also the strong association found between protein intake and

frailty might have been due to the added affects of the various amino acids since the amino acids were not calculated into total protein intake values. However, in Japan it is uncommon to consume supplements with high amounts of protein so an influence on supplementation was probably minimal. Another limitation was the most common frailty criteria was not used, which is based on five characteristics of unintentional weight loss greater than 10 pounds over the past year, self-reported exhaustion, weakness utilizing handgrip strength, slow walking speed, and low physical activity (Fried et al., 2001). Instead this study used an equally appropriate method proposed by Woods et al. (2005), which defined frailty status as physical functioning at the 25th percentile, which is strongly associated with poor grip strength and low walking speed (Woods et al., 2005). Lastly, grandmothers of dietetic students may not be representative of the general older population since these individuals tend to be of a higher economic status and have better dietary intakes. Therefore, these results may not be generalizable for other older Japanese women since the recruitment process may have formulated some selection bias. The rate of grandmother responses was low and therefore may have favored those with healthier dietary habits. In the end, no cause and effect relationship was established due to the cross-sectional study design. Nevertheless, this study signifies the importance of dietary protein while providing further background information on the specific types of high protein sources and amino acids that frail individuals may be lacking in. Furthermore, when comparing frail individuals against healthy older adults, this could help figure out which dietary nutrients may have the greatest impact in delaying or inhibiting frailty development, which could stem from sarcopenia advancements (Kobayashi et al., 2013).

From these three studies it is evident analyzing protein intake can be highly variable when it comes to total protein intake, protein distribution, and physical activity outcomes of

older adults. Collaboratively, each study focused on a slightly different older adult population, but each evaluated some level of frailty. The main outcome on whether protein intake increased an elder's risk for frailty was mixed. Bollwein et al. (2013) found that frailty risk was not reduced from a higher protein intake whereas, Kobayashi et al. (2013) made the opposite conclusion that a higher protein intake could decrease the prevalence of frailty in older adults. Also Tieland et al. (2012) and Bollwein et al. (2013) both evaluated protein distribution and found similar associations that an unequal protein distribution could play into an elder's frailty risk. In addition, Tieland et al. (2012) and Kobayashi et al. (2013) examined which protein sources made up the majority of an elder's total protein consumption. Tieland et al. (2012) found dairy and meat products were the most consumed in their study population while Kobayashi et al. (2013) found fish, shellfish, and cereal to be the majority of their frail older adult's diet; however, these types of foods could be subjective to each of the study's geographical areas. Furthermore Bollwein et al. (2013), the only study to evaluate muscle strength and physical activity against protein intakes, found a positive association between dietary protein, walking speed, and hand grip strength. Due to a lack of research study comparison, future studies need to be conducted with sarcopenic elders and these variables. Future research should also analyze the key elements of protein intake (i.e. overall intake, protein distribution, level of physical activity, types of protein sources consumed, etc.) that are different between healthy and sarcopenic individuals to accurately establish what puts an elder at higher risk nutritionally.

Dietary Protein with Emphasis on Muscle Mass

Gaining more insight into the dietary protein consumption of frail and sarcopenic individuals will help provide more empirical evidence towards a more appropriate dietary

intervention. However, how inadequate protein consumption could potentially impact an older adult's overall quality of life requires more informative research. Morris and Jacques (2013) used protein consumption data, from a nationally representative sample of Americans, to address the question: does dietary protein enhance the benefits when combined with exercise and what are the effects of protein intake on lean muscle mass?

The purpose of this cross-sectional study was to evaluate reported protein intake data, from 2003-2006 National Health and Nutrition Examination Survey (NHANES), among participants 50 years and older. The main outcome was to determine if any correlations existed between total protein intake, animal protein intake, and physical activity relative to muscle mass composition. A total of 2,425 individuals with a mean age of 63 years were reported in this study. Whole body DXA scan data was available for participants in the 2003-2004 survey (n = 1,639), but not from those participating in the 2005-2006 survey (Morris & Jacques, 2013).

The results showed beef consumption was significantly correlated to total protein intakes in all free-living participants ($r = 0.19$; $p < 0.001$). There was a positive correlation found in the appendicular skeletal muscle mass (aLM) of non-obese subjects who performed vigorous aerobic physical activity. Also an increase in aLM was significantly related to higher beef intakes and was exceptionally strong and significant in non-obese subjects who participated in muscle-strengthening exercise. In comparison, obese subjects who consumed < 70 g/day of protein and participated in muscle-strength exercises had a significantly lower aLM than physically inactive individuals ($p = 0.013$). Adjustments were made for total caloric intake when protein intake was analyzed against aLM index measures of obese and non-obese subjects. As a result, obese, physically active subjects had a stronger association between total protein intake and aLM when compared to physically active non-obese subjects ($P_{interaction} = 0.07$ for vigorous physical activity

and $P_{interaction} = 0.049$ for muscle-strength training). For protein recommendations, it did not matter whether a non-obese individual met the RDA for protein or not as long as they performed vigorous aerobic activities. Conversely, for obese subjects, muscle-strength training seemed to be more effective in improving aLM, but only if the RDA for protein was met or exceeded (Morris & Jacques, 2013).

All in all, the authors concluded that higher intakes of good quality protein sources, like beef, may be helpful when trying to increase aLM in older individuals whether obese or not. Exceeding the RDA for protein was only beneficial in obese subjects whereas, non-obese individuals showed no added benefits when exceeding this recommended amount. These findings from this present study recapped that non-obese subjects only had skeletal muscle enhancements when meeting the RDA for protein. This was especially true when non-obese subjects combined adequate protein intake with muscle-strength exercises. Aerobic activity was also helpful in preserving aLM, but a diet in high quality protein should be added to see additional benefits (Morris & Jacques, 2013).

Study limitations included using a cross-sectional study design to illustrate one time period and the lack of DXA measurements for aLM for all study participants. Furthermore since physical activity measures were based on observations and this study design did not involve a formal exercise program, subjects could have reported inaccurate results that unintentionally swayed the study results. There was no indication of whether these subjects were sarcopenic or not so these results were restricted to the information provided by the NHANES surveys. This could have minimized the strength of evidence relative to this research question since the prevalence of sarcopenia was not part of the inclusion criteria. A major strength of this study was the use of a large nationally representative sample population and that adjustments were included

to control for total caloric intake when analyzing protein consumption. This allowed investigators to assess usual intakes of specific high-protein foods and detect significant relationships between strength and physical activity, dietary protein, and beef intakes (Morris & Jacques, 2012). Morris and Jacques (2012) were also able to identify the types of most frequent physical activities older adults participated to determine which activities can be easily attainable for this population.

As a continuation of addressing some of these knowledge gaps between protein intake and body composition changes, a nested, prospective, case control study conducted by Gray-Donald et al. (2014), assessed the relationship between protein intake and the rate of one year incidence of $\geq$ 5% weight loss, in community-dwelling healthy older adults for 2 years. All participants were recruited from a previous cohort nutrition study called NuAge and matched by sex and age categories (70 $\pm$ 2, 75 $\pm$ 2, and 80 $\pm$ 2 years old) (Gray-Donald et al., 2014).

Healthy, well-functioning participants (n = 422) were selected based on substantial weight loss criteria and separated into cases (n = 211) and control groups (n = 211) depending on the percentage of weight lost in one year. Cases represented subjects who had a $\geq$ 5% weight loss over one year, between baseline and one year (n = 129) or from the one year to the two year follow-up (n = 82). The controls accounted for subjects with a $\leq$ 2% weight loss between baseline and the one year follow-up. Controls and cases were then randomly matched with an eligible weight stable participant (1:1) from the corresponding time period. Protein intakes were categorized as low (< 0.8 g/kg/day), moderate (0.8-1.0 g/kg/day), high (1.0-1.2 g/kg/day), and very high ($\geq$ 1.2 g/kg/day was the reference standard) after three dietary recalls from each participant were analyzed. Since protein intake has been associated with changes in body composition, Gray-McDonald et al. (2014) decided to gather a subsample of 60 participants to

determine if an association existed; these were the only participants with body composition measures at both baseline and two year. Furthermore to determine if chronic inflammation was a modifiable risk factor for unintentional weight loss in older adults, C-reactive protein (CRP) levels were assessed (Gray-Donald et al., 2014).

The sample size was 121 (57.3%) female and 90 male older adults. After controlling for energy intake, protein was the only macronutrient to remain significantly different between the case and control groups. As a result, there was a significantly high correlation between protein intake and weight loss ($p = 0.005$). Respectively, the most significant finding was participants had a 2.56 and 2.15 times greater chance of losing weight in the low and moderate protein intake categories when compared to the high and very high intake categories. These results were prevalent after adjusting for all covariates (energy intake, BMI, smoking, physical activity, physical function, dieting to lose weight, appetite, number of medications, number of chronic diseases, depressive symptoms, and serum albumin concentrations). The average two year loss of lean muscle mass was 1610 $\pm$ 1680 g (1.61 $\pm$ 1.68 kg) for the case group and 1050 $\pm$ 1200 g (1.05 $\pm$ 1.2 kg) for the control group. This indicated a significant difference of 560 g of lean muscle mass ($p = 0.034$). Participants who consumed < 0.8 kcal/kg/day of protein compared to $\geq$ 1.2 kcal/kg/day were also twice as likely to lose weight ($p = 0.018$). While those in the highest protein intake group were 70% less likely to lose weight compared to the highest protein intake group of < 0.8 kcal/kg/day ($p = 0.039$) (Gray-Donald et al., 2014).

Based on these results, the researchers concluded that protein intake may play a protective role against unintentional weight loss since weight status was significantly associated with the amount of protein an elder consumed over just one year. Compared to the highest protein intake group, older adults who consumed moderate amounts of protein (< 1.0 g/kg/day)

were twice as likely to lose weight. Additionally, those who consumed < 0.8 g/kg/day were 2.5 times at greater risk for weight loss compared to the highest protein intake group as well. However, while this study did not emphasize the impact protein intake had on lean muscle mass losses, the chosen subsample (n = 60) lost 0.5 kg more lean mass than the control group at two time points during the study. Individuals who lost ≥ 5% of their body weight over one year consumed 0.1 g/kg less protein per day than those who remained weight stable (Gray-Donald et al., 2014). Considering a significant portion of lean muscle mass was lost due to inadequate protein consumption, the researchers assumed protein intake may effectively guard against weight loss. Accordingly, these researchers supported the notion that protein may play a protective role against weight loss and body composition changes, commonly experienced in sarcopenic individuals (Gray-Donald et al., 2014).

Figuring out if lean mass changes were directly correlated to protein intake quantities was limited to the selected subsample so these results may not be applicable to frail, ill, or other older individuals. Another weakness was inflammation was only shown to have a minimal influence on muscle loss, which indicated the need for more research to determine its level of significance. The strengths included the use of precise and valid measuring tools to obtain measurements of anthropometrics, weight stability markers, diet, and biomarkers while controlling for potential cofounders when protein intake was analyzed. Participants were highly diverse so these results could be applicable to other healthy older adults. As a result, these researchers were supportive of increasing total protein intake to > 1.0 g/kg/day RDA for healthy older individuals since this may help to reduce an older individual's risk of weight loss and lean mass declines (Gray-Donald et al., 2014).

While the previous study found inadequate dietary protein may increase an older adult's risk for greater weight loss, this is only one aspect of sarcopenia. Therefore, a recent cross-sectional study hypothesized that dietary protein and leisure-time physical activity were predictive of an older adult's lean body mass. The overall purpose was to determine if the current RDA (0.8 g/kg/day) for protein was sufficient for this age group. Subjects, whom were greater than 65 years old, were recruited from a previous 12-week intervention resistance training program (Geirsdottir et al., 2013).

A complete statistical analysis was conducted for a total of 237 healthy older adults with a mean age of 73.6 $\pm$ 5.7 years and consisted of 58% older females. On average, participants significantly consumed dietary protein quantities above the RDA of 0.8 g/kg-bw/day ($p < 0.001$) after each three day 24-hour recall was analyzed. Men consumed about 90.3 $\pm$ 26.7 g/day of protein (0.98 g/kg/day) while women consumed 69.6 $\pm$ 19.1 g/day (0.95 $\pm$ 0.29 g/kg/day). As hypothesized by Geirsdottir et al. (2013), the results from the linear model found daily protein intake was considered a positive predictor of lean body mass after controlling for different factors and covarties (sex, protein intake quartiles, BMI categories, age, number of drugs, and physical activity). In regards to physical activity reports, about two-thirds of participants met the current physical activity requirement of 30 minutes/day (Geirsdottir et al., 2013).

As a result, the researchers concluded the amount of dietary protein consumed was positively associated with lean body mass in community-dwelling older adults and may help delay the progression of sarcopenia. This was indicative of the average participant consuming greater than the RDA for protein, which correlated to a higher lean body mass. After separating participants into different protein intake quartile groups, there was a significant difference of 2.3 kg of lean body mass between the fourth (1.36 $\pm$ 1.19 g/kg of protein) and first (0.63 $\pm$ 0.08 g/kg

of protein) quartile with a significant trend towards 2.0 kg among the fourth (1.36 ± 1.19 g/kg of protein) and second (0.85 ± 0.05 g/kg of protein) quartiles. In contrast, there was no correlation between leisure-time physical activity and lean body mass so endurance exercise many not be useful for maintaining lean body mass for community-dwelling older adults (Geirsdottir et al., 2013).

This study was limited since it did not analyze the types of protein sources participants consumed from their dietary assessments even though it was noted that a majority of the protein predominately came from animal sources. Therefore, these investigators questioned the impact of plant proteins had on lean body mass, but this information needed to be further analyzed to determine how each type of protein source affected lean body mass. Another limitation was the use of cross-sectional study design since it did not reflect a cause-and-effect relationship or evaluate prolonged behaviors impacting lean body mass. Nonetheless, this study had strengths of accounting for covariates and variables during statistical analysis, using appropriate inclusion and exclusion criteria, and comparing gender diversity to produce stronger outcomes. Finally, subjects were observed on routine physical activities without implementing an intervention; this provided a more realistic scenario for an older community-dwelling age group (Geirsdottir et al., 2013).

In accordance, emerging evidence has shown there may be a link between dietary protein intake and fat mass and lean mass during intentional weight loss in older adults. This is of high importance since weight declines usually become more prevalent as a person ages. The Health, Aging, and Body Composition (Health ABC) Study was published in 2008 by Houston et al. It was a 3-year prospective cohort study design. The main objective was to address the association between dietary protein consumption and alterations in lean muscle mass and aLM in

community-dwelling older adults, 65 years and older. The secondary aim was to examine the functionality of dietary protein in preserving lean mass in those who were considered at high risk for lean muscle mass losses (Houston et al., 2008).

A total of 2066 healthy, community-dwelling older adults were eligible to participant in this study. Participants in the highest protein quintile groups significantly lost about 43% less LMM and 39% less aLM compared to those in the lowest protein quintile groups ($p < 0.01$ trend). These associations became attenuated after adjusting for fat mass over 3 years. Energy and protein intakes were associated with significant changes in lean muscle mass [β (SE): 8.76 (3.00) to 8.82 (3.01); $p = 0.004$, respectively] and aLM [β (SE): 5.31 (1.64) to 5.26 (1.65); $p = 0.001$, respectively] throughout the 3-year study period. In regards to sex differences, men typically consumed more dietary protein (70.8 g/kg/day) compared to women (60.9 g/kg/day). There were significant associations between total protein and animal protein intakes on lean muscle mass ($p < 0.01$) and aLM ($p < 0.01$). This became evident after adjustments were made for total energy intake, age, sex, race, study site, baseline lean mass or aLM, height, smoking, alcohol use, physical activity, oral steroid use, prevalent disease, and interim hospitalizations. Additionally, the same variables were controlled for when it was determined that protein intake was associated with aLM changes in participants who gained or lost weight, but not for those who were weight stable (Houston et al., 2008).

According to Houston et al. (2008), this was the first longitudinal study to examine the role protein has on body composition characteristics. Within this cohort study, protein intake was associated with significant lean muscle mass changes, even after adjusting for fat mass, and may have affected overall aspects of body composition in community-dwelling older adults. Although there were small lean muscle mass changes over 3-years, the investigators hypothesized that if

this study was prolonged there may be more significant lean muscle mass changes than were currently presented. Overall, low intake of dietary protein may be an adjustable factor in preventing sarcopenic losses in the elderly population (Houston et al., 2008).

A constraint of this study was more animal-based protein sources were consumed compared to plant-based protein foods so this may have minimized the significant association found between vegetable-based proteins and lean muscle mass variations. Another weakness was dietary information was obtained by a single FFQ so this may have impeded the results by not representing the typical diet of older adults over 3-years. The choice of study design also inhibited the researchers from developing a direct cause-and-effect relationship between protein intake and body composition (Houston et al., 2008).

An advantage of this study was protein intake sources were evaluated, in addition to, total protein intake, which is nonexistent in other studies. The inclusion of a large diverse sample of black and white community-dwelling older adults, over a long study period, is an added strength. FFQs are common in cohort studies, but the researchers modified it specifically for the Health ABC study and its demographic making this tool more proficient when the results were analyzed. Other strengths consisted of adjusting for multiple cofounders that were prevalent in this population and the use of trained interviewers to increase reliability and quality of data to ensure less errors were reported (Houston et al., 2008)

Within current sarcopenia research, minimal knowledge exists on total dietary protein intake of older adults, but there is even less collaborative evidence on protein consumption during mealtimes. So far it has been reported that older adults may require 1.0-1.3 g/kg of protein to achieve adequate balance and should consume approximately 25-30 g of protein at each meal (Symons, Sheffield-Moore, Wolfe, & Paddon-Jones, 2009; Houston et al., 2008;

Morais et al., 2006). Prior to the pilot study conducted by Ruiz Valenzuela et al. in 2013, there was only one other study published that involved older adults affected by sarcopenia. Therefore this convinced these investigators to assess the dietary protein intake and distribution during mealtimes while exploring the association with aLM in healthy older adults (Ruiz-Valenzuela et al., 2013).

Participants were split into two groups depending on how much protein was consumed per meal based on three 24-hour diet recalls. Group A consisted of those with an intake < 25 g at each mealtime while Group B comprised of those who consumed > 25 g of protein for at least one meal. A total of 78 non-Caucasian older adults (60% female) with a mean age of 68.7 $\pm$ 6.3 years old were involved in this study. Men consumed significantly more protein than women ($p < 0.05$) with the average daily protein consumption at 0.9 g/kg/day. Based the recommended protein intake distribution per meal, 81% of participants were below the recommended 30 g of protein for breakfast while 86% failed to meet this recommendation for dinner. The amount of protein consumed per meal was significantly associated with the amount of lean muscle mass a participant had. Through this cross-sectional analyses, the researchers observed participants who consumed < 25 g of protein at each meal had significantly less aLM compared to those who ingested > 25 g of protein for at least one mealtime (15.9 $\pm$ 0.9 kg versus 19.1 $\pm$ 0.6 kg, $p < 0.01$) (Ruiz Valenzuela et al., 2013).

The overall conclusion was even though the majority of participants consumed above the RDA of 0.8 g/kg-bw/day of protein, these older adults still failed to achieve the higher levels reported to offset the effects of sarcopenia (1.0-1.3 g/kg/day). The inadequate protein consumption during breakfast and dinner meals was associated with a significant loss of aLM in older adults. With this evidence, the researchers suggested both low protein intake and

inadequate protein distribution may lead to increased muscle mass loss and risk of sarcopenia (Ruiz Valenzuela et al., 2013).

Some weaknesses and limitations were a small sample size, no detailed information on when measurements were reported and taken at certain follow-ups, and pertinent background demographics on ethnicity were unavailable. The nature of this study design did not include an intervention, which would have been useful when comparing those with or without sarcopenia and protein consumption. As a result, this could have reduced the validity of the associations found regarding protein intake quantities since these variables could have affected these outcomes. This would have provided more information on what high-quality protein sources would be the most effective in preserving lean muscle mass in these sarcopenic individuals. On the contrary, the advantages of this pilot study were that it focused on non-Caucasian older adults, in addition to, protein intake distribution at mealtimes. Also potential cofounders of body weight, sex, and height was controlled for when comparing protein intake against aLM changes.

Few longitudinal studies have focused on protein intake and bone and muscle health in post-menopausal women although aging has been associated with a higher risk of falls and fractures and reduced muscle strength. A 5-year prospective cohort randomized controlled cohort trial conducted by Meng et al. (2009) determined if there was an association between dietary protein intake and bone-free lean mass and muscle size at baseline compared to bone mass at 5-years, in community-dwelling older women (Meng et al., 2009).

A total of 862 healthy older women, with a mean age of 75 $\pm$ 3 years old and a mean BMI of 26.8 $\pm$ 4.4 kg/m^2, were randomly placed into two groups: 1) the calcium treatment group, which required a daily intake of 1.2 g calcium carbonate supplement (n = 450), or 2) a matched placebo group (n = 412). Dietary assessments, BMI, and demographic and lifestyle data was

obtained at baseline and at 5-years while upper arm muscle area (UAMA), triceps skin fold, and other body composition measurements (bone-free lean mass, BMC, lean muscle mass, and appendicular BMC) were only obtained at year-5. The analyzed results from the self-administered FFQ found the average total daily protein consumed was 80.6 $\pm$ 27.6 g with 771 (89%) of subjects consuming > 0.75 g/kg/day of protein and 615 (71%) consuming > 0.94 g/kg/day. Women in the highest protein quartile group (> 87 g/day of protein) were significantly higher in weight, BMI, and physical activity levels when compared to the lowest protein quartile group (> 66 g/day of protein). Whole body lean mass had the strongest correlation with protein intake ($r = 0.18$, $p < 0.001$) compared to the other macronutrients. There was also a positive association between protein intake and aLM and BMC ($r = 0.14$-0.18, $p < 0.001$), UAMA ($r = 0.08$, $p < 0.05$), and whole body FM. Those in the highest quartile group had significantly higher whole lean body mass, aLM, and UAMA when compared to the lower protein intake quartile groups even after adjusting for age, height, energy intake, and physical activity levels. After controlling for baseline age, height, energy intake, physical activity, and calcium treatment the strongest correlation was found between whole body BMC and protein intake. There were no associations between the calcium treatment and lean muscle mass, UAMA, and FM when comparatively analyzing these groups (Meng et al. 2009).

This study concluded that a higher protein intake was associated with greater benefits of bone health due to its maintenance and size of lean mass. However, providing a 1.2 g calcium supplement may not have any additional benefits to body composition measures. Women in the highest protein quartile group had significantly higher whole body and appendicular BMC compared to those in the lowest quartile group. This occurred independently from the potential covarties except for lean body mass, which suggests that protein intake may have affected bone

health may be arbitrated by its effect on muscle. The highest protein intake quartile of > 87 g/day (1.6 g/kg/day) was the most beneficial in maintaining lean muscle mass and BMC for women > 70 years old, even after adjusting for energy intake, body size, age, and physical activity levels. Therefore a higher protein intake may have beneficial effects on BMC values since this may be mediated by great lean muscle mass improvements (Meng et al., 2009).

A weakness was no baseline body composition data of UAMA, triceps skin folds, and whole body DXA scans were gathered so Meng et. al (2009) could not determine when high protein intakes had an impact on body composition in these elderly women. This cohort study design could also not establish a cause-and-effect relationship. Nonetheless, a unique strength from this study was other important variables like BMC, UAMA, and triceps skin fold tests, were accounted for within the methodology. Lastly another strength was having a large sample size to help establish a stronger association between protein intake and lean mass and BMC in post-menopausal women (Meng et al., 2009).

These six studies mainly evaluated protein intake against body composition measures of either of lean body mass, aLM, and/or weight loss in community-dwelling, healthy older adults who were primarily Caucasian women. Therefore, future studies need to address a more diverse older adult population ranging from healthy to sarcopenic, community-dwelling to institutionalized, involve more men, and different ethnic groups. Nevertheless, there was a universal consensus that a protein intake above the RDA was positively associated with lean muscle mass and/or aLM changes although Houston et al. (2008) found no aLM changes in weight stable subjects. All of the studies controlled for important potential cofounders. The most commonly adjusted variables were total caloric intake, BMI, smoking, physical activity levels, and age, which meant these variables did not factor into the protein intake results during

analysis. Even though the Gray-Donald et al. (2014) mainly focused on overall weight loss, the selected subsamples produced the same results between these two variables as well. Geirsdottir et al. (2013) was the only study to evaluate leisure-time physical activity and lean body mass changes, but no association was found. Meng et al. (2009) also found positive effects between protein intake and body health so adequate protein intake could play a protective role not only in preserving muscle mass, but bone health in older adults. The results from Geirsdottir et al. (2013) and Meng et al. (2009) do not provide strong enough evidence to establish a reliable conclusion since these associations were only from one study.

Adding the Outcome of Muscle Strength

Using a 3-year prospective cohort study design, Scott, Blizzard, Fell, Giles, and Jones (2010) evaluated if there was a correlation between dietary nutrient intake and the development of sarcopenia in community-dwelling older adults. However, Scott et al. (2010) also analyzed aLM and muscle strength. To evaluate protein intake, qualified participants were stratified into two protein intake groups depending on the FFQ dietary assessment as followed: 1) failing to meet the current RDI for protein and 2) meeting or exceeding the RDI for protein based on the RDIs in Australia. The recommended RDIs for men between the ages of 51-70 years old are 64 g of protein and 81 g of protein for those > 70 years old whereas women, in the same age groups, should consume 46 and 57 g/day. All measurements were obtained at baseline, year 2, and year 3 for data analysis (Scott et al., 2010).

A total of 740 healthy participants (50% female) with a mean age of 62 $\pm$ 7 years old, were included for analysis. At baseline and follow-up, 89 (12%) and 106 (14.2%) failed to meet the RDI for protein. When the FFQs from each participant were compared against the Australian

dietary protein RDI, participants below the RDI for protein had significantly lower aLM at baseline and follow-ups (-0.81 kg, 95% CI (-1.54 to -0.08); p = 0.03 and -0.79 kg, 95% CI (-1.42 to -0.17); p = 0.01, respectively). Utilizing the stepwise regression model, at baseline aLM predicted about 20% of the changes in aLM throughout the 2.6 years. Out of all the macronutrients, protein was the only one to be a significantly independent predictor of aLM changes after total energy intake was controlled for (p = 0.007). This is after whole-body DXA scans were conducted at baseline, year 2, and year 3. Given that energy-adjusted protein was the sole nutrient significantly independently associated with aLM changes, protein intake may be an important modifiable risk factor to improve muscle mass in older adults. Hence, a higher long-term protein intake may reduce age-related muscle mass declines, but statistical analyses showed no difference in muscle strength between the protein intake groups. Other nutrients associated with significantly positive aLM changes were iron, magnesium, phosphorus, and zinc even after protein and other nutrients like magnesium and phosphorous were fixed (Scott et al., 2010).

In summary, protein intake in addition other micronutrients, were positively associated with changes in lean muscle mass and rates in muscle losses in older adults, except for muscle strength. The researchers indicated that diet alone may not be able to offset age-related muscle strength without some form of physical activity, but it is evident diet plays an important role in maintaining muscle function. There could potentially also be many different nutrients that delay the progression of sarcopenia as a person ages. Many of the nutrients positively associated with aLM are within many animal meats, such as iron and zinc, so it is important to consume an adequate amount of high-quality protein to maintain muscle mass (Scott et al., 2010).

However, there were several limitations within the chosen methodology and study design. During recruitment, although the retention rate at follow-up was high (82%), the initial

the response rate was low (57%) and there was a lack of nutrient intake and lean muscle mass differences between participants who completed the studies verses those who did not. Both of these study characteristics may have limited the scope of potential participants included in the study. All the dietary nutrients were reported, but only emphasis was placed on nutrients that had a continuous significant relationship with aLM, which may have limited the presented data. Overall, the results from this sample of Caucasian community-dwelling, healthy older adults between the ages of 70 and 79 years old may not be generalizable for the rest of this population, specifically those with sarcopenia (Scott et al., 2010).

Although this study did not include any older adults with sarcopenia, a strength of this study was these results provided background information on how protein intake was associated with characteristics of sarcopenia (including muscle mass and strength). Therefore, gaining insight into the body composition changes in healthy older adults will help figure out whether protein intake is beneficial before applying this type of intervention on those more vulnerable with sarcopenia. Another strength of this study was that participant's dietary assessments were extensively analyzed beyond protein intake, totaling 28 different nutrients, while maintaining emphasis on those positively associated with aLM changes. Another major strength was this study consisted of a large sample size over a prolonged period of time. Both of these study elements helped develop greater insight into determining how much protein may be associated with the effects of sarcopenia, along with other possible dietary interventions. As a whole, this study constructed a better idea as to the correlation between nutrient intakes and aLM, but how dietary protein influences physical performance was not assessed (Scott et al., 2010).

Gray-Donald et al. (2014) found a minimal influence of inflammation and protein consumption on muscle strength, and Scott et al. (2010) found no association between muscle

strength and protein consumption. More sarcopenia research is prudent on how muscle strength is reflective of dietary protein intake so a study conducted by Bartali et al. (2013) focused on the effectiveness of increased protein intake on muscle strength in the general older adult population. A pro-inflammatory state has been shown to promote greater reductions in muscle strength, muscle wasting, and protein catabolism (Ferrucci et al., 2002; McNurlan & Garlik, 2000). Therefore, a secondary outcome was to investigate how inflammation affected dietary protein utilization. A total of 598 (53% women) participants were recruited from the InCHIANTI Study, which was a study that previously evaluated various risk factors thought to contribute to impairments in mobility for older adults in the Florence, Italy (Bartali et al., 2013).

This retrospective cohort study established dietary intake from a FFQ, muscle strength from a hand-held dynamometer three times to determine the average value, markers of inflammation (CRP, IL-6, and TNF-α) from fasting blood samples, and other potential variables (presence of chronic diseases, smoking habits, physical activity level, and BMI). The timeline for when the background questionnaire and the FFQ data were gathered was unspecified, but all other measurements were recorded at baseline and during the 3-year follow-up.

After the 3-year study period, the results showed no significant associations between protein intake and muscle strength changes after age, sex, BMI, physical activity, energy intake, chronic conditions, smoking, and muscle strength were adjusted for in a linear model at baseline. However, there was a significant difference between protein intake and inflammatory biomarkers (CRP, IL-6, and TNF-α) on muscle strength ($p = 0.003$; $p = 0.050$; $p = 0.019$, respectively). Those with elevated levels of inflammatory markers were more likely to consume less protein and have greater declines in muscle strength. However, these results were not attributable to the presence of chronic conditions so this analysis was repeated from a selective subsample of 188

participants without chronic diseases to see if these individuals had the same results. Muscle strength compared to protein intake and inflammatory markers only changed slightly, except for IL-6; these results could have occurred from the lack of statistical power within the adjusted general linear models. The interaction between protein intake and inflammatory markers on muscle strength were not dependent upon a participant's muscle strength at baseline (Bartali et al., 2013).

In conclusion, there was a significant association between protein intake and inflammatory markers in regards to muscle strength at the 3-year follow-up. Participants with higher amounts of inflammation were also discovered to consume lower amounts of protein, which was a negative indicator of muscle strength. Overall, Bartali et al. (2013) suggested that older adults with higher inflammation may experience more insufficient protein metabolism and impaired protein utilization, but to confirm this assumption more research has to be conducted specifically evaluating metabolic changes. With this in mind, older adults with chronic diseases may require more protein than the average healthy older adult to counteract protein catabolism. Overall, these results may help determine the etiology as to why aging muscle eventually weakens so that a well established dietary intervention can be formulated to prolong the consequences of sarcopenia (Bartali et al., 2013).

According to Bartali et al. (2013), this was the first study to analyze the impact of inflammation may have on protein synthesis and muscle strength in independent older adults greater than 65 years old. Consequently another strength was that muscle strength was measured objectively to increase testing reliability. Additionally, statistical adjustments were made for total energy intake to ensure a lower protein intake was not affiliated simply from a loss of appetite. These adjustments were also incorporated to independently compare protein intake and muscle

strength separate from other intake energy sources. In contrast, a weakness was the older more sedentary participants, with a higher prevalence of chronic diseases and inflammatory markers, were generally excluded when compared to participants who completed this study. However, high attribution rates were expected with this type of population. Another weakness was that protein intake was obtained from a FFQ, which could have developed report bias and underreporting. For research purposes, the foremost limitation of this study was that muscle strength was the only measure compared to protein intake, without involving measurements of muscle loss or efficiency. Furthermore, the RDA for protein was not utilized as a reference point against the participant's current protein intake. Therefore, it was unclear how these participant's protein intakes compared to the RDA, in regards to, muscle strength (Bartali et al., 2013).

Sarcopenia has been an important contributor for declines in muscle strength and functional capacity in older adults so it is pertinent to evaluate other studies that focus on this association. Therefore, to help clarify this relationship between protein intake, muscle mass, and physical performance further, a cross-sectional study conducted by Gregorio et al. in 2013 evaluated the association between these variables in community-dwelling, post-menopausal women. It was hypothesized that if an older woman consumed more protein, this would result in better physical function outcomes compared to lower protein consumers. Subjects were stratified into low (< 0.8 g/kg/day) and high (> 0.8 g/kg/day) protein intakes groups depending on their dietary assessments (Gregorio et al., 2013).

Eligible subjects (n = 387) involved within this study had a mean age of 72.7 $\pm$ 7.0 years and were 95.5% Caucasian. On average, subjects consumed 1.1 g/kg/day of protein and 72.2 g of protein/day with 25% (97) of subjects consumed less than the RDA whereas, 75% (290) consumed above the RDA. Subjects in the low protein intake group had more incidence of

hypertension, osteoarthritis, and bone fractures compared to the high protein intake group (46% versus 36%, $p = 0.050$; 40% versus 20%, $p = 0.001$; 48.9% versus 35%, $p = 0.014$; respectively). Women in the high protein intake group also had lower BMIs, body weight, FM and lean muscle mass, and incidence of bone fractures compared to the low protein intake group. For physical fitness, women who consumed more protein performed better on a majority of the physical performance tests, but muscle strength was similar between groups. To ensure these results were not influenced by the differences in BMI between the protein intake groups, an analysis of covariance was conducted to control for this variable. Additionally, a linear regression analyses was conducted for the physical functioning tests, but no variables were controlled for. When compared against the low protein intake group, the high protein group scored significantly higher on the Physical Performance Test (PPT), Short Physical Performance Battery (SPPB), and chair rise time. Specifically, women who consumed low amounts of protein scored inferior in the single leg stance ($p < 0.002$) and walking speed tests ($p = 0.006$) (Gregorio et al., 2013).

In conclusion, the older women who consumed less protein had significantly more bone fractures, which suggested physical performance and muscular health may be an outcome of adequate protein consumption. However, while there was no association found between lean muscle mass and protein intake, there was a correlation between a higher dietary protein intake and improved walking speed and single leg stance time. Similarly, muscle strength did not differ between high and low protein intake groups so protein intake may not be a predictive factor for this particular outcome. Dietary protein intake may be a modifiable risk factor towards ensuring better health outcomes of functional status and bone composition in older post-menopausal females for delaying sarcopenia (Gregorio et al., 2013).

The primary limitation was that subjects were grouped into two broad protein intake categories, high and low, making it difficult to determine what the optimal amount of protein is required for healthy, elderly females to prevent sarcopenia and improve physical performance. Also total caloric intake and physical activity were not controlled for when protein intake was analyzed, which could have reduced the validity of these results and the conclusions drawn from this study. This may have caused muscle strength to be indifferent between protein intake groups so multiple variables like these should be controlled for in future sarcopenia studies. Another limitation was only total protein intake was assessed, without considering the types of protein sources (animal- or plant-based), to figure out which is more effective at increasing lean muscle mass, physical function, and strength. This study population may also not be generalizable for other older adults since only older community-dwelling, Caucasian females were involved. However, some strengths were the use of validated tools and food records and the involvement of trained registered dietitians. Both observational and self-reported data was used to determine physical function, which increased data reliability and limited bias. In addition, by focusing on post-menopausal females, this may have helped decipher if gender differences existed between protein intake amounts and physical function (Gregorio et al., 2013).

In agreement with the previous studies that evaluated the association between protein intake and lean muscle mass improvements, Scott et al. (2010) and Gregorio et al. (2013) also had significantly positive results. However, Scott et al. (2010) found no difference in muscle strength and protein intakes compared to Gregorio et al. (2013) while Bartali et al. (2013) had significant differences between protein intake and muscle strength when inflammation was elevated. Thus the higher the inflammation, the less protein these individuals consumed and a greater reduction in muscle strength becomes prevalent. Nonetheless, Scott et al. (2010) did find

protein intake below the RDI was associated with declines in aLM and Gregorio et al. (2013) found a higher protein intake was affiliated with better physical performance and bone health similar to Meng et al. (2009). All three studies included healthy, community-dwelling elders so these results may not have the same outcome with sarcopenic elders. Gray-Donald et al. (2013) controlled for BMI, age, sex, physical activity, energy intake, chronic diseases, and smoking whereas, Scott et al. (2010) controlled for total energy intake, magnesium, and phosphorous. Furthermore, Gregorio et al. (2013) solely adjusted for BMI. For these reasons, only Gray-Donald et al. (2013) increased the likelihood that there was more of a direct relationship between aLM and protein intake since this study exclusively made multivariable adjustments.

Dietary Protein Intake Interventions

From the literature, it is apparent that weight loss becomes more prevalent as a person ages, yet, uncertainty remains as to how much lean muscle mass versus FM is lost as a result of sarcopenia advancements. One research theory is the variation of dietary protein intake may contribute to greater lean body mass losses since the current RDA of 0.8 g/kg/day for protein is under debate for being inadequate. However, few studies have incorporated a dietary protein intervention even though this area of practice is considered of high clinical relevance.

One study investigated whether a higher protein hypocaloric weight loss diet could reduce an older adult's risk for significant lean mass losses. The purpose was to determine if a protein intake > 1.2 g/kg/day, during hypocaloric feedings of higher protein, would minimize lean mass losses compared to a hypocaloric in low protein (< 0.8 g/kg/day) in older, healthy overweight/obese women (Gordon et al., 2013).

Twenty-four post-menopausal obese women, with a mean age of 58 ± 6.6 years and a BMI of 33 ± 3.6 kg/m^2, completed this 20-week intervention trial. Each subject was assigned an individualistic diet that included about a 400 kcal/day energy deficit, provided by the researchers, and a vitamin D supplement. Fifteen women were assigned to the low protein diet (0.5-0.7 g/kg/day of protein) while nine older women were prescribed a high protein diet for a study comparison (1.2-1.5 g/kg/day of protein). The women in the high protein group were additionally assigned a daily protein supplement (comprised of 90 calories and 23 g of protein) to help maintain a higher protein intake (Gordon et al., 2013).

The results from Gordon et al. (2013) showed that the high protein intake group had a mean total weight loss of 8.4 ± 4.5 kg verses 11.4 ± 3.8 kg in the low protein intake group; this difference was not statistically significant. However, the low protein intake group lost significantly more lean mass (4.1 ± 2.0 kg versus 2.3 ± 1.4 kg) and aLM (2.1 ± 1.8 kg versus 1.1 ± 1.07 kg), which equated to almost twice as much as the high protein intake group. The average percent of total body mass lost in the high protein intake group was significantly less than the low protein intake group (17.3% ± 27.8% versus 37.7% ± 14.6%; $p = 0.03$). Since weight loss from hypocaloric diets has resulted in reductions in both lean muscle mass and FM, the benefits of weight reduction in older adults has remained unclear. Therefore, Gordon et al. (2013) wanted to determine if protein intake was a significant predictor of fat free mass retention by analyzing lean mass losses while controlling for FM losses. As a result, the high protein intake group lost 2.2 ± 0.6 kg and the low protein group lost 4.1 ± 0.5 kg of lean mass ($p = 0.03$). This relative loss of lean mass was approximately equal to 37% in the low protein intake group, but the amount lost in the high protein intake group was not mentioned (Gordon et al., 2013; Houston et al., 2008).

In conclusion, consuming protein above the RDA (1.2-1.6 g/kg/day) may help protect against the amount of lean mass lost in older women undergoing intentional weight loss. Although a higher protein diet did not totally prevent the loss of lean mass, but these individuals were more successful in retaining their lean mass so protein intake should be of high importance when elders are trying to lose weight. A high protein hypocaloric diet may be a safe dietary intervention without negatively affecting lean body mass and fat mass. Failure to maintain adequate total protein intake during weight loss may lead to unnecessary loss of lean mass, which could lead to increased mortality and disabilities in the long term (Gordon et al., 2013).

Subjects were not blinded to their diet assignments, which is the main limitation within this study design. Even though this sample size provided Gordon et al. (2013) with an appropriate statistical power for results analysis, 24 post-menopausal women was limiting in determining if these results were generalizable. Although this study added a dietary protein supplement and was a weight loss intervention, which are aspects beyond this literature review, this hypocaloric dietary intervention was highly controlled and involved a long term follow-up. Finally, subjects reported high compliance and there was good subject retention during this 20 week intervention period (Gordon et al., 2013).

While a high protein diet may be beneficial to inhibit sarcopenia, understanding the impact of adding high protein food source to an elder's typical diet is essential in figuring out whether or not this macronutrient intervention can actually influence body composition characteristics. Daly, O'Connell, Mundell, Grimes, Dustan, & Nowson (2014) evaluated the IL-6 inflammatory marker, like Bartali et al. (2012), but conducted a 4-month cluster randomized controlled trial that assessed the effects of progressive resistance training (PRT) and a protein-enriched diet. To determine the effectiveness of PRT and a protein-enriched diet, these variables

were analyzed against lean muscle (mass, size, strength, and functionality), inflammatory markers, blood pressure, and lipid level changed in elderly women. The main outcome was to determine whether apparently healthy older women, who consumed lean red meat two times per day in combination with PRT in a vitamin D depleted state, would experience greater increases in total body and regional lean tissue mass (LTM), muscle size and strength, functional performance, and decreases in inflammatory markers compared to elderly women who solely participated in PRT. The dependent variables consisted of bone mineral density (BMD), muscle density, intermuscular adiposity, blood pressure, and blood lipid concentrations (Daly et al., 2014).

One-hundred older women, with a mean age of 73 years old and a BMI of 28, were randomly clustered by retirement village into either the intervention or control group. The intervention group (RT+Meat group; n = 53) consisted of PRT plus two 80 g portions of cooked lean red beef per day. The control group (CRT group; n = 47) involved controlled progressive resistance training (CRT) combined with consuming $\geq$ 1 serving of cooked rice and/or pasta daily (equivalent to about 75 g portion sizes and 25-35 g of carbohydrates). Diet was assessed at baseline and every four weeks by telephone-facilitated 24-hour dietary recalls. Participants in the intervention group were required to record all the meat consumed/day on a compliance calendar, which was collected monthly; the control group was assigned similar instructions. In small groups, all women participated in a 4-month, progressive resistance and balance-agility training program that comprised of 45-60 minute sessions (32 sessions total/person), two times/week. Throughout the study all were prescribed a 1000-IU vitamin D_3 supplement (Daly et al., 2014).

Results from the dietary assessment showed that significantly higher amounts of dietary protein were consumed by the RT+Meat group compared to the CRT group. On average the

RT+Meat group consumed 1.29 ± 0.30 g/kg/day of protein whereas, the CRT group consumed significantly less protein at 1.15 ± 0.35 g/kg/day ($p < 0.05$). The CRT group consumed 20-40 g more carbohydrates and leisure time physical activity significantly increased compared to the intervention group. After 4-months, women in the RT+Meat group experienced significantly greater average total body LTM gains of 0.5 kg, particularly in leg LTM, than the CRT group. Participants in the RT+Meat group also had significant average declines of 0.5 kg for fat mass and a 0.8% reduction in percentage of body fat. For muscle strength, the RT+Meat group experienced an estimated 18% greater muscle power from the leg-extension test compared to the CRT group. Compared to the CRT group, the RT+Meat group had significant decreases in the IL-6 inflammatory marker after 4-months. The RT+Meat group had a significant reduction of 7.8% for TNF-α while these same participants had significant increases in IGF-I at 2-months and 4-months compared to the control group (Daly et al., 2014).

At the conclusion of this intervention, Daly et al. (2014) found that older community-dwelling women who consumed a protein-rich diet of lean red beef, equal to about 1.3 g/kg/day of protein, had positive enhancements in total body and leg LTM, leg muscle strength, and serum IGF-I from PRT. This increase in daily meat consumption did not have any negative health consequences for these older women and inferred no increases in inflammation, saturated fat intake, blood pressure, blood lipid concentrations, or kidney dysfunction. For these reasons, a higher dietary protein intake of red meat may be a safe, well-tolerated, and effective intervention to help capitalize the anabolic affects of resistance training (Daley et al., 2014).

A limitation was the red meat portions were controlled in regards to portion size and packaging, but investigators only made recommendations for when the meat should be consumed. As such, the timing and size of each serving was monitored to maintain report

consistency. This could have also fundamentally affected the participant's anabolic response when combined with the work out and created unequal protein distribution. The diet adherence decreased from 86% to 75% after 2-months, which may have minimized the intervention's effectiveness. A diet high in lean red meat may not be sustainable for many older women, but could be more acceptable in older men. Another limitation is not all investigators could be blinded throughout the study so this may have produced some observational basis. In contrast, two strengths were the utilization of a large sample size and a long study duration (Daly et al., 2014). Daly et al. (2014) implied this study provided the strongest evidence on how effective higher red meat consumption, combined with PRT, could be in improving functionality, body composition, and inflammatory biomarkers in older women.

It can be a general challenge to find a high-protein food source that is not high in calories, fat, and cholesterol. In Sonora, Mexico in 2012, researchers Aleman-Mateo, Macias, Esparza-Romero, Astiazaran-Garcia, and Blancas illustrated this relationship by testing whether adding ricotta cheese, a well-known protein-rich food item, to the habitual diet of sarcopenic elderly individuals would increase total aLM and strength.

After subjects were selected based on various inclusion and exclusion criteria, 40 older individuals (23 women and 17 men) were randomly placed into either a control or intervention group. Subjects in the control group continued their habitual diet (HD) while those in the intervention group (RCH + HD) added 210 g of ricotta cheese to every meal (breakfast, lunch, and dinner) for 3 months. This amount of ricotta cheese was equivalent to 15.7 g protein and 267 extra kcal/day. Baseline measurements and the beginning of this intervention process were taken two weeks apart with follow-up measurements taken on the last day of ricotta cheese consumption (Aleman-Mateo et al., 2012).

Following study completion, only 12 subjects in the RCH+HD group and 17 in the HD group passed all study protocol. After 3 months of total aLM measurements, the percent of relative change showed subjects in the RCH+HD group had a positive tendency towards significance ($p = 0.06$). Men in the RCH+HD group gained 1.6 kg and 490 g of total aLM while men in the HD group, gained 220 g of TASM and lost weight (equivalent to 2.2 lbs.). In contrast, women in the RCH+HD group gained 260 g of total aLM whereas, women in the HD group only gained 220 g of aLM, respectively; both groups lost an estimated 800 g of total body weight. Overall, the men had a significantly positive trend in body weight, lean muscle mass and strength, but women in the RCH+HD group only showed a slight gain in muscle strength (Aleman-Mateo et al., 2012).

According to Aleman-Mateo et al. (2012), adding 210g of ricotta cheese to an elder's habitual diet, did not prohibit the loss of TASM in free-living sarcopenic individuals, but was positively associated with muscle strength in males and females within the intervention group. The elderly male subjects received the most substantiated benefits when consuming a protein-rich food item since men in the RCH+HD group gained 270 g more total aLM than those in the HD group and had better improvements in muscle strength, lean muscle in the arms, and body weight. However, older men may have experienced more positive results since 25% of women in the intervention group complained of early satiety after ingesting the ricotta cheese. Therefore implementing a high protein intervention, such as ricotta cheese, may help increase total protein consumption, but may not be completely tolerable for sarcopenic elders. Although there were gender differences in muscle strength, lean tissue in the arms, and body weight, this study showed the potential of implementing dietary protein as an intervention against sarcopenia (Aleman-Mateo et al., 2012).

The primary limitation of this study was a dietary assessment was not included within the methodology to determine if nutrient intakes were different between groups for a more in-depth nutrient analysis. As a consequence, the additional protein from the ricotta cheese and a general habitual diet were the only dietary measures to compare against body composition characteristics. Also adjustments of total caloric intake and BMI were not included within this study to ensure there variables did not influence body composition changes when protein intake and lean muscle changes were analyzed. Another limitation was the calculated sample size was based on gains in lean mass, but not for total aLM or strength; the reasons for this were unknown. Considering elderly women reported greater difficulty when consuming the large portions of ricotta cheese compared to the men, this may have skewed the results. The statistical analyses included subjects who did not meet all study protocol, which could be a weakness as well. However, despite the small sample size, this was the only study known to emphasize the effects of a dairy protein-rich food item on sarcopenic elderly individuals. Overall this was a well-controlled intervention trial with unified samples and minimal bias. Finally utilizing ricotta cheese, was an ideal dietary protein intervention for this population since it can be readily ingested, is highly accepted, and can be easily incorporated into many recipes (Aleman-Mateo et al., 2012).

Out of these three dietary protein intervention studies, Aleman-Mateo et al. (2013) was the only study that included sarcopenic older adults. Every study showed positive outcomes with a higher protein diet whether it was in the form of a high protein diet with a protein supplement, lean red beef, or ricotta cheese. Gordon et al. (2013), Daly et al. (2012), and Aleman-Mateo et al. (2012) found older adults who consumed more protein had improvements in various body composition measures compared to each of the lower protein intake groups. Daly et al. (2012)

also found older adults who consumed more protein had better physical capabilities. Daly et al. (2012) and Aleman-Mateo et al. (2012) both discovered some form of muscle strength enhancements similar to Bartali et al. (2013), but ultimately this differed from Scott et al. (2010) and Gregorio et al. (2013). Aleman-Mateo et al. (2012) showed older men had more positive outcomes from a higher protein food than older women, but Gordon et al. (2013) and Daly et al. (2012) only evaluated women so it is unclear if outcomes differ by gender. Future research should focus on increasing the number of older men and sarcopenic individuals in these studies to determine which methods are most effective in delaying the consequences of sarcopenia.

Conclusion

Overall, the evidence consistently shows that dietary protein intake above the RDA is positively associated with muscle mass and physical performance benefits in healthy older adults, but the improvements in muscle strength seem to be more inconsistent among studies. Only one study involved sarcopenic individuals so it is difficult to conclude whether increasing dietary protein intake may help delay and/or prevent the development of sarcopenia. Although every study controlled for at least one cofounding variable, not every variable was adjusted for, such as total energy intake, BMI, age, sex, and physical functional level. As a result, this may have unintentionally influenced the strength of associations found between protein intake and the characteristics of sarcopenia. Nonetheless, recommendations for essential preventative measures, including an adequate diet, should be established to counteract this unclear aging process since benefits of a higher protein intake were found. Additionally, the current RDA may be inadequate to offset age-related muscle losses, which means older adults are being prescribed nutrition interventions that do not meet their higher dietary protein demands. Thereby increasing dietary protein to greater than 0.8 g/kg/day may become the first line of defense against sarcopenia in

the form of protein-rich foods other than prescribing protein supplementation. A higher protein intake could be achieved by evenly distributing protein throughout the day to ensure greater endogenous protein synthesis (Paddon-Jones and Rasmussen, 2009). Therefore, if an older adult consumes dietary protein greater than the RDA, this could lead to increases in lean muscle mass and possibly muscle strength that will help maintain independence in daily life activities and prevent this syndrome.

CHAPTER 3: METHODOLOGY

For any health care professional it is a continuous challenge to keep up with new scientific literature while remaining efficient in day-to-day practice. To assist dietitians in deciphering this overwhelming amount of information, the Academy of Nutrition and Dietetics (AND) developed a systematic method in 2004 known as the evidence analysis process (AND, 2015a). The purpose of this process is to analyze current research and establish evidence-based practice (EBP) to assist dietitians in making appropriate nutritional decisions by providing strongly rated supportive evidence (AND, 2015d). Expert workgroups, from the nutrition and dietetics field, work together to identify, analyze, and rate the strength of current materials (AND, 2015a).

Once this process is completed, the disease-specific guidelines are summarized on the Evidence Analysis Library (EAL) webpage for AND members (AND, 2015b). The EAL is an easily accessible resource for dietitians to refer to when developing their own conclusions and providing creditable nutrition care (AND, 2015d). The methodology involved in developing these guidelines is explained in detail in the *Evidence Analysis Manual: Steps in the Academy Evidence Analysis Process*. There are five steps involved in the EAL process: 1) Formulate the Evidence Analysis Question, 2) Gather and Classify Evidence, 3) Critically Appraise Each Article, 4) Summarize Evidence, and 5) Write and Grade the Conclusion Statement (AND, 2012c).

Step 1- Formulate the Evidence Analysis Question

The initial phase of the EAL process is to formulate a research question that can be utilized for evidence-based guidelines. The question should focus on a certain aspect of the

nutritional care process, which includes nutrition assessment, diagnosis, intervention, and monitoring and evaluation. To assist in formulating a focused research question, the PICO format is incorporated using the following four elements: (P) for *population* of interest that has the current problem, (I) for *intervention*, procedure, or approach such as the treatment, cause, or prognostic factor, (C) for *comparison intervention* such as the other approaches to care in comparison to the intervention, procedure, or approach, and (O) for *outcome* of interest (AND, 2012c). The PICO format for the current EAL project is presented in Table 1 followed by the established research question:

Table 2: *PICO Format.* This is how the current research question was formulated using PICO.

Population (Patient or Problem)	Intervention (cause, treatment, or prognostic factor)	Comparison Intervention (if necessary)	Outcomes
Older Adults	Adequate or higher dietary protein intake (≥ 0.8 g/kg of body weight protein)	Inadequate or lower protein intake (< 0.8 g/kg of body weight)	Does this affect body composition and/or muscle strength

(AND, 2012c)

Step 2- Gather and Classify Evidence

The second step is to conduct a systematic search to find all the literature pertaining to the research question. The main goal of this step is to gather all the relevant evidence pertaining to this question and decide which articles should be included or excluded for further analysis. Several actions are involved in this step. First, it is essential to plan a research strategy by identifying the inclusion and exclusion criteria, search terms, and search databases. The second part of the analysis is to use this research strategy to find articles and review the titles and abstracts of each to determine which ones best match the inclusion criteria. Finally, after the

articles are gathered and fit within the criteria, each must be classified based on the article's study design to more proficiently determine the level of evidence available; see Table 2 (AND, 2012c).

Table 3: *Hierarchy and Classification of Studies*
Classification system developed by the AND to determine the level of evidence depending on the type of study design.

Primary Reports		Secondary Reports	
A	Randomized Controlled Trial Cluster Randomized Trial Randomized Crossover Trial	**M**	Meta-analysis or Systematic review Decision analysis Cost-benefit analysis Cost-effectiveness study
B	Prospective Cohort Study Retrospective Cohort Study		
C	Non-Randomized Controlled Trial Non-Randomized Crossover Trial Case-Control Study Time Series Study Diagnostic, Validity or Reliability Study	**R**	Narrative review (Review article) Consensus statement Consensus report
D	Non-Controlled Trial Case Study or Case Series Other Descriptive Study Cross-Sectional Study Trend Study Before-After Study	**X**	Medical opinion

(AND, 2012c)

Research Question

The research question used for the literature review and the evidence analysis process was, "Does dietary protein intake improve lean muscle mass losses in older adults?" After reviewing

the current literature and learning more about sarcopenia, it was evident that this condition goes beyond solely lean muscle mass losses. Therefore, the original research question was later revised to, "Does dietary protein intake, greater than the RDA, delay the consequences of sarcopenia in older adults?" The consequences of sarcopenia include greater than normal losses of muscle, strength, and physical performance, which are all main pathophysiological changes that older adults develop.

Inclusion Criteria

Certain inclusion criteria were set to ensure relevant and scholarly journals were utilized for this EAL project. Initially primary, peer-reviewed research articles published in 2011 or newer were included within the critical analyses and used to establish a graded conclusion statement, but less than five articles were found pertinent to the research question. As a result, this timeline extended to 2008 or newer to ensure a substantial amount of literature to review for study analysis. Each study had to include age specific participants, determined as $\geq$ 50 years old, but any health status was acceptable. At minimum, each article had to incorporate measurements of lean body mass and dietary protein intake (observationally or as an intervention) and be in English.

Exclusion Criteria

Articles were excluded if they only included measurements of frailty and weight status changes without integrating body composition measurements within the methodology. Primary articles that involved protein supplementation as an intervention study or an observation and/or incorporated physical activity as an intervention were also excluded. Studies that incorporated a

meta-analysis or review study design and are animal-based were excluded. Although secondary reports were included to provide more background information on sarcopenia, these studies are not accepted for establishing evidence-based practice. Comparatively, animal studies are also not accepted by EAL standards so these types of articles will be excluded as well.

Search Terms

After few primary research articles were found simply using the term sarcopenia, the search terms were expanded to cover words that included, "protein intake", "body composition measurements", and "aging". The specific search terms used for protein were as followed: "protein intake", "dietary protein", "protein-rich sources", "high protein foods", and "protein consumption". These terms are intermixed in combination with various muscle terminologies such as "lean muscle mass", "muscle mass loss", "age-related muscle losses", "sarcopenia", "improvements in muscle mass" and "physical performance".

Search Databases

To ensure pertinent articles were included in the literature review, a couple search databases were utilized to input the combination of search terms. The date of the literature review was March 2015 using the search databases EBSCOhost (including Academic Search Premier, ERIC, CINAHL, MEDLINE, and Health Source: Nursing/Academic Edition) and PubMed. All searches were limited to studies that were peer-reviewed, in the English language, used human subjects, and published 2008 to current; see Table 3. Thereafter *Table 4: Summary of Articles in Literature Review* is presented below, which is followed by the list of included and a list of articles excluded with the reasons why.

Table 4: Search Terms and Results

Represents the number of articles that were displayed after various search terms to input into different search databases.

Search Databases	Search Terms	Number of Hits
EBSCOhost	"protein intake and sarcopenia"	129
	"protein intake and lean muscle mass"	149
	"protein intake and muscle mass loss"	188
	"protein intake and age-related muscle losses"	4
	"protein intake and improvements in muscle mass"	32
	"protein intake and physical performance"	2
	"protein intake and aging"	521
	"dietary protein and sarcopenia"	124
	"dietary protein and lean muscle mass"	153
	"dietary protein and muscle mass loss"	164
	"dietary protein and age-related muscle losses"	5
	"dietary protein and improvements in muscle mass"	32
	"dietary protein and physical performance"	296
	"protein-rich sources and sarcopenia"	1
	"protein-rich sources and lean muscle mass"	0 (7,627)*
	"protein-rich sources and muscle mass loss"	0 (9,012)*
	"protein-rich sources and age-related muscle losses"	0 (13,743)*
	"protein-rich sources and improvements in muscle mass"	0 (54,263)*
	"protein-rich sources and physical performance	1
	"high protein foods and sarcopenia"	20
	"high protein foods and lean muscle mass"	55
	"high protein foods and muscle mass losses"	8
	"high protein foods and age-related muscle losses"	2
	"high protein foods and improvements in muscle mass"	9
	"high protein foods and physical performance"	124
	"protein consumption and sarcopenia"	8
	"protein consumption and lean muscle mass"	32
	"protein consumption and muscle mass loss"	37
	"protein consumption and age-related muscle losses"	0 (10,276)*
	"protein consumption and improvements in muscle mass"	5
	"protein consumption and physical performance"	130
PubMed	"protein intake and sarcopenia"	90
	"protein intake and lean muscle mass"	88
	"protein intake and muscle mass loss"	132
	"protein intake and age-related muscle losses"	1
	"protein intake and improvements in muscle mass"	10
	"protein intake and physical performance"	165
	"protein intake and aging"	594

	"dietary protein and sarcopenia"	119
	"dietary protein and lean muscle mass"	112
	"dietary protein and muscle mass loss"	136
	"dietary protein and age-related muscle losses"	2
	"dietary protein and improvements in muscle mass"	9
	"dietary protein and physical performance"	223
	"protein-rich sources and sarcopenia"	0
	"protein-rich sources and lean muscle mass"	1
	"protein-rich sources and muscle mass loss"	1
	"protein-rich sources and age-related muscle losses"	0
	"protein-rich sources and improvements in muscle mass"	0
	"protein-rich sources and physical performance	0
	"high protein foods and sarcopenia"	34
	"high protein foods and lean muscle mass"	40
	"high protein foods and muscle mass loss"	48
	"high protein foods and age-related muscle losses"	1
	"high protein foods and improvements in muscle mass"	6
	"high protein foods and physical performance"	111
	"protein consumption and sarcopenia"	17
	"protein consumption and lean muscle mass"	45
	"protein consumption and muscle mass loss"	4
	"protein consumption and age-related muscle losses"	0
	"protein consumption and improvements in muscle mass"	12
	"protein consumption and physical performance"	241

*** After the initial search inquiry no results were yielded. The numbers in parentheses are the number of articles that were displayed after the search database used SmartText. These were not reviewed to maintain search result consistency since the displayed documents were not required to include all search terms.**

Table 5: Summary of Articles in Literature Review. This summarizes how many articles were reviewed compared to how many articles that remained included.

Summary of Articles Identified to Review	Number of Articles
Number of Primary Articles Identified	9
Number of Review Articles Identified	0
Total Number of Articles Identified	9
Number of Articles Reviewed but Excluded	6

The Following are the List of Included Articles for Analysis:

Aleman-Mateo, H., Macias, L., Esparza-Romero, J., Astiazaran-Garcia, H., Blancas, & A.L. (2012). Physiological effects beyond the significant gain in muscle mass in sarcopenic

elderly men: evidence from a randomized clinical trial using a protein-rich food. *Clinical Interventions in Aging*, 7, 225-234. doi: 10.2147/CIA.S32356

Geirdottir, O.G., Arnarson, A., Ramel, A., Jonsson, P.V., & Thorsdottir, I. (2013). Dietary protein intake is associated with lean body mass in community-dwelling older adults. *Nutrition Research*, 33(8), 608-612. doi: 10.1016/j.nutres.2013.05.014

Gray-McDonald, K., St-Arnaud-McKenzie, D., Gaudreau, P., Morais, J.A., Shatenstein, B., & Payette, H. (2014). Protein Intake Protects against Weight Loss in Healthy Community-Dwelling Older Adults. *The Journal of Nutrition*, 144, 321-326. doi: 10.3945/jn.113.184705

Gregorio, L., Brindisi, J., Kleppinger, A., Sullivan, R., Mangano, K.M., Bihuniak, J.D... & Insogna, K.L. (2013). Adequate Dietary Protein is Associated with Better Physical Performance Among Post-Menopausal Women 60-90 Years. *The Journal of Nutrition, Health & Aging*, 18(2), 155-160. doi: 10.1007/s12603-013-0391-2

Houston, D.K, Nicklas, B.J., & Ding, J. (2008). Dietary protein intake is associated with lean muscle mass change in older, community-dwelling adults: the Health, Aging, and Body Composition (Health ABC) Study. *American Journal of Clinical Nutrition*, 87(1), 150-155. Retrieved from http://ajcn.nutrition.org/content/87/1/150.full.pdf+html

Meng, X., Zhu, K., Devine, A., Kerr, D.A., Binns, C.W., & Prince, R.L. (2009). A 5-Year Cohort Study of the Effects of High Protein Intake on Lean Mass and BMC in Elderly Postmenopausal Women. *Journal of Bone and Mineral Research*, 24, 1827-1834. doi: 10.1359/JBMR.090513

Morris, M.S. & Jacques, P.F. (2012). Total protein, animal protein and physical activity in relation to muscle mass in middle-aged and older Americans. *British Journal of Nutrition*, 109, 1294-1303. doi: 10.1017/S0007114512003133

Ruiz Valenzuela R.E., Ponce J.A., Morales-Figueros G.G., Muro K.A., Carreon V.R., & Aleman-Mateo H. (2013). Insufficient amounts of inadequate distribution of dietary protein intake in apparently healthy older adults in a developing country: implications for dietary strategies to prevent sarcopenia. *Clinical Interventions in Aging*, 8, 1143-1148. doi: 10.2147/CIA.S49810

Scott D., Blizzard L., Fell J., Giles G., & Jones G. (2010). Associations Between Dietary Nutrient Intake and Muscle Mass and Strength in Community-Dwelling Older Adults: The Tasmanian Older Adult Cohort Study. *Journal of the American Geriatrics Society*, 58(11), 2129-2134. doi: 10.1111/j.1532-5415.2010.03147.x

Articles Excluded:

Excluded for: including a protein supplement and intentional weight loss as an intervention

Gordon, M.M., Bopp, M.J., Miller, G.D., Lyles, M.F., Houston, D.K., Nicklas, B.J., & Kritchevsky, S.B. (2008). Effects of Dietary Protein on the Composition of Weight Loss in Post-Menopausal Women. *The Journal of Nutrition Health and Aging*, 12(8), 505-509. Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3629809/

Excluded for: including resistance training element

Daly, R.M., O'Connell, S.L., Mundell, N.L., Grimes, G.A., Dunstan, D.W., & Nowson, C.A. (2014). Protein-enriched diet, with the use of lean red meat, combined with progressive resistance training enhances lean tissue mass and muscle strength and reduces circulating IL-6 concentrations in elderly women: a cluster randomized controlled trial. *American Journal of Clinical Nutrition*, 99, 899-910

Excluded for: not assessing lean body mass

Bartali, B., Frongillo, E.A., Stipanuk, M.H., Bandinelli, S., Salvini, S., Palli, D.,…& Ferrucci, L. (2013). Protein Intake and Muscle Strength in Older Persons: Does Inflammation Matter?. Journal of American Geriatric Society, 60(3), 480-484. doi: 10.1111/j.1532-5415.2011

Tieland, M., Borgonjen, K.J., den Berg, V., van Loon, L.J.C., & de Groot, L.C.P.G.M. (2012). Dietary protein intake in community-dwelling, frail, and institutionalized elderly people: scope for improvement. *European Journal of Nutrition*, 51, 173-179. doi: 10.1007/s00394-011-0203-6

Excluded for: measures only frailty against protein intake

Kobayashi, S., Asakura, K., Suga, H., Sasaki, S., & the Three-generation Study of Women on Diets and Health Study Group. (2013). High protein intake is associated with low prevalence of frailty among old Japanese women: a multicenter cross-sectional study. *Nutrition Journal*, 12, 164. doi: 10.1186/1475-2891-12-164

Bollwein, J., Diekmann, R., Kaiser, M.J., Bauer, J.M., Uter, W. Sieber, C.C., & Volkert, D. (2013). Distribution but not amount of protein intake is associated with frailty: a cross-sectional investigation in the region of Nurnberg. *Nutrition Journal*, 12, 109. doi: 10.1186/1475-2891-12-109

Step 3- Critically Appraise Each Article

Once articles are deemed relevant and each study design is classified, the third step is to complete the evidence analysis worksheets for each inclusion article. The purpose of the worksheets are to paraphrase key information so it can be used as a quick reference, identify study details that will determine study quality, summarize major findings and the author's

conclusions, provide notes on reviewer's comments (i.e. strengths, weaknesses, and limitations), and funding sources. Primary and secondary research articles involve separate worksheets so the information is specific to the types of subjects involved. Following the summary of worksheet information, a Quality Criteria Checklist must be completed to assign a rating to each study. This checklist is written in the form of yes/no questions that focus on study relevancy and validity. At the end of the worksheet, the study is given an overall rating indicative of a positive (+), neutral (∅), or negative (-) symbol. This signifies the strength of scientific evidence and helps the analyst recognize an invalid or possible threat that may undermine sound research towards answering the question. All of the answers from the checklists are then summarized in a single table so the answers can quickly be reviewed in a side-by-side comparison (AND, 2012c).

Step 4- Summarize Evidence

In the fourth step, the information from the Evidence Analysis worksheets are included in an overview table that includes author, year, study design, class rating (+,∅, or - symbols), study type, purpose, study populations, intervention, outcomes (and measurements of interest), and limitations of the relevant articles. This overview table is a quick tool that allows the studies to be evaluated on the most important aspects so each can be critically analyzed against one another in an efficient manner. Thereafter specific findings from each study, written in a brief statement, capture the following information: author(s) and publication year, outcomes (and measurements) of interest, important sample characteristics and comparison factors (i.e. sex, age, etc.) implications for practice, and limitations of findings (AND, 2012c).

Step 5- Write and Grade the Conclusion Statement

The last step in the evidence analysis process is to pull all the information together and create a conclusion statement on what the current evidence is in response to the research question. This conclusion statement is then graded depending on the strength of evidence found throughout the literature review. The grading system consists of Grade I (Good), Grade II (Fair), Grade III (Limited), Grade IV (Expert Opinion Only), and Grade V (Not Assignable); a detailed description of each grade is mentioned in Table 5. The overall conclusion statement is considered strong if it receives a Grade I or II while a weaker conclusion is indicated by grades III, IV, or V. A weaker study emphasizes the need for more valid evidence from research studies before a stronger conclusion can be drawn for evidence-based practice; Table 6 explains the key elements to investigate when deciding on an appropriate grade for the conclusion (AND, 2012c).

Table 6: *Conclusion Statement Grading System*

Grade Definitions: Strength of the Evidence for a Conclusion Statement

Grade I: Good—The evidence consists of results from studies of strong design for answering the question addressed. The results are both clinically important and consistent with minor exceptions at most. The results are free of serious doubts about generalizability, bias, and flaws in research design. Studies with negative results have sufficiently large sample sizes to have adequate statistical power.

Grade II: Fair—The evidence consists of results from studies of strong design answering the question addressed, but there is uncertainty attached to the conclusion because of inconsistencies among the results from different studies or because of doubts about generalizability, bias, research design flaws, or adequacy of sample size. Alternatively, the evidence consists solely of results from weaker designs for the questions addressed, but the results have been confirmed in separate studies and are consistent with minor exceptions at most.

Grade III: Limited—The evidence consists of results from a limited number of studies of weak design for answering the questions addressed. Evidence from studies of strong design is either unavailable because no studies of strong design have been done or because the studies that have been done are inconclusive due to lack of generalizability, bias, design flaws, or inadequate sample sizes.

Grade IV: Expert Opinion Only—The support of the conclusion consists solely of the statement of informed medical commentators based on their clinical experience, unsubstantiated by the results of any research studies.

Grade V: Not Assignable*— There is no evidence available that directly supports or refutes the conclusion.

(AND, 2012c)

Table 7: *AND's Grade Scale Definitions*

Strength of Evidence Elements	Grades				
	I Good/Strong	II Fair	III Limited/Weak	IV Expert Opinion Only	V Grade not Assignable
Quality (scientific rigor/validity , considers design and execution	Studies of strong design for question. Free of design flaws, bias and execution problems	Studies of strong design for question with minor methodological concerns, OR Only studies of weaker study design for question	Studies of weak design for answering the question OR Inconclusive findings due to design flaws, bias or execution problems	No studies available Conclusion based on usual practice, expert consensus, clinical experience, opinion, or extrapolation from basic research	No evidence that pertains to question being addressed
Consistency Of findings across studies	Findings generally consistent in direction or size of effect or degree of association, and statistical significance with minor exceptions at most	Inconsistency among results of studies with strong design, OR Consistency with minor exceptions across studies of weaker design	Unexplained inconsistency among results from different studies OR single study unconfirmed by other studies	Conclusion supported solely by statements of informed nutrition or medical commentators	NA
Quantity (Number of studies, Number of subjects in studies)	One to several good quality studies Large number of subjects studied Studies with negative results have sufficiently large sample size for adequate statistical power	Several studies by independent investigators Doubts about adequacy of sample size to avoid Type I or Type II error	Limited number of studies Low number of subjects studied and/or inadequate sample size within studies	Unsubstantiated by published research studies	Relevant studies have not been done
Clinical Impact (Importance of studied outcomes, Magnitude of effect)	Studied outcomes relates directly to the question. Size of effect is clinically meaningful Significant (statistical) difference is large	Some doubt about the statistical or clinical significance of the effect	Studied outcomes is an intermediate outcome or surrogate for the true outcome of interest OR Size of effect is small or lacks statistical and/or clinical significance	Objective data unavailable.	Indicates area for future research
Generalizability (To population of interest)	Studied population, intervention and outcomes are free from serious doubts about generalizability	Minor doubts about generalizability	Serious doubts about generalizability due to narrow or different study population, intervention or outcomes studied	Generalizability limited to scope of experience	NA

(AND, 2012c)

Subsequently, the primary articles Morris and Jacques (2012), Geirsdottir et al. (2013), Houston et al. (2008), Ruiz Valenzuela et al. (2013), Meng et al. (2009), Scott et al. (2010), Gregorio et al. (2013), Aleman-Mateo et al. (2012), and Morris and Jacques (2013) will be critically analyzed using the evidence analysis process above. Details of Step 3 can be found in Appendix A. Chapter 4 Results reports Step 4 and Step 5 and Chapter 5 describes the evidence summary.

CHAPTER 4: RESULTS

Nine primary research articles fit within the established inclusion criteria and provide relevant evidence towards answering the research question, "Does dietary protein intake, greater than the RDA, delay the consequences of sarcopenia in older adults?" Narratives of each research article are summarized in detail below along their established rating. A collaborative summary table depicting the keys aspects from each inclusionary primary article is displayed thereafter; see Table 7. All of the studies required a P-value of $p < 0.05$ in order to be considered significant unless otherwise specified. The finalized Quality Criteria Checklists and EAL Article Worksheets are in Appendices section with the results of the Quality Criteria Checklists in Table 8.

Articles Relative to Research Question

Morris & Jacques (2013)

Morris & Jacques (2013) conducted a cross-sectional study to find answers to some of the unknown questions regarding age-related muscle losses and strength declines, by evaluating the National Health and Nutrition Examination Survey (NHANES) data from 2003-2006, on participants $\geq$ 50 years old. According to the Quality Criteria Checklist, this study received a neutral rating; refer to Appendix A. For an individual to be involved in the NHANES survey, they must go to a mobile examination center (MEC) and participate in a physical exam (including body composition measures of a DXA scan) and interview (included information on physical activity, medical history, and smoking data) while completing two 24-hour recalls and a

FFQ. Therefore, using this NHANES survey data from 2003-2004 and 2005-2006, a secondary analysis was conducted for this current study.

After excluding subjects who did not complete the MEC assessment, a total of 2,425 older adults were included in this current study analysis. However, only subjects in the 2003-2004 survey underwent DXA scans so out of the 2425 subjects, only 557 men and 625 women were included in this part of the study. These DXA scan results were combined with another 290 men and 167 women, all of whom were excluded prior for missing some of the MEC data. Each participant had measurements from four DXA scans, which were used to determine aLM by separating men from women. Also beef intakes were estimated using the 24-hour recalls and the FFQ along with assessments of protein intake and other micronutrients (ususal protein and energy intakes) and physical activity status (specifically muscle-strength activities). Protein intake was separated into three categories to compare against aLM: < 0.8 g/kg (below the RDA for protein; n = 468), 0.8-1.0 g/kg (meeting the RDA; n = 806), and > 1.0 g/kg (exceeding the RDA; n = 1,151).

A total of 2,425 subjects were identified for analysis, with a mean age of 63 years old and one-quarter were considered obese. Sixty-five percent of subjects reported engaging in some form of physical activity with 58% participating in moderate aerobic activities and about 50% in vigorous physical activities or muscle-strength training. On average, subjects consumed about 260 g/day of beef (318 g/week for men and 210 g/week for women). For total protein intake, a majority of non-obese subjects consumed > 1.0 g/kg/day of protein whereas, obese individuals mainly consumed < 0.8 g/kg/day of protein. There were significant associations between total protein intake and beef consumption ($r = 0.19$, $p < 0.0001$). In regards to aLM, the mean value was 19.1 kg for non-obese men and 15.4 kg for non-obese women whereas, obese men (28.6 kg)

and women (24.4 kg) on average had significantly greater aLM indexes ($p < 0.001$). Those who were older, female, of Mexican American racial ethnicity, and smoked had a lower aLM. There was only a positive association found between vigorous physical activity and aLM in non-obese subjects. Regardless of protein intake quantities, non-obese subjects who engaged in vigorous physical activity had a significantly modest higher aLM, which was related to a higher beef consumption. A stronger, more significant association was found between aLM and muscle-strengthening exercises for non-obese individuals compared to inactive non-obese subjects. In contrast, physically active obese subjects had a stronger association between aLM and total protein intake compared to non-obese subjects. Beef intake was also positively associated with aLM, but only in obese individuals who performed vigorous aerobic activities. Nonetheless, obese subjects had the most aLM increases when the RDA for protein was exceeded.

In conclusion, a diet with a high-quality protein, such as beef, and a higher total protein intake were associated with greater aLM values. When comparing protein intakes between obese and non-obese individuals, obese subjects were least likely to consume higher than the RDA. However, exceeding the RDA for protein was only beneficial on aLM values for obese subjects. Instead non-obese subjects who met the RDA for protein showed the highest aLM values. Interestingly, obese subjects had a lower aLM when strengthening exercises were combined with lower-protein intakes, or below the RDA for protein, compared to obese participants who were inactive. It was apparent muscle-strengthening exercises may benefit aLM indexes, but only if supported by an adequate protein intake. Overall, obese older adults may be at greatest risk for inadequate protein intake and should be targeted for higher protein interventions.

This article received a neutral rating as a result of many study limitations. Therefore, these results should be recommended with some caution. Cross-sectional study designs are

ranked lower on the AND's study design classification scale so many of the relevance and validity questions were non-applicable. Blinding was not implemented as part of study to prevent bias. There could have been a conflict of interest since the National Cattleman's Beef Association provided funding, which could have been why only beef consumption was analyzed.

Gray-Donald et al. (2014)

A positive rating was determined for this nested prospective, case control study after completing the Quality Criteria Checklist; see Appendix B for more detail. This study was based off of a previous longitudinal population-based cohort study called NuAge. The main objective was to assess the relationship between protein intake and rate of a one year incidence of $\geq$ 5% weight loss in community-based older adults. Participants were randomly recruited from a Quebec provincial health database and matched (1:1) based on sex and age, against subjects from a previous longitudinal population-based cohort study. Exclusion criteria consisted of physical disabilities such as inability to walk 100 meters or climb 10 steps without rest and incapacity to perform daily activities. Participants were further excluded for health disparities including cognitive deficits, class II heart failure, inflammatory intestinal diseases, cancer requiring treatment within five years of enrollment, and chronic obstructive pulmonary disease requiring oxygen or oral steroid therapies. Subjects were placed into three age categories: 70 $\pm$ 2 years, 75 $\pm$ 2 years, and 80 $\pm$ 2 years. Annual data was collected via in-person interviews.

Participants (n = 422) were selected based on substantial weight loss criteria and separated into case (n = 211) and control groups (n = 211) depending on the percent of weight lost in one year. Cases were subjects who had a $\geq$ 5% weight loss over one year, either between baseline to 1st year (n = 129) or 1st year to 2nd year follow-up (n = 82). Subjects were switched

if 1st year controls were selected as second year cases (n = 8). The controls accounted for subjects with a ≤ 2% weight change from baseline to the first year follow-up. Thereafter, controls and cases were randomly matched with an eligible weight stable participant (1:1) from the corresponding time period.

All variables were gathered at baseline and at the 1st year or 2nd year follow-up for analysis. A detailed three day nonconsecutive 24-hour dietary recall was conducted by a trained registered dietitian (one face-to-face and two telephone interviews). Macronutrients and total energy intake were evaluated separately using unadjusted models for statistical analysis. Protein intakes were categorized as low (< 0.8 g/kg/day), moderate (0.8-1.0 g/kg/day), high (1.0-1.2 g/kg/day), and very high (> 1.2 g/kg/day was the reference standard). Anthropometric measurements included BMI, percent weight change, fat mass, and fat free mass. Lifestyle habits (appetite, dieting for weight loss, smoking status, physical activity level, and physical function), depressive symptoms, chronic disease burden, polypharmacy use, and biochemical measures (low serum albumin and elevated C-reactive protein) were also measured. The researchers wanted to also evaluate the effects protein intake had on weight loss, in regards to body composition, since this was a major topic of interest. A subsample of 60 participants were consequently analyzed on the effects protein intake had on lean since these participants had body composition measures at both baseline and year 2.

A total of 422 participants, 121 female (57.3%) and 90 male, completed the study. On average, when comparing baseline measures, case subjects had significantly more chronic diseases and medications, diminished physical function, and lower serum albumin scores than the control group. After using separate models of mean energy, protein, and lipid intakes, it was disclosed that the case group consumed 0.10 g/kg/day fewer grams of protein than the control

group, one year prior to losing weight. Only protein intake remained significant between the case and control groups ($P < 0.05$) considering 51.9% of participants in the case group had protein intakes < 1.0 kcals/kg/day compared to 39.6% of controls. This indicated a highly significant correlation between protein intake and weight loss ($p = 0.005$). For the unadjusted model, participants who consumed > 1.2 kcal/kg/day of protein compared to < 0.8 kcal/kg/day, were twice as likely to lose weight ($p = 0.018$), while those in the moderate category were 70% more likely to lose weight ($p = 0.039$). For the adjusted model, participants were at 2.56 and 2.15 times greater chance of losing weight in the low- and moderate-protein-intake categories, respectively. The average 2 year loss of lean muscle mass was 1610 $\pm$ 1680 g (1.61 $\pm$ 1.68 kg) for the case group and 1050 $\pm$ 1200 g (1.05 $\pm$ 1.2 kg) for the control group; there was a significant difference of 560 g of lean mass ($p = 0.034$).

Based on the results, the researchers concluded that protein intake impacted the weight status and protein mass of older adults within year 1, putting those with lower protein intakes at further weight loss risk. Compared to the highest protein intake group, older adults who consumed moderates amount of protein (< 1.0 g/kg/day) were twice as likely to lose weight and those consuming < 0.8 g/kg/day were 2.5 times at greater risk for weight loss. This further demonstrates the importance of higher protein consumption to maintain lean muscle mass. However, despite the fact that this study did not emphasize the impact protein intake can have on lean muscle mass losses, the chosen subsample lost 0.5 kg greater lean muscle mass compared to the control group at two time points, 2 years apart. Moreover, those who lost > 5% of body weight over 1 year consumed 0.1 g/kg/day less protein than those who remained weight stable. Therefore, in order for protein intake to effectively guard against weight loss, the researchers assumed that a significant portion of lean mass was lost due to inadequate protein consumption.

Limitations found in this study included a lack of direct impact of protein intake on lean muscle mass and correlated evidence that may not apply to frail or ill older adults. This study did not focus mainly on how protein intake effected changes in lean muscle mass so the only selected subsample of 60 subjects was relative to the research question on muscle mass changes. In addition, inflammation was shown to have minimal influence on muscle loss indicating the need for more research to determine its level of significance.

Geirsdottir et al. (2013)

Another cross-sectional study was conducted by Geirsdottir et al. (2013), which received a neutral rating after completing a Quality Criteria Checklist; see Appendix C. The purpose was to test whether there was a link between dietary protein consumption and leisure-time physical activity by assessing lean body mass in community-dwelling, healthy older adults. The secondary objective was to determine whether the current RDA for protein was sufficient for this age group. The overall purpose of was to improve muscle mass and strength by analyzing measurements from a previous 12-week intervention resistance training program. Participants (n = 237) were recruited by advertisements in the Reykjavik area and had to be $\geq$ 65 years old; final age range was 65 to 92 years old. Subjects were excluded for low cognitive function, major orthopedic diseases, musculoskeletal disorders, disorders affecting muscle mass, and involvement in pharmacologic interventions affecting muscle mass or testosterone levels. Assessments included dietary intake, body composition, leisure-time physical activity, other covariates, and physical function.

Subjects weighed and reported dietary food recalls for three nonconsecutive days, which was used to group subjects based on protein intake. Anthropometric measurements were

obtained (body composition and weight, height, and two waist circumference measures) along with categorized BMIs (BMI < 25 kg/m2, 25< BMI >30 kg/m2, 30 < BMI > 35 kg/m2, and BMI > 35 kg/m2). For leisure-time physical activity, participants recorded the hours and types of activities done over the past year. As for physical function, subjects participated in a "Timed Up and Go" test in which participants were instructed to rise from a chair while in a seated position, walk three meters, return to the chair, and sit down again while being timed; the faster the time, the better the subject's functional ability. The second fitness test was the "Six-Minute Walk" test, which required subjects to walk back and forth in a gym hallway for six minutes while the number of laps were recorded; the more laps recorded the greater the subject's fitness level (ATS, 2002). The demographic characteristics assessed alcohol and smoking behavior, background on health concerns, and medications. Activity records and demographics were recorded by formulated questionnaires.

Participants (n = 237) were 58% female and 42% male (mean age of 73.6 $\pm$ 5.7 years). Eighty-two percent of participants reported regular leisure-time physical activity with two-thirds at a recommended level of 30 min/day. The average total energy intake was 1682 $\pm$ 494 kcal/day and participants consumed quantities of protein significantly above the RDA ($p < 0.001$). Based on gender averages, men consumed 90.3 $\pm$ 26.7 g/day (0.98 g/kg/day) and women consumed 69.6 $\pm$ 19.1 g/day (0.95 $\pm$ 0.29 g/kg/day). In regards to the linear model, daily protein intake was considered a positive predictor of lean body mass as hypothesized by the researchers.

Accordingly, the researchers concluded dietary protein intake was positively associated with lean body mass in community-dwelling older adults through validation that the average participant consumed protein levels above the current RDA (0.8 g/kg), indicative of a higher lean body mass. This reiterates a significant difference within this linear relationship. When

evaluating the protein quartiles, there was a significant difference of 2.3 kg for lean body mass between the fourth (1.36 ± 1.19 g/kg of protein) and first (0.63 ± 0.08 g/kg of protein) quartile and trending towards significance of 2.0 kg among the fourth (1.36 ± 1.19 g/kg of protein) and second (0.85 ± 0.05 g/kg of protein) quartiles. Increasing energy intake was also considered for differences in protein intake, but remained a minor part of the discovered relationship. In contrast, there was no correlation between leisure-time physical activity and lean body mass, which suggested that endurance exercises may not be connected to lean body mass for this population regime.

One limitation is this sample population may not have been representative of the general older adult population because this study only consisted of highly functional community-dwelling older adults. Choosing a cross-sectional study design was a weakness since it did not reflect a cause-and-effect relationship or evaluate prolonged behaviors impacting lean body mass; measurements were only taken at one point in time. Physical activity was self-reported, likely resulting in overestimation and subject bias. All of these reasons are why this study received a neutral rating instead of a positive rating.

Houston et al. (2008)

Houston et al. (2008) conducted a prospective cohort study known as, the Health ABC (Aging and Body Composition) Study, to determine if there was an association between dietary protein consumption and alterations in lean mass and aLM in the community-dwelling older adults. The results from the Quality Criteria Checklist indicated a positive rating; refer to Appendix D. Participants were recruited from a selected sample of Medicare-eligible residents within the metropolitan areas of Pittsburg, PA and Memphis, TN if they were between the ages

of 70 to 79 years old and Black or Caucasian. Older adults were eligible if they had no issues walking ¼ of a mile, climbing 10 steps, or performing basic ADLs. No life-threatening illnesses, current participation in other lifestyle intervention trials, and willingness to stay in the study's geographical region for the 3 year study, were also part of the inclusion criteria. If the FFQ at year 2 was not completed or had > 2 errors, then the older adult could not participate since this was considered the baseline dietary measurement. Other ineligible conditions were caloric intake reports < 500 kcals/day or > 3500 kcals/day for women and < 800 kcals/day or > 4000 kcals/day for men. Those that missed any of the study variables, including lean muscle mass measurements at year 2 or year 5, could not be included for analysis.

Participants were measured on body composition (lean mass, aLM, and weight) annually. Dietary assessment was obtained through a FFQ at year 2, specifically developed for this study, and focused on total protein intake along with the protein source (animal- or vegetable-based). For analysis, protein intake was evaluated as both a continuous and categorical variable by using sex-specific quintiles. Consistency and higher-quality data collection was of high importance so trained interviewers monitored participants periodically throughout the study period. Other potential cofounders were demographic characteristics (age, sex, race, and study site), smoking status, alcohol consumption, physical activity level, prevalence of health conditions at baseline (ischemic heart disease, diabetes, CHF, cerebrovascular disease, cancer, and COPD), use of oral steroids, and occurrence of interim hospitalizations.

A total of 2066 community-dwelling older adults completed the study. Participants in the highest protein quintile groups significantly lost about 40% less lean mass and aLM in comparison to those in the lowest protein quintile groups ($p < 0.01$ trend). After completely adjusting for potential cofounders and energy intake, protein intake was associated with body

composition changes throughout the study's 3 year process; lean mass was $p = 0.004$ and aLM was $p = 0.001$, respectively. In regards to sex differences, men typically consumed more dietary protein (70.8 g/kg/day) compared to the women (60.9 g/kg/day). Total protein and animal protein intakes were significantly associated with lean mass ($p < 0.01$) and aLM ($p < 0.01$). Protein intake was also associated with aLM changes in participants who gained or lost weight, but not for those who had weight stability.

In summary, this was the first longitudinal study conducted to examine the role protein has on body composition characteristics. Protein intake was found to be significantly associated with lean mass changes, even after adjusting for FM, and may affect overall aspects of body composition in community-dwelling older adults. Although, there were small lean mass changes over 3 years, if this study was prolonged there may be even more significant lean muscle mass changes. Therefore, low intake of dietary protein may be a modifiable factor in preventing sarcopenic losses in the elderly population.

Some constraints of this study were the larger intakes of animal protein compared to plant protein, which may have minimized the significant association found between vegetable-based proteins and lean muscle mass variations. Also, dietary information was obtained by a single FFQ so this may have impeded the results by not representing the typical consumption of older adults over 3 years. The choice of study design inhibited the researchers from developed a casual association between protein intake and body composition. Future research should emphasis the impact of different protein sources.

Ruiz Valenzuela et al. (2013)

Healthy older adults may require anywhere between 1.0-1.5 g/kg of protein to achieve adequate nitrogen balance, according to some prior research (Morley, 2008; Morais, Chevalier, & Gougeon, 2006; Campbell, Crim, Dallal, Young, & Evans, 1994). Therefore, Ruiz Valenzuela et al. (2013) conducted a cross-sectional pilot study to assess the intake of dietary protein and distribution during mealtimes while exploring their association with aLM in healthy older adults. A neutral rating was given for this study after completing the Quality Criteria Checklist; see Appendix E. An older adult was invited to participate based on home visits and active participation through telephone calls and posted announcements. All subjects had to be physically independent according to the scale of Lawton and Brody and had to be deemed "healthy" based on the results from self-reports and biochemical analyses during their clinical examinations. In detail, the Lawton and Brody Scale combined aspects of the Physical-Self Maintenance Scale (PSMS). Subjects were asked on competency levels of daily behaviors like toileting, feeding, dressing, grooming, locomotion, and bathing, and the Instrumental Activities of Daily Living (IADL) scale, which encompassed tasks such as shopping, cooking, and laundry abilities (Lawton & Brody, 1969). The Pfeiffer scale was also utilized to establish whether a participant had full intellectual functioning, determined by a 10-item Short Portable Mental Status Questionnaire (SPMSQ) (Pfeiffer, 1975a). The SPMSQ consisted of questions such as, "What is the date today?" and "What day of the week is it?" Therefore the fewer number of errors, the better the subject's mental status (Pfeiffer, 1975b). Other inclusion criteria consisted of being free of major chronic diseases (heart disease, stroke, cancer, chronic respiratory disease and diabetes) by reviewing their clinical history for confirmation. To prevent dietary data discrepancies, all three 24-hour recalls had to be completely free of errors and have no dietary restrictions or evidence of protein supplementation. Participants could not have any recent

weight loss, physical disabilities, and body composition exceeding margins of the DXA machine. All measurements were gathered baseline.

Every participant underwent a medical assessment (biochemical analyses that involved eight hours of fasting, body composition by DXA, anthropometry including body weight, height, waist circumference, and BMI). The 24-hour recalls determined average daily protein intake and how much protein was consumed per meal (indicated as g/breakfast, g/lunch, and g/dinner). The main food sources were assessed by looking at average protein intake (grams), quality of protein provided from each food and frequency of consumption. All procedures were performed by trained, standardized personnel to ensure appropriate data completion. Thereafter, participants were split into two groups depending on how much protein was consumed per meal; this was based on the current recommendations of 25-30 g/meal. Group A consisted of those with an intake < 25 g at each mealtime while Group B consisted of those who consumed > 25 g of protein for at least one meal.

A total of 78 non-Caucasian older adults (60% female) with a mean age of 68.7 $\pm$ 6.3 years old completed the study. Men consumed 13.4 more grams of protein/day than women ($p < 0.05$) with the average daily protein consumption at 0.9 g/kg/day. Overall, only 28% of subjects reached 100% of the DRI for protein. At breakfast 81% of older adults consumed below the RDA for protein while 86% consumed less than the RDA for protein at dinner meals compared to the recommended 25-30 g per meal ($p < 0.05$). There was a significant effect between the amount of protein consumed per mealtime and muscle mass. It was observed that participants in Group A differed in aLM compared to Group B (15.9 $\pm$ 0.9 kg versus 19.1 $\pm$ 0.6 kg, $p < 0.01$).

The overall conclusion was that while protein intake was generally higher than the recommended amount for healthy older adults, participants still failed to achieve the higher

levels reported to offset the effects of sarcopenia (1.0-1.3 g/kg/day). Moreover, the inadequate protein consumption during breakfast and dinner mealtimes showed a significant difference between protein consumption per meal and loss of aLM in older adults throughout this study. For this reason, both low protein intake and inadequate protein distribution may lead to increased muscle mass loss and risk of sarcopenia.

This study was given a neutral rating due to its many weaknesses and limitations found with the methodology. Although this was the only study to focus on sarcopenic Hispanic older adults, the sample size was small, which may not have been representative of the aging population. There was also no mention of blinding to prevent research bias so the protein intake distribution groups may not have been comparable. The dietary information was gathered using self-reports, which could have limited data reliability. The nature of this study design did not include an intervention, which would have be useful when comparing those with or without sarcopenia and protein consumption. This would have provided more information as to what high-quality protein sources were effective in preserving muscle mass in these sarcopenic individuals; therefore a neutral rating was given for this study.

Meng et al. (2009)

Few longitudinal studies have focused on the effects protein intake can have on both bone health and muscle in elderly women so Meng et al. (2009) conducted a 5 year prospective cohort trial. The purpose was to determine if there was an association between dietary protein intake and bone-free lean mass and muscle size at baseline compared to bone mass at year 5, in community-dwelling older women. This study received a neutral rating from the Quality Criteria Checklist; for more detail refer to Appendix F. Older women between the ages of 70 and 85

years old, were recruited from the Western Australian population of white origin by sending letters to women from the voting registry. Although this sample favored women of higher economic status, the authors assured they still represented the general population. Women were included if they completed a FFQ at baseline and had their whole body composition and BMC evaluated at year 5. However, women were excluded if they had a medical condition preventing them from living for the full length of the study and were taking bone active medications (calcium, estrogen, bisphosphonates, and vitamin D). Subjects were randomly placed into two groups, the calcium treatment group, which required a daily intake of 1.2 g calcium carbonate supplement (n = 450), or a matched placebo group (n = 412).

A self-administered FFQ was used to evaluate daily energy, protein, carbohydrate, fat and calcium consumption from the previous year, was completed at baseline. BMI was obtained at baseline and year 5; whereas, upper arm muscle area, calculated using right upper arm muscle area (UAMA) and triceps skin folds, were only measured at the year 5 follow-up. Also at year 5, body composition measurements of lean muscle mass, referred to as bone-free lean mass, and BMC of the arms and legs were added together to determine lean muscle mass and appendicular BMC. Demographic and lifestyle factors were obtained from a questionnaire to categorize subjects into sedentary and active groups.

The final sample size comprised of 862 older women for statistical analysis. Initially, the elderly women had a mean age of 75 $\pm$ 3 years old and a BMI of 26.8 $\pm$ 4.4 kg/m2 (representing 23% obese, 40% overweight, 35% normal weight, and 2% underweight). Subjects consumed 80.6 $\pm$ 27.6 g of protein/day (19 $\pm$ 3% of their total energy intake). A total of 771 (89%) subjects consumed $\geq$ 0.75 g/kg/day of protein daily and 615 subject (71%) consumed > 0.94 g/kg/day. Subjects in the highest protein quartile (> 87 g/day) had significantly higher weight, BMI, and

physical activity levels than those in the lowest quartile (< 66 g/day). Women in the highest quartile (> 87 g/day) also consumed higher amounts of energy, fat, carbohydrates, and calcium.

At year 5, whole body lean mass represented approximately 55 $\pm$ 5% total body mass while aLM and fat mass was 34 $\pm$ 6% of total body mass and UAMA represented approximately 45.5 $\pm$ 12.4 cm^2. Total body and aLM were positively correlated to baseline protein intake, weight, height, energy intake, and physical activity at year 5. Whole body lean mass had the strongest correlation with protein intake ($r = 0.18$, $p < 0.001$) compared to the other macronutrients. Protein intake also had a positive association between aLM and BMC ($r = 0.14$-0.18, $p < 0.001$), UAMA ($r = 0.08$, $p < 0.05$), and whole body fat mass. After adjusting for potential confounding factors, women in the highest quartile group had significantly higher whole body lean muscle mass and aLM compared to the lower protein intake groups. Furthermore, the highest quartile for UAMA had significantly greater muscle mass. At baseline, positive associations were discovered between bone, whole body BMC and appendicular BMC and protein and energy intakes, weight, height, and physical activity; conversely, age had a negative impact. The strongest correlation was between whole body BMC and protein intake. Women consuming the most protein had 5.3% higher BMC values than the other intake groups, but this did not remain significant at year 5.

In conclusion, protein intake can have a positive impact on bone health due to its maintenance of lean muscle mass. The highest protein intake quartile of > 87 g/day (1.6 g/kg/day) showed favorable effects on lean muscle mass and BMC for women > 70 years old. Overall, these researchers suggested that the RDA for protein should be between 1.0 to 1.25 g/kg/day to offset the aging metabolism.

As a result of a neutral rating, there were some important limitations and weaknesses to address. This study may not be feasible due to the extensive anthropometric measurements used. Additionally, no baseline body composition data was gathered. As a result, the researchers were unable to determine the time frame between high protein intakes and changes in body composition in elderly women. Within the methodology, subjects were separated into two groups, but this seemed unnecessary since minimal statistical analyses were conducted to compare groups. No potential study populations were mentioned in the discussion part of this study, although demographic and lifestyle factors and FFQ were self-administered. Also, a protein-rich food intervention was not included to express a cause-and-effect relationship.

Scott et al. (2010)

Scott et al. (2010) evaluated whether or not there were any correlations between dietary nutrient intake and the development of sarcopenia using a prospective cohort design. This study received a neutral rating from the Quality Criteria Checklist; see Appendix G. Participants were selected by electoral rolls that used random sex-stratified sampling replacement. If an older adult was between the ages of 50-79 years old, community-dwelling, and resided in South Tasmania they were included in the study. However, if they were institutionalized or had contraindications with magnetic resonance imaging they were not able to participate.

Participants were evaluated on BMI, leg strength, physical activity using a pedometer (baseline was considered the average steps over 7 days), and dietary intakes using a self-administered FFQ that evaluated total energy and 28 nutrients. To ensure consistency and completion, the FFQ was checked by interviewers so each could be efficiently analyzed. All measurements were obtained at baseline and follow-ups, 2 year and 3 year. Participants were

later stratified into two protein intake groups as followed: 1) failing to meet the current RDI for protein (< 0.75 g/kg/day) and 2) meeting or exceeding the RDI for protein (≥ 0.75 g/kg/day) (Commonwealth of Australia, 1991). The Australian RDIs for men, aged 51-70 years old, were 64 g of protein and 81 g of protein for those > 70 years old while women, in the same age groups, should consume 46 and 57 g/day.

A total of 740 participants (50% female) with a mean age of 62 ± 7 years old, were included for analysis. Eighty-nine (12%) of older adults failed to meet the RDI for protein, at baseline, while 106 (14%) consumed inadequate amounts of protein at follow-ups. Participants below the RDI for protein had significantly lower aLM at baseline and follow-ups [-0.81 kg, 95% CI (-1.54 to -0.08); p = 0.03 and -0.79 kg, 95% CI (-1.42 to -0.17); p = 0.01, respectively] as well. However, muscle strength did not differ between the two protein intake groups. The nutrients found to have a positive correlation to aLM were included in a forward stepwise regression model; a significance level of $p < 0.10$ was required for inclusion. As a result, protein was the only macronutrient to be a significant independent predictor of aLM changes ($p = 0.007$) hence, a higher long-term protein intake may reduce age-related muscle mass declines. After further protein intake adjustments, other nutrients also found to be significantly related to positive aLM changes were iron, magnesium, phosphorus, and zinc.

In conclusion, protein intake in addition to other nutrients, were positive predictors of changes in muscle mass and rates in muscle losses in older adults, except for muscle strength. Diet alone may not be able to offset age-related muscle strength without some form of physical activity, but it was evident diet plays an important role in maintaining muscle function. It was suggested that there could potentially be many different nutrients that delay the progression of sarcopenia as a person ages. Many of the positively associated nutrients can be found within

many animal meats, such as iron and zinc, which reflects the importance of consuming adequate high-quality protein to maintain aLM. Therefore, future studies should evaluate dietary associations and the progression of sarcopenia in younger individuals to examine how these nutrients influence initial muscle mass declines.

There were several limitations within this methodology and study design, considering the neutral rating. During recruitment the initial response rate was low (57%), although the retention rate at follow-up was high (82%), which may have minimized subject diversity. It was unclear whether or not protein intake impacted muscle strength since no significant association was found. There was also a lack of nutrient intake and muscle mass differences between participants who completed the study and those who did not comply up to the follow-up. Both of these study characteristics may have limited the scope of potential participants included in the study. The subjects and investigator may have been bias since blinding was not described. Additionally, emphasis was solely placed on nutrients positively associated with aLM. Overall, the results were not generalizable to all older adults since the majority of participants were 70-79 year old white, community-dwelling older adults.

Gregorio et al. (2013)

Gregorio et al. (2013) evaluated the relationship between protein intake, body composition, and physical performance in community-dwelling, independent post-menopausal females. The researcher's hypothesized physical function would be higher in subjects who consumed greater amounts of protein compared to those with lower protein intakes. After completing the Quality Criteria Checklist, this study was given a neutral rating. Refer to Appendix H. For study analysis, baseline measurements were obtained from healthy females

between 60-90 years old who participated in one of three intervention trials. The first study evaluated dehydroepiandosterone (DHEA) plus gentle aerobic or yogic exercise, the second compared 1.2 g fish oil supplementation against a placebo, and the third study was undergoing enrollment so the only information provided was women were included based on reports of lower protein intake. Females being treated for osteoporosis and other diseases, consuming medications affecting bone metabolism, or had a life expectancy < 2 years were excluded from all studies. In the current study, all three studies were combined to increase the sample size and demonstrate a wider variety of protein intakes and physical abilities; this combined sample size assessed body composition, in addition to lean mass.

The assessment of lean muscle mass included total lean mass, appendicular skeletal mass aLM, and FM measurements. Muscle strength was assessed using the Physical Performance Test (PPT) to measure strength of the upper extremities, motor function, mobility, and coordination when participating in simple functional tasks. To measure physical function, the Short Physical Performance Battery (SPPB) was implemented to measure lower extremity function (balance, walking speed, and strength). The higher the scores for the PPT and SPPB tests, the greater the subject's physical performance and functional abilities. The Physical Activity Scale in the Elderly (PASE) and the Medical Outcomes Survey Short-form 8 (MOS SF-8), utilized in the first and third study, were implemented in the current study as well. The PASE questionnaire assessed physical activity; a higher score equated to greater activity. The MOS SF-8 questionnaire evaluated participants' health related to quality of life; a higher score represented better well-being. A registered dietitian interviewed the subjects to obtain a four-day food record.

A total of 387 post-menopausal females, with a mean age was 72.7 $\pm$ 7.0 years and 95.5% were Caucasian. Subjects in the lower protein intake group had more incidences of hypertension,

osteoarthritis, and bone fractures. For BMI, 43% of subjects were in the normal range, 33% were overweight, and 23.5% were considered obese; however, 0.5% of individuals were unaccounted for. The mean protein consumption was 72.2 g/day (1.1 g/kg/day), ranging from 0.31 g/kg/day to 3.16 g/kg/day. Ninety-seven (25%) subjects consumed < 0.8 g/kg/day of protein, whereas 290 (75%) ate > 0.8 g/kg/day; these represented the low and high protein intake groups. Females in the higher protein group were lower in weight, BMI, and fat and lean mass than those who consumed low levels of protein. After adjusting for BMI, bone fractures were more prevalent in the low protein intake group. There were significant differences between physical performance measures and protein intake groups when evaluating mean $\pm$ SD scores. On average, women in the high protein group performed better on five out of six physical performance tests (PPT, SPPB, single leg stance, timed 8 foot walk, and average handgrip strength), compared to those who consumed less protein. After adjusting for IBW and lean mass, chair rise time became statistically significant and there were significant differences between SPPB and PPT test scores. In particular, females in the low protein group performed significantly inferior in the single leg stance part of the SPPB test ($p < 0.002$) and walking speed ($p = 0.006$) when groups were compared.

The authors concluded on average healthy older post-menopausal females consumed 1.1 g/kg/day of protein with only 25% of subjects consuming below the RDA for protein. However, those who consumed less protein experienced significantly more bone fractures, which suggests physical performance may be an outcome for adequate protein, in addition to, muscular health. Equally important, higher protein intake was representative of better performance on the self-reported and physical function tests compared to those in the low protein group. However, despite the lack of association between lean muscle mass and protein intake, this study

demonstrated how dietary protein may influence walking speed and single leg stance time. Therefore, dietary protein may be a predictive factor for physical performance, bone, and muscular health for older post-menopausal females in delaying sarcopenia.

A limitation was the researchers did not evaluate what protein sources, animal-or plant-based, the subjects consumed to establish which is more effective at increasing lean muscle mass, physical function, and strength. Protein intake was only separated into two broad categories, high and low intakes, which may have made it difficult to determine what the optimal amount of protein was for elderly females to prevent sarcopenia and improve physical performance. This sample was not generalizable since it focused on community-dwelling older Caucasian females. This study was also not free of bias since blinding was not mentioned and it was unclear how withdrawals were handled. Despite the fact this study emphasized females, one of the previous trials involved only older males; therefore, it is unclear how this study was included in this sample population. A cause-and-effect relationship could not be determined, which weakened the association between protein intake and physical performance. As a result, this study received a neutral rating on the Quality Criteria Checklist.

Aleman-Mateo et al. (2012)

The Aleman-Mateo et al. (2012) study was given a positive rating after the Quality Criteria Checklist was utilized; see Appendix I. The purpose was to test whether adding a protein-rich food item, ricotta cheese, into the habitual diet of elderly individuals, would increase their total appendicular skeletal muscle mass (TASM) and strength. Participants were recruited from home visits and phone calls within Sonora, Mexico to undergo medical screenings, including DXA measurements, to determine the presence of sarcopenia. Participants were further

excluded if they were < 60 years old, not physically independent, had type 2 diabetes (or glucose > 126 mg/dL), had microalbuminuria, refused to eat ricotta cheese or fully participate, or reported a lactose intolerance or allergy. If diagnosed with sarcopenia, another medical examination involving biomarkers of hemoglobin, fasting glucose, lipid profile, hepatic profile, and glomerular filtration rate for kidney function was conducted. All measurements were obtained at baseline and after the 3 month intervention to eliminate adverse effects of additional protein needed for kidney function. Other measurable variables were the insulin-like growth factor and insulin resistance assessment and various anthropometric data (body weight, BMI, TASM, total body mass, and muscle strength using the handgrip strength test).

Subjects (n = 40) were randomly selected for the control or intervention group (1:1). In detail, this represented 23 women and 17 men with a mean age of 76 $\pm$ 5.4 years for age and BMI of 26.3 $\pm$ 3.8 kg/m2. Those in the control group continued their habitual diet (HD) while the intervention group (RCH + HD) added 210 g of ricotta cheese to every meal (breakfast, lunch, and dinner). The 210 g of ricotta cheese (equated to 15.7 g protein and 267 extra kcal/day) were previously portioned, weighed, and packaged. Personnel visited participants three times per week for diet compliance and to ensure participants continued their typical daily physical activities. After taking baseline measurements, participants had two weeks until starting the intervention, whereas follow up measurements were taken after the last day of ricotta cheese consumption. However, those who were unable to adhere to the intervention were still used for statistical analysis. A sample size of 40 was calculated to provide an 80% study power, which allowed lean body mass differences to be identified between the two equally dispersed groups (20 subjects per group). Correspondingly, intergroup outcomes were analyzed under the intention-to-treat strategy.

However after study completion, only 12 subjects in the RCH + HD group and 17 in the HD group passed all protocol. After 3 months TASM measurements indicated the percent of relative change was not significantly different between groups, but the RCH + HD group showed a positive tendency towards significance ($p = 0.06$). Men in the RCH + HD group gained 1.6 kg and 490 g of TASM while men in the HD group, gained 220 g of TASM and lost weight (equivalent to 2.2 lbs.). In contrast, women in the RCH + HD group gained 260 g of TASM whereas in the HD group women only gained 220 g, respectively. Women in both groups lost an estimated 800 g of total body weight. Overall, body weight, lean muscle mass, and muscle strength showed a significantly positive trend for the men, whereas women in the RCH + HD group only showed slight positive tendency for muscle strength. For biological measures, fasting insulin levels decreased by 10.1% for men in the intervention group while men in the control group had an increase of 5.1% ($p = 0.05$).

According to the author's conclusion, adding 210g of ricotta cheese to a habitual diet, did not prohibit the loss of TASM in free-living sarcopenia elderly, but showed a positive correlation for muscle strength in males and females in the intervention group. In particular, elderly men received the most substantiated benefits of consuming a high-quality protein food since men in the RCH + HD group gained 270 g of TASM more than those in the HD group. Men in the intervention group also improved in muscle strength, lean mass in the arms, and body weight. However, older men could have received greater positive results since 25% of women in the RCH + HD group reported adverse effects of early satiety after ingesting the ricotta cheese. Therefore implementing a high protein intervention, such as ricotta cheese, may help increase total protein consumption, but may not completely be effective for sarcopenic elders. While there

may be gender differences in regards to muscle strength, lean tissue in the arms, and body weight, there may be potential benefits as more studies are conducted.

The primary limitation was the calculated sample size was based on gains in lean body mass and not TASM or strength. The elderly women reported difficulty consuming the large ricotta cheese portions, whereas most of the men had higher adherence. Consequently, this may have skewed the results, in addition to, the fact that the statistical analyses included subjects who did not meet all study protocols.

Table 8: *Article Summarizes.* This table summarizes the key aspects of each inclusionary article with details specifically pertaining to the research question.

Author, Year, Study Design, Class Rating	Study Purpose	Study Population	Intervention/Methodology	Outcomes	Conclusion	Limitations
Gray-Donald K, St-Arnaud-Mckenzie D et al. 2014 **Study Design:** Prospective case-control study **Class:** C **Rating:** +	To assess the relationship between protein intake and rate of a one year incidence of ≥ 5% weight loss in community-based, healthy older adults.	Quebec, Canada From previous NuAge Study n = 422 (57.3% female) Cases (n = 211) Controls (n = 211) Subsample (n= 60)	2 year study Separated subjects into 4 protein intake categories to determine differences in weight loss: Low (< 0.08 g/kg) Moderate (0.8-1.0 g/kg) High (1.0-1.2 g/kg) Very high (≥ 1.2 g/kg) Subsample: analyzed effect PRO intake on LM by evaluating body composition (baseline and 2-yr)	Highly significant correlation between protein intake and weight loss (p = 0.005). Participants who consumed ≥ 1.2 kcal/kg/day of PRO compared to those who consumed < 0.8 kcal/kg/d, were twice as likely to lose weight (P = 0.018), while those in the moderate category were 70% more likely to lose weight (P = 0.039). Participants were 2.56 and 2.15 times at greater chance of losing weight in the low- and moderate-protein-intake	Compared to the highest protein intake group, older adults who consumed a moderate amount of protein (0.8- 1.0 g/kg/day) were twice as likely to lose weight and those who consumed < 0.8 g/kg/day were 2.5 times at greater risk for weight loss. Protein intakes > 1.0 g/kg are protective against weight loss in healthy older adults.	Lack of direct impact of protein intake on LM mass May not be applicable to frail or ill elderly Minimal influence between inflammation and LM mass.

				categories than the higher protein intake groups.	Subsample lost 0.5 kg greater LM compared to the control group at two time points, 2 years apart.	
Scott D, Blizzard L et al. 2010 **Study Design:** Prospective Cohort **Class:** B **Rating:** ø	To evaluate whether or not there were any correlations between dietary nutrient intake and the development of sarcopenia in healthy, community-dwelling older adults.	South Tasmania, Australia Community-dwelling older adults n = 740 (50% female) 62 ± 7 yrs old	3 year study Separated subjects into 2 protein intake categories for analysis: 1) Failing to meet the Australian RDI for protein (< 0.75 g/kg/day) 2) Meeting or exceeding the Australian RDI for protein (≥ 0.75 g/kg/day)	At baseline, 89 (12%) of older adults failed to meet the RDI for protein. 106 (14%) consumed inadequate amounts of protein at follow-up. Participants below the RDI for protein had significantly lower aLM at baseline and follow-ups (-0.81 kg, 95% CI (-1.54 to -0.08); p = 0.03 and -0.79 kg, 95% CI (-1.42 to -0.17); p = 0.01, respectively). Muscle strength did not significantly differ.	Protein intake and several other nutrients, were positive predictors of changes in muscle mass and rates in muscle losses in older adults, except for muscle strength.	During recruitment, the response rate was low (57%), although the retention rate at follow-up was high (82%). Results were not generalizable to all older adults since the majority of participants were 70-79 year old white, community-dwelling older adults. Every analysis untaken was reported since no adjustments were made for the multiple comparison tests so emphasis was placed only on nutrients that had a continuous significant relationship with aLM.
Gregorio L, Brindisi J et al. 2013	To evaluate the relationship between	From Central Connecticut area	2 year study Women chosen from previous 3 trials to obtain	Mean protein consumption was 72.2 g/day (1.1 g/kg/day), ranging	Healthy females consumed 1.1 g/kg/day of protein with only	No evaluation of protein sources (animal-or

Study Design: Cross-sectional, observational **Class:** D **Rating:** ø	protein intake, body composition, and physical performance in community-dwelling, healthy post-menopausal females.	n = 387 Mean age: 72.7 ± 7.0 yrs old 95.5% Caucasian Post-menopausal females BMI: 43% normal 33% overweight 23.5% obese (0.5% was unaccounted for)	current sample size: 1) DHEA + gentle aerobic or yogic exercise 2) 1.2 g fish oil supplement again placebo 3) Women with low reports of protein intake (recruitment was going on) All participants were separated into low (< 0.08 g/kg/day) and high (≥ 0.8 g/kg/day) protein intake groups for study analysis.	from 0.31 g/kg/day to 3.16 g/kg/day. 97 (25%) subjects consumed < 0.8 g/kg/day of protein, whereas 290 (75%) ate ≥ 0.8 g/kg/day. Females in the low protein group performed significantly inferior in the single leg stance part of the SPPB test ($p < 0.002$) and walking speed ($p = 0.006$) when groups were compared. Women in the high protein group performed better on five out of six physical performance tests (PPT, SPPB, single leg stance, timed 8 foot walk, and average handgrip strength). No significant different in muscle strength.	25% of subjects consuming below the RDA for protein. Women who consumed less protein experienced significantly more bone fractures, suggesting physical performance may be an outcome for adequate protein, in addition to, muscular health. Higher protein intake was representative of better performance on the self-reported and physical function tests compared to those in the low protein group. No association was found between LM and protein intake, but those with higher BMI and fat/lean ratio were more likely to consume < 0.8 g/kg/day of protein.	plant-based) were obtained. Protein intake only separated into 2 broad categories. Sample might not be generalizable since all Caucasian community-dwelling post-menopausal females. Cause-and-effect relationship could not determined. Total caloric intake was not controlled for when total protein intake was analyzed. This could have affected the validity of results.
Geirsdottir L, Arnarson A et	To determine	Reykjavik, Iceland	Participant were recruited from a previous 12-week	82% reported regular physical	Dietary protein intake was	A cause-and-effect

al. 2013 **Study Design**: Cross-sectional **Class:** D **Rating:** ø	whether there was a link between dietary protein consumption and leisure-time physical activity by assessing lean body mass (LBM) in community-dwelling, healthy older adults. Secondary objective was to determine whether the current RDA for protein was sufficient for this age group.	Volunteered n = 237 (58% female) Between 65-92 years old Mean age: 73.6 ± 5.7 yrs old	intervention resistance training program. For analysis, subjects were grouped into 4 different protein intake quartile groups; quartile groups not specified.	activity. Significant differences of 2.3 kg of LBM between the 4th quartile (1.36 ± 1.19 g/kg of protein) and 1st quartile (0.63 ± 0.08 g/kg of protein) and trending towards significance of 2.0 kg between the 4th quartile (1.36 ± 1.19 g/kg of protein) and second (0.85 ± 0.05 g/kg of protein) quartiles. Daily protein intake was considered a positive predictor of LBM as hypothesized.	positively associated with LBM in community-dwelling older adults through validation that the average participant consumed protein levels above the current RDA (0.8 g/kg), indicative of a higher LBM. No correlation between leisure-time physical activity and LBM, which suggested that endurance exercises may not be connected to LBM for this population regime.	relationship or evaluate prolonged behaviors impacting LBM. Majority of the protein intake was predominately from animal sources, questioning the impact of plant proteins on LBM. Since participants were volunteered they may not be representative of general population.
Houston DK, Nicklas BJ et al. 2008 **Study Design:** Prospective Cohort **Class:** B **Rating**: +	To determine if there was an association between dietary protein consumption and alterations in LM and aLM in healthy, community-dwelling older adults.	Medicare-eligible residents within the metropolitan areas of Pittsburg, PA and Memphis, TN. n = 2066 (53.2% female) Community-dwelling Black/Caucasian Between ages	3 year study After dietary assessment separated participants into 5 sex-stratified protein intake categories depending on dietary assessments depicted as g/kg/day of protein (median total protein intake as a percentage of total energy intake): Q1: 0.7 g/kg/day (11.2%) Q2: 0.7 g/kg/day (12.7%) Q3: 0.8 g/kg/day (14.1%) Q4: 0.9 g/kg/day (15.8%)	Participants in the highest protein quintile group (1.1 g/kg/day) significantly lost about 40% less LM and aLM in compared to those in the lowest protein quintile groups ($p < 0.01$ trend). Men typically consumed more dietary protein (70.8 g/kg/day) in comparison to the	Protein intake was associated with significant LM changes, even after adjusting for FM, and may affect overall aspects of body composition in community-dwelling older adults. There were small LM changes over 3	Larger intakes of animal protein were consumed compared to plant protein so this may have minimized the significant association found between vegetable-based proteins and LM variations. No cause-and-

		70-79 yrs old Mean age: 74.5 yrs old	Q5: 1.1 g/kg/day (18.2%)	women (60.9 g/kg/day). Total protein and animal protein intakes were significantly associated with LM ($p < 0.01$) and aLM ($p < 0.01$). Protein intake was associated with body composition changes throughout the study's 3 year process; LM was $p = 0.004$ and aLM was $p = 0.001$. Protein intake was associated with aLM changes in participants who gained or lost weight, but not for those who had weight stability.	years, if this study was prolonged there may be even more significant LM mass changes. Low protein intake may be a modifiable factor in preventing sarcopenic losses in the elderly population.	effect relationship could be established.
Meng X, Zhu K et al. 2009 **Study Design:** Prospective randomized controlled cohort **Class:** B **Rating:** ø	To determine if there was an association between dietary proteinintake and bone-free lean mass and muscle size at baseline compared to bone mass at 5-yr, in community-dwelling, healthy older women.	Western Australian population n = 862 70-85 yrs old Mean age: 75 ± 3 yrs Caucasian All females Mean BMI: 26.8 + 4.4 kg/m^2 Majority of higher economic status (researchers assured this is not affect results).	5 year study Separated into 2 groups: 1) (n = 450) 1.2 g calcium carbonate supplement 2) (n = 412) matched placebo group Participants were separated into 3 protein intake groups depending on dietary assessment: 1st tertile: (n = 287) < 66 g/day (0.84 ± 0.19 g/kg/day) 2nd tertile: (n = 287) 66-87 g/day (1.17 ± 0.22 g/kg/day) 3rd tertile: (n = 288) > 87 g/day (1.64 + 0.44	Subjects consumed 80.6 ± 27.6 g of protein/day (19 ± 3% of their total energy intake). A total of 771 (89%) subjects consumed > 0.75 g/kg/day of protein daily and 615 subject (71%) consumed > 0.94 g/kg/day. Subjects in the highest protein quartile (> 87 g/day) had significantly higher weight, BMI, and physical activity levels than those in	Protein intake can have a positive impact on bone health due to its maintenance of LM mass. The highest protein intake quartile of > 87 g/day (1.6 g/kg/day) showed favorable effects on LM mass and BMC for women > 70 years old. Researchers	No baseline body composition data was gathered. Researchers were unable to determine the time frame between high protein intakes and changes in body composition in elderly women. No cause-and-effect relationship can be established.

			g/kg/day)	the lowest quartile (< 66 g/day). At the 5-yr, whole body LM represented approximately 55 ± 5% total body mass while aLM and fat mass was 34 ± 6% of total body mass. Whole body lean mass had the strongest correlation with protein intake ($r = 0.18$, $p < 0.001$) compared to the other macronutrients. Protein intake also had a positive association with aLM and BMC ($r = 0.14$-0.18, $p < 0.001$), UAMA ($r = 0.08$, $p < 0.05$), and whole body fat mass. Women consuming the most protein had 5.3% higher BMC values than the other intake groups, but this did not remain significant at 5-yr.	stated the RDA for protein should be between 1.0 to 1.25 g/kg/day to offset the aging metabolism.	
Aleman-Mateo Macias L et al. 2012 **Study Design:** RCT	To test whether adding a protein-rich food item, namely ricotta cheese, into the habitual	From Sonora, Mexico n = 40 (23 women, 17 men) Ethnicity unspecified	3 month study Randomly selected (1:1) for the control or intervention group. **Control:** HD Following habitual diet (HD)	TASM (total appendicular skeletal muscle) measurements indicated the percent of relative change was not significantly different between	Adding 210g of ricotta cheese to a habitual diet, did not prohibit the loss of TASM in free-living sarcopenic elderly, but	Sample size was calculated based on gains in LBM and not TASM or strength. Women had difficulty

Class: A **Rating:** +	diet of sarcopenic elderly individuals, would increase their total appendicular skeletal muscle mass (TASM) and strength.	mean age: 76 ± 5.4 yrs Mean BMI: 26.3 ± 3.8 kg/m^2 After study completion n = 29, but all 40 subjects were included in analyses.	(n = 12) **Intervention:** RCH + HD Following habitual diet (HD) + adding 210g of ricotta cheese to breakfast, lunch, and dinner (RCH) (n = 17)	groups, but the RCH + HD group showed a positive tendency towards significance (p = 0.06). Men in the RCH + HD group gained 1.6 kg and 490 g of TASM while men in the HD group, gained 220 g of TASM and lost weight (equivalent to 2.2 lbs.). Women in the RCH + HD group gained 260 g of TASM whereas in the HD group women only gained 220 g, respectively. Women in both groups lost an estimated 800 g of total body weight. Body weight, LM mass, and muscle strength showed a significantly positive trend for the men, whereas women in the RCH + HD group showed only slight positive tendency for muscle strength.	showed a positive correlation for muscle strength in males and females in the intervention group. Men in the intervention group showed the most substantiated benefits with improved muscle strength, LM in arms, TASM weight gain, and body weight. Men could have received greater positive results since 25% of women in the RCH + HD group reported adverse effects of early satiety after ingesting the ricotta cheese. A high protein intervention, such as ricotta cheese, may help increase total protein consumption, but may not be completely effective for sarcopenic elders.	consuming large ricotta cheese portion so may have skewed results. Statistical analyses included subjects who did not meet all study protocols. Small sample size. Cofounding variables were not controlled for along with assessing other dietary components between groups.
Ruiz	To assess	Northwest	Participants were	Men consumed	While protein	Small sample

Valenzuela RE, Morales-Figueroa GG et al. 2013 **Study Design**: Non-probability Cross-sectional **Class:** D **Rating:** ø	dietary proteinintake and distribution during mealtimes while exploring their association with aLM in healthy older adults.	Mexico residents. n = 78 (60% female) Non-Caucasian Mean age: 68.7 ± 6.3 yrs old	separated into 2 protein intake groups: **Group A:** < 25 g of protein intake at each meal **Group B:** > 25 g of protein intake for at least one meal.	13.4g of protein/day more than women ($p < 0.05$) with the average daily proteinconsumption at 0.9 g/kg/day. 28% of subjects did reach 100% of the DRI for protein. Breakfast and dinner meals had the least amount of protein when compared to the recommended 25-30 g per meal ($p < 0.05$). There was a significant effect between the amount of protein consumed per mealtime and muscle mass. It was observed that participants in Group A differed in aLM compared to Group B (15.9 ± 0.9 kg versus 19.1 ± 0.6 kg, $p < 0.01$).	intake was generally higher than the recommended amount for healthy older adults, participants still failed to achieve the higher levels reported to offset the effects of sarcopenia (1.0-1.3 g/kg/day). Throughout this study, inadequate protein consumption during breakfast and dinner mealtimes showed a significant difference between protein consumption per meal and loss of muscle mass in older adults. Both low protein intake and inadequate protein distribution may lead to increased aLM loss and risk of sarcopenia.	size. Lack of timeline between certain measurements (between baseline and follow-up). Only depicts protein intake at one point in time due to the nature of the study.
Morris & Jacques 2013 **Study Design:** Cross-	To figure out the answers to some of the unknown questions regarding	Tufts University Boston, MA n = 2425 (55.6% female)	Secondary analysis from participants NHANES Survey Data from 2003-2004 and 2005-2006. Participants > 50 years old	Beef consumption significantly correlated to total protein intake ($r = 0.19$; $p < 0.001$)	The higher good quality protein sources consumed, like beef, may be helpful when	This study design only illustrated one time period so results may not be

sectional **Class**: D **Rating:** ø	age-related muscle losses and strength declines, by evaluting the National Health and Nutrition Examination Survey (NHANES) data from 2003-2006, on participants ≥ 50 years old.	84.7% Non-Hispanic White Mean age: 63 years old	DXA scan measurements were only available from the 2003-2004 survey, A total of 847 men and 792 women were used for measurement comparison. Participants separated into 3 protein intake groups: 1) < 0.8 g/kg 2) 0.8-1.0 g/kg 3) > 1.0 g/kg	An increase in aLM was significantly related to higher beef intakes; this was exceptionally strong and significant in non-obese subjects who participated in muscle-strengthening exercises. For protein recommendations, it did not matter whether an non-obese individual met the RDA for protein as long as they performed vigorous aerobic activities. Obese subjects who performed muscle-strength training seemed to have greater improvements in aLM, but only if the RDA for protein was met or exceeded.	increasing aLM in older individuals whether obese or not. Exceeding the RDA for protein was only beneficial in obese subjects whereas, non-obese individuals showed no added benefits when exceeding this recommended amount. Muscle strength training exercises preserves muscle mass when added with adequate dietary protein consumption. Aerobic activity may be helpful in preserving aLM, but a diet in high quality protein should be added to see the benefits.	representative of typical dietary intake and/or physical activity in older adults. DXA scans were only available for subjects in the 2003-2004 NHANES survey. Physical activity was based on observation only so subjects may have reported inaccurate results that unintentionally swayed these results. Could have potential conflict of interest since the beef cattle association provided funding so could be the reason behind only evaluating beef intake.

Table 9: *Quality Criteria Article Summarizes.* This table summarizes the results from the relevant and validity questions relative to the Quality Criteria Checklists.

	Morris & Jacques (2013)	Gray-Donald et al. (2014)	Geirsdottir et al. (2013)	Houston et al. (2008)	Ruiz Valenzuela et al. (2013)	Meng et al. (2009)	Scott et al. (2010)	Gregorio et al. (2013)	Aleman-Mateo et al. (2012)
Rating	ø	+	ø	+	ø	ø	ø	ø	+
Relevant Questions									
1	N/A	N/A	N/A	Unclear	Unclear	Yes	N/A	N/A	Yes
2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	Unclear	N/A	Yes	Yes	Unclear	N/A	Yes	Yes	Yes
Validity Questions									
1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
3	N/A	Yes	N/A	Yes	No	Yes	N/A	Yes	Yes
4	N/A	Yes	N/A	Yes	Yes	Yes	Yes	No	No
5	N/A	No	N/A	No	No	No	No	No	Yes
6	N/A	Yes	N/A	Yes	N/A	Yes	N/A	Yes	Yes
7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	Unclear	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	Yes	No	Yes	No	Yes	No	Unclear	Unclear	Yes
10	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Conclusion Statement and Grade

Dietary protein intake is inversely associated with the consequences of sarcopenia (muscle mass, muscle strength, and/or functionality) in older adults. Therefore, meeting or exceeding the RDA for dietary protein intake may play a protective role in preserving muscle mass, which may delay advancements of sarcopenia. Eight of out nine studies found a positive association for muscle mass changes, but the impact on muscle strength and physical

performance characteristics were less consistent. While the ninth study did not find a correlation between protein intake and lean muscle mass, a higher protein intake was affiliated with a healthier body composition of lower fat and higher lean muscle mass. However due to the inconsistencies in defining sarcopenia and diagnostic methods, it is difficult to determine if these results can be deemed as clinically significant. Until a clearer understanding of sarcopenia becomes evident, the benefits of a higher dietary protein consumption can only be recommended to healthy older adults. The lack of sufficient evidence involving sarcopenic elderly makes this dietary recommendation inapplicable until more research pertains to these individuals. Since one study followed an international RDI value as a reference for protein intake and another found no significant association between protein intake and lean muscle mass, seven studies discovered older adults who consumed dietary protein above the RDA of 0.8 g/kg was associated with optimal muscular health. An increase in lean muscle and aLM, have been linked to better health outcomes in older adults by reducing their risk for falls and injuries that could have led to disabilities or bone fractures in the future (Fielding et al., 2011). Therefore, a higher protein intake may be a preventative strategy in the future against the development of sarcopenia as research expands on this topic. However, a six out of nine studies received a neutral rating due to common issues of not controlling for bias, research design flaws, and generalizability limitations.

Grade II: Fair. The evidence is from a majority of weaker study designs, but the results are confirmed and consistent in multiple studies with only minor issues.

CHAPTER 5: DISCUSSION

After analyzing articles using the evidence analysis protocol, nine primary research articles (one case-control, three prospective cohorts, four cross-sectional, and one randomized control trial) pertained to the research question, "Does dietary protein intake, greater than the RDA, delay the consequences of sarcopenia in older adults?" Each study provided an important viewpoint into some of the pathophysiological changes between dietary protein and age-related muscle characteristics. The provided evidence was initially weakened from the lack of randomized controlled trials considering this is the "gold standard" methodology for establishing nutrition interventions (AND, 2012c). Aleman-Mateo et al. (2012) was the only study that depicted a cause-and-effect relationship between a protein-rich food, body composition, and muscle strength. Morris and Jacques (2013) was another relevant study that analyzed a protein-rich food source, but only an association between beef intake and improvements in lean muscle and appendicular skeletal muscle mass could be established; this was due to the nature of the chosen study design.

Despite the lack of randomized controlled trials, according to Bradford Hill's criteria for causation, result consistency can also provide credence towards establishing a stronger causation between protein intake and sarcopenia risk (Schiinema, Hill, Guyatt, Akl, & Ahmed, 2011). Considering all studies, except for Gregorio et al. (2013), found greater lean muscle mass improvements and greater health outcomes were associated with a higher protein intake, this form of causation is applicable. The results from each cohort and cross-sectional study, should not be overlooked for evidence-based practice considering these are the preferred methods when determining the etiology, diagnosis, causation, and/or harm pertinent to sarcopenia research.

Taking into account that these are the clinical and biological aspects of sarcopenia that have remained unclear, these studies attributed pertinent information that could lead towards figuring out an effective dietary intervention to possibly prevent diagnosis.

Every study had a sufficient sample size greater than one-hundred older participants, except for Aleman-Mateo et al. (2012) and Ruiz Valenzuela et al. (2012). However, this is a commonly anticipated challenge for older adult studies since this age group tends to have a higher dropout rate and greater study incompleteness. One of the most prominent issues was the lack of subject diversity since the majority of older adults were community-dwelling, healthy, older Caucasian women. Only Ruiz Valenzuela et al. (2012) focused on Hispanic older adults, whereas Aleman-Mateo et al. (2012) was the sole study that involved sarcopenic elders within their inclusion criteria. Considering one out of nine studies solely involved sarcopenic elders, proves sarcopenia research is extremely lacking. As a result, the evidence may not be applicable to those who are ill, low-functioning, or have sarcopenia. The evidence supported a positive association between a higher protein intake and possibly delaying the consequences of sarcopenia, but sarcopenic elders were not adequately represented to formulate that sarcopenic elders will have these same associations. It should become a priority for future investigators to add sarcopenic elders to their inclusion criteria since this is the target population for primarily increasing the RDA. Those diagnosed with sarcopenia may not experience the same positive outcomes found within these relevant studies and instead may require higher protein than what was found to be beneficial in healthy older adults. As the prevalence of sarcopenia increases and further research on diagnostic criteria is established, as more studies involve these individuals, it will help to strengthen the current associations found when higher dietary protein is consumed.

Another factor that differentiated between studies was how protein intake was measured. Either a FFQ or dietary recall was given to participants to analyze protein consumption, but most were self-reported and may have produced bias, as well as, over and underestimation. Also this may not be the most reliable dietary tool to utilize for this population given that an intact memory is required to ensure reliable results. Therefore, these dietary methods may pose reliability issues for those with different degrees of dementia. This information should be obtained through in-person interviews to minimize some of these data report concerns. The amount of protein that showed the greatest benefits in preserving muscle mass, above the RDA, per study was > 1.0 g/kg/day for Gray-Donald et al. (2014), $\geq$ 0.8 g/kg/day for Gregorio et al. (2013), 1.36 $\pm$ 1.19 g/kg/day for Geirsdottir et al. (2013), 1.1 g/kg/day for Houston et al. (2008), 1.6 g/kg/day for Meng et al. (2009), 0.99 g/kg/day for Ruiz Valenzuela et al. (2013), and 0.8 g/kg/day for non-obese subjects and $\geq$ 0.8 for obese subjects in Morris and Jacques (2013). Thus, a protein intake ranged from > 0.8 to 1.6 g/kg/day may help increase an older adult's quality of life by maintaining a healthier body composition. Although this protein intake range was too board to empirically apply into dietetic practice for healthy older adults, this could be the starting range to compare to against the RDA for future research. This could allow researchers to gain a more thorough understanding on what specific protein quantities may be required for healthy older adults in preventing sarcopenia.

In particular, greater lean muscle mass was associated with higher protein intake as indicated by Gray-Donald et al. (2014), Geirsdottir et al. (2013), Houston et al. (2008), Meng et al. (2009), and older men in the Aleman-Mateo et al. (2012) study, but no differences were found in Gregorio et al. (2013). In comparison, Ruiz Valenzuela et al. (2013) found this association as well, but specifically evaluated protein distribution. Regarding appendicular skeletal muscle

mass changes, Scott et al. (2010), Houston et al. (2008), and Morris and Jacques (2013) discovered a positive correlation with higher protein consumption. However, Morris and Jacques (2013) found this association only in non-obese individuals so BMI may be an influential factor for protein intake utilization. Comparatively, Gray-Donald et al. (2013), Meng et al. (2009), and Aleman-Mateo et al. (2012) linked a greater dietary protein intake to less weight loss although, Aleman-Mateo et al. (2012) only found this in older men. The only studies to evaluated BMC were Meng et al. (2009) and Gregorio et al. (2013) and both found a positive correlation with protein intake. Additionally, a higher dietary protein intake may help improve bone health in older adults beyond sarcopenia advancements so this should be evaluated further.

Relative to muscle strength, Scott et al. (2010) did not find any significant differences between protein intake groups while Gregorio et al. (2013) found muscle strength improvements in women who consumed more protein. Similarly, Aleman-Mateo et al. (2012) discovered a positive correlation in the older adults who consumed added ricotta cheese with their diet while only women experienced a positive tendency towards significance. For physical performance, Morris and Jacques (2013) found obese individuals only benefited from a higher protein intake of > 0.8 g/kg/day, when combined with muscle strength exercises. Better physical performance was also associated with a higher protein intake for Gregorio et al. (2013) whereas, Geirsdottir et al. (2013) found no such correlation for leisure-time physical activity. Nonetheless, more studies focused on muscle mass than muscle strength or physical performance, which may have prevented a stronger association from being established.

There are many other research limitations that should be addressed when expanding sarcopenia research in the future. It was evident from the Ruiz Valenzuela et al. (2013) study that adequate protein distribution may play a role in the progression of sarcopenia. This should be

further analyzed to see if these results are valid. Another limitation was dietary protein was not divided into animal-based or plant-based protein sources to determine if certain types of protein sources influenced muscle mass, strength, and/or functionality differently. Additionally, physical activity was not the focus on this literature review although it is a common intervention to delay the consequences of sarcopenia. In the future, studies should combine a higher dietary protein intake with physical activity to see if this may produce more consistent results for muscle strength and physical performance. Finally, not one study included all three characteristics of sarcopenia including muscle mass, strength, and functionality so it was challenging to determine if dietary protein could fully improve sarcopenia completely. However, given the complex nature of the aging process and the current clinical uncertainties on sarcopenia, it can be difficult to capture all of the consequences of this condition consistently among studies for a stronger conclusion. Ideally the future, muscle mass, strength, and functionality should all be analyzed within every study design, utilizing the same diagnostic tools. Furthermore, it was also unclear why some studies included measurements of appendicular muscle mass and/or lean muscle mass so this was another research inconsistency that requires further investigation to figure out which is a better predictor of sarcopenia.

Overall, the evidence suggests meeting or exceeding the RDA for protein may be a beneficial modifiable risk factor against the onset of sarcopenia in healthy older adults, but may not be applicable to those diagnosed with sarcopenia. Nonetheless, increasing the current RDA to > 0.8 to 1.6 g/kg/day may more sufficiently meet the needs of the aging population and shows promise for potentially becoming a nutrition intervention against sarcopenia, once more research is conducted. While it is important future research focuses on study designs of randomized control trials, it is equally vital that standardized diagnostic criteria is established so more

reliable and valid measurement tools are formulated. Establishing advanced protein measures are necessary to better understand this condition including the most common protein sources and eating patterns among older adults. In general it is essential more research is conducted on sarcopenia to gain further understanding on the fundamental etiology, pathophysiology, and preventative strategies as a stronger causation is recognized.

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APPENDIX A

Citation:	Morris, MS. & Jacques, PF. Total protein, animal protein and physical activity in relation to muscle mass in middle-aged and older Americans. *Br J Nutr.* 2013; 109: 1294-1303. doi: 10.1017/S0007114512003133
Study design:	Cross-sectional
Study Class (A,B,C,D)	D
Research Quality Rating	**NEUTRAL (ø)**
Purpose/Population Studied/Practice Studied	
Research purpose:	To figure out the answers to some of the unknown questions regarding age-related muscle losses and strength declines, by evaluting the National Health and Nutrition Examination Survey (NHANES) data from 2003-2006, on participants > 50 years old.
Inclusion criteria:	> 50 years old.
Exclusion criteria	If participant did not complete all the medical examination center (MEC) data required for the NHANES survey.
Recruitment	Not required since this was a secondary analysis of the NHANES survey data.
Description of study protocol	NHANES data from the 2003-2004 and 2005-2006 surveys contains information on body composition measures from a medical exam (including DXA scan), an interview assessment performed by NHANES personnel (including physical activity, medical history, and smoking status), and a dietary assessment (including two 24-hour recalls and a FFQ). From this information, this current study mainly investigated how physical activity status and usual beef intakes and total protein intake interact. However, DXA measurements were only available for participants involved in 2002-2004 survey so only part of the participants were analyzed for this part of the study. Participants who were excluded previous from missing MEC data were added back in to increase aLM measurement comparisons.
Statistical analysis:	**Multiple Linear Regression Model:** to compare physical activity and dietary factors to appendicular muscle mass. Appendicular muscle mass was a continuous outcome variable. **Residuals Method:** to relate usual protein intakes to appendicular muscle mass after adjusting for estimates of total energy intakes. **Multivariate model:** to separate participants into three-levels of usual protein intake groups (not meeting the RDA (< 0.8 g/kg), meeting the RDA (0.8-1.0 g/kg), and exceeding the RDA (> 1.0 g/kg)). **Significance level:** $p < 0.05$ using a two-sided tests
Timing of measurements:	Not applicable.
Dependent variables:	Appendicular muscle mass

Independent variables	Leisure-time physical activity and protein quality (beef intake)
Control Variables	Total caloric intake
Initial n	n = 4724
Final n	n = 2425 (55.6% female) DXA scan measurements were only available from participants in the 2003-2004 NHANES survey so only 557 men and 625 women were included in this part of the analysis. An additional 290 men and 167 women were added after being excluded before to increase this subsample.
Age	**Mean age:** 63 years old
Ethnicity	**Non-Hispanic White:** 84.7% **Non-Hispanic Black:** 6.3% **Mexican American:** 2.9% Only adds up to 93.9%, but other ethnicity background information not given.
Other relevant demographics:	Not mentioned.
Anthropometrics:	There were no groups. **BMI (> 30 kg/m^2):** 26.6% of the total study population
Location:	Secondary analysis was conducted at Tufts University in Boston, MA
Summary of Results:	Beef consumption was significantly correlated to total protein intakes ($r = 0.19$; $p < 0.001$). A positive correlation found in the appendicular skeletal muscle mass (aLM) of non-obese subjects who performed vigorous aerobic physical activity. An increase in aLM was significantly related to higher beef intakes; this was exceptionally strong and significant in non-obese subjects who participated in muscle-strengthening exercises. Obese subjects who consumed < 70 g/day of protein and participated in muscle-strength exercises had a significantly lower aLM than physically inactive individuals ($p = 0.013$). Furthermore, obese individuals who were physically active had a stronger association between total protein intake and aLM when compared to physically active non-obese subjects. For protein recommendations, it did not matter whether an non-obese individual met the RDA for protein as long as they performed vigorous aerobic activities. Conversely, for obese subjects, muscle-strength training seemed to be more effective in improving aLM, but only if the RDA for protein was met or exceeded.
Author's Conclusions	
Author conclusion:	The higher good quality protein sources consumed, like beef, may be helpful when increasing aLM in older individuals whether obese or not. Exceeding the RDA for protein was only beneficial in obese subjects whereas, non-obese individuals showed no added benefits when exceeding this recommended amount. Muscle strength training exercises

	preserves muscle mass when added with adequate dietary protein consumption. Aerobic activity was also helpful in preserving aLM, but a diet in high quality protein should be added to see the benefits.
Reviewer comments:	***Strengths:*** • *A large collected data set was used from the four years of NHANES surveys to provide a better estimate of which high-protein foods are consumed long-term on a national level.* • *A large representative sample of older adults were involved to determine the strength between physical activity, dietary protein, and beef intakes.* • *An accurate statistical power was obtained* • *The different types of physical activities older adult's typically participated in were able to be further explored instead of implementing an unrealistic physical activity intervention.* ***Limitations:*** • *This study design only illustrated one time period so results may not be representative of typical dietary intake and/or physical activity in older adults.* • *DXA scans were only available for subjects in the 2003-2004 NHANES survey.* • *Physical activity was based on observation only so subjects may have reported inaccurate results that unintentionally swayed these results.*

RELEVANCE QUESTIONS					
Citation: Morris, MS. & Jacques, PF. Total protein, animal protein and physical activity in relation to muscle mass in middle-aged and older Americans. *Br J Nutr.* 2013; 109: 1294-1303. doi: 10.1017/S0007114512003133		**YES**	**NO**	**UNCLEAR**	**NA**
1. Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	1				√
2. Did the authors study an outcome (dependent variable) or topic that the patients/clients/ population group would care about?	2	√			
3. Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to dietetics practice?	3	√			
4. Is the intervention or procedure feasible (NA for some epidemiological studies)?	4			√	
If the answers to all of the above relevance questions are "yes", the report is eligible for designation with a plus (+) on the Evidence Quality Worksheet, depending on answers to the following validity questions.					

VALIDITY QUESTIONS					
1. Was the <u>research question</u> clearly stated?		**YES** √	**NO**	**UNCLEAR**	**NA**
1.1 Was the specific intervention(s) or procedure (independent variable(s)) identified?	1.1	√			

1.2 Was the outcome(s) (dependent variable(s)) clearly indicated?	1.2	√			
1.3 Were the target population and setting specified?	1.3	√			
2. Was the selection of study subjects / patients free from bias? As per answers to subquestions below, selection was free from bias, but groups were not comparable (and thus study was biased)		YES √	NO	UNCLEAR	NA
2.1 Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	2.1	√			
2.2 Were criteria applied equally to all study groups?	2.2				√
2.3 Were health, demographics, and other characteristics of subjects described?	2.3	√			
2.4 Were the subjects /patients in a representative sample of the relevant population?	2.4	√			
3. Were study groups comparable?		YES	NO	UNCLEAR	NA √
3.1 Was the method of assigning subjects / patients to groups described and unbiased? (Method of randomization identified if RCT)	3.1				√
3.2 Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	3.2				√
3.3 Were concurrent controls used? (Concurrent preferred over historical controls.)	3.3				√
3.4 If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	3.4				√
3.5 If case control study, were potential confounding factors comparable for cases and controls? If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.	3.5				√
3.6 If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g. "gold standard")?	3.6				√
4. Was method of handling withdrawals described?		YES	NO	UNCLEAR	NA √
4.1 Were follow up methods described and the same for all groups?	4.1				√
4.2 Was the number, characteristics of withdrawals (i.e. dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80 %.)	4.2				√
4.3 Were all enrolled subjects/patients (in the original sample) accounted for?	4.3	√			
4.4 Were reasons for withdrawals similar across groups?	4.4				√
4.5 If diagnostic test, was decision to perform reference test not dependent on results of test under study?	4.5				√
5. Was blinding used to prevent introduction of bias?		YES	NO	UNCLEAR	NA √

5.1 In intervention study, were subjects, clinicians / practitioners and investigators blinded to treatment group, **as appropriate**?						√
5.2 Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	5.2		√		
5.3 In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	5.3		√		
5.4 In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	5.4				√
5.5 In diagnostic study, were test results blinded to patient history and other test results?	5.5				√
6. Were intervention / therapeutic regimens / exposure factor or procedure and any comparison(s) described in detail? Were intervening factors described?		YES	NO	UNCLEAR	NA √
6.1 In RCT or other intervention trial, were protocols described for all regimens studied?	6.1				√
6.2 In observational study, were interventions, study settings, and clinicians / provider described?	6.2				√
6.3 Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	6.3				√
6.4Was the amount of exposure and, if relevant, subject / patient compliance measured?	6.4				√
6.5Were co-interventions (e.g., ancillary treatments other therapies) described?)	6.5				√
6.6 Were extra or unplanned treatments described?	6.6				√
6.7 Was the information for 6.4, 6.5, 6.6 and 6.7 assessed the same way for all groups?	6.7				√
6.8 In diagnostic study, were details of test administration and replication sufficient?	6.8				√
7. Were outcomes clearly defined and the measurements valid and reliable?		YES √	NO	UNCLEAR	NA
7.1 Were primary and secondary endpoints described and relevant to the question?	7.1	√			
7.2 Were nutrition measures appropriate to question and outcomes of concern?	7.2			√	
7.3 Was the period of follow-up long enough for important outcome(s) to occur?	7.3				√
7.4 Were the observations and measurements based on standard, valid, and reliable data collection instruments / tests / procedures?	7.4	√			
7.5 Was the measurement of effect at an appropriate level of precision?	7.5	√			
7.6 Were other factors accounted for (measured) that could affect outcomes?	7.6		√		
7.7 Were the measurements conducted consistently across groups?	7.7				√
8. Was the statistical analysis appropriate for the study design and type of outcome indicators?		YES	NO	UNCLEAR √	NA
8.1 Were statistical analyses adequately described and the results reported appropriately?	8.1	√			

8.2 Were correct statistical tests used and assumptions of test not violated?	8.2			√	
8.3 Were statistics reported with levels of significance and/or confidence intervals?	8.3	√			
8.4 Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	8.4				√
8.5 Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g. multivariate analyses)?	8.5			√	
8.6 Was clinical significance as well as statistical significance reported?	8.6				√
8.7 If negative findings, was a power calculation reported to address type 2 error?	8.7				√
9. Are <u>conclusions</u> supported by results with biases and limitations taken into consideration?	**YES**	**YES** √	**NO**	**UNCLEAR**	**NA**
9.1 Is there a discussion of findings?	9.1	√			
9.2 Are biases and study limitations identified and discussed?	9.2	√			
10. Is bias due to study's <u>funding or sponsorship</u> unlikely?	**YES**	**YES**	**NO** √	**UNCLEAR**	**NA**
10.1 Were sources of funding and investigators' affiliations described?	10.1	√			
10.2 Was there no apparent conflict of interest?	10.2		√		
SYMBOL NEUTRAL (ø)					
NEUTRAL (ø) *If the answers to validity criteria questions **2, 3, 6, and 7** do not indicate that the study is exceptionally strong, the report should be designated with a neutral* (ø) *symbol on the Evidence Quality Worksheet.*					

APPENDIX B

Citation:	Gray-Donald K, St-Arnaud-McKenzie D, Gaudreau P, Morais JA, Shatenstein B, Payette B. Protein Intake Protects against Weight Loss in Healthy Community-Dwelling Older Adults. *J Nutr*. 2014; 144(3): 321-326. doi: 10.3945/jn.113.184705
Study design:	Prospective case-control
Study Class (A,B,C,D)	C
Research Quality Rating	**PLUS/POSITIVE (+)**
Purpose/Population Studied/Practice Studied	
Research purpose:	To evaluate the relationship between protein intake and the incidence of ≥5% weight loss, over a 1-year period, in community-dwelling older adults.
Inclusion criteria:	Not specified.
Exclusion criteria:	• Unable to climb 10 steps or walk 100m without stopping to rest • Disabilities in daily living activities • Any cognitive deficits • Presence of class II heart failure, chronic obstructive pulmonary disease that requires at-home oxygen or oral steroids, inflammatory digestive diseases, and/or cancer that requires treatment during the 5 year study period • Missing any weight measurements for study analysis
Recruitment	• **Current study and comparative (NuAge) study for matching:** Random sample of Quebec provincial health database from 2003 to 2008 by finding participants in three age categories ((70±2, 75±2, and 80±2 years old)
Description of study protocol	Participants were placed into cases and control groups by having participants who experienced severe weight loss were matched and separated into cases (those with weight loss of ≥5% over 1-year) or controls (those with weight loss of ≤2% over 1-year). Stratified sampling method was conducted to select cases and control further. One year cases selected from 1st year follow-up (weight loss ≥5%; n=129) and 2nd year follow-up visits (weight loss ≥ 5%; n = 82)**.** Second year controls could be considered for 2nd year cases (n = 8). Cases were matched (1:1) by sex and age against randomly selected controls who were weight stable from the same time period. After participants were placed into cases and control groups, they were assessed on the following measurements: **Body Weight:** determined percentage of weight change over the study period over one year. **Body Composition:** involved measurements of whole body DXA scan to determine FM and FFM. Total lean body mass was also measured. **Energy and Macronutrient Intakes:** At baseline, 3 nonconsecutive 24-h dietary recalls were obtained (1 face-to-face and 2 telephone interviews).

	Interviews were conducted by trained RDs (included graduated utensils and food portion visuals). Average daily total caloric intake and protein were further analyzed**.** Participants were also analyzed on different protein intake categorizes (low [0.8g/kg/day], moderate [0.8-<1.0g/kg/day], high [1.0-1.2g/kg/day], and very high [≥1.2g/kg/day]). **Potential Cofounders:** involved measurements of BMI (<25, 25≤BMI<30, and BMI ≥30), total energy intake, appetite, dieting to lose weight, smoking status, prevalence of chronic disease, polypharmacy, and biomarkers of low serum albumin and high CRP (C-reactive protein) concentrations.
Statistical analysis:	t**-test, Mann-Whitney, or chi-square tests:** used when appropriate for baseline characteristics (aka potential cofounders) **Chi-square test**: used for protein intake categories and linear trends **Conditional logistic regression model** used to control for sex and the 3 age categories for the 1-year weight loss. **Unadjusted models**: used to evaluate energy and macronutrient intakes separately with dummy variables created for protein intake categories **Statistical significance:** using 2-tailed p-values, representative of $p < 0.05$
Timing of measurements:	**At baseline:** body weight, energy and macronutrient intakes, potential cofounders from subjects from the 1st year follow-up (BMI, total energy intake, appetite, dieting to lose weight, smoking status, prevalence of chronic disease, polypharmacy, and biomarkers). **Year 1:** body weight, potential cofounders from participants selected for the 2nd year follow-up (BMI, total energy intake, appetite, dieting to lose weight, smoking status, prevalence of chronic disease, polypharmacy, and biomarkers). **Year 2 (only if eligible):** body composition for 60 matched pairs who had lean body mass measurements at both time points (baseline and 2-y) since this is when the previous study measured these components.
Dependent variables:	Body weight (to determine percentage of 1-y weight change)

RELEVANCE QUESTIONS					
Citation: Gray-Donald K, St-Arnaud-McKenzie D, Gaudreau P, Morais JA, Shatenstein B, Payette B. Protein Intake Protects against Weight Loss in Healthy Community-Dwelling Older Adults. *J Nutr*. 2014; 144(3): 321-326. doi: 10.3945/jn.113.184705		**YES**	**NO**	**UNCLEAR**	**NA**
1. Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	1				√
2. Did the authors study an outcome (dependent variable) or topic that the patients / clients / population group would care about?	2	√			

3. Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to dietetics practice?	3	√			
4. Is the intervention or procedure feasible (NA for some epidemiological studies)?	4				√
If the answers to all of the above relevance questions are "yes", the report is eligible for designation with a plus (+) on the Evidence Quality Worksheet, depending on answers to the following validity questions.					
VALIDITY QUESTIONS					
1. Was the research question clearly stated?		YES √	NO	UNCLEAR	NA
1.1 Was the specific intervention(s) or procedure (independent variable(s)) identified?	1.1	√			
1.2 Was the outcome(s) (dependent variable(s)) clearly indicated?	1.2	√			
1.3 Were the target population and setting specified?	1.3	√			
2. Was the selection of study subjects / patients free from bias?		YES √	NO	UNCLEAR	NA
2.2 Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	2.1	√			
2.3 Were criteria applied equally to all study groups?	2.2	√			
2.4 Were health, demographics, and other characteristics of subjects described?	2.3	√			
2.4 Were the subjects /patients in a representative sample of the relevant population?	2.4	√			
3. Were study groups comparable?		YES √	NO	UNCLEAR	NA
3.1 Was the method of assigning subjects / patients to groups described and unbiased? (Method of randomization identified if RCT)	3.1		√		
3.2 Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	3.2		√		
3.3 Were concurrent controls used? (Concurrent preferred over historical controls.)	3.3	√			
3.4 If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	3.4				√
3.5 If case control study, were potential confounding factors comparable for cases and controls? If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.	3.5	√			
3.6 If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g. "gold standard")?	3.6				√
4. Was method of handling withdrawals described?		YES √	NO	UNCLEAR	NA

4.1 Were follow up methods described and the same for all groups?	4.1	√			
4.3 Was the number, characteristics of withdrawals (i.e. dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group?	4.2	√			
4.3 Were all enrolled subjects/patients (in the original sample) accounted for?	4.3	√			
4.5 Were reasons for withdrawals similar across groups?	4.4	√			
4.5 If diagnostic test, was decision to perform reference test not dependent on results of test under study?	4.5				√
5. Was <u>blinding</u> used to prevent introduction of bias?		YES	NO	UNCLEAR	NA
			√		
5.4 In intervention study, were subjects, clinicians / practitioners and investigators blinded to treatment group, **<u>as appropriate</u>**?					√
5.5 Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	5.2		√		
5.6 In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	5.3				√
5.7 In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	5.4	√			
5.5 In diagnostic study, were test results blinded to patient history and other test results?	5.5				√
6. Were intervention / therapeutic regimens / exposure factor or procedure and any comparison(s) described in detail? Were intervening factors described?		YES	NO	UNCLEAR	NA
		√			
6.1 In RCT or other intervention trial, were protocols described for all regimens studied?	6.1				√
6.2 In observational study, were interventions, study settings, and clinicians / provider described?	6.2				√
6.3 Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	6.3	√			
6.4 Was the amount of exposure and, if relevant, subject / patient compliance measured?	6.4	√			
6.5 Were co-interventions (e.g., ancillary treatments other therapies) described?	6.5				√
6.6 Were extra or unplanned treatments described?	6.6				√
6.7 Was the information for 6.4, 6.5, 6.6 and 6.7 assessed the same way for all groups?	6.7	√			
6.8 In diagnostic study, were details of test administration and replication sufficient?	6.8				√
7. Were <u>outcomes</u> clearly defined and the measurements valid and reliable?		YES	NO	UNCLEAR	NA
		√			
7.2 Were primary and secondary endpoints described and relevant to the question?	7.1	√			
7.2 Were nutrition measures appropriate to question and outcomes of concern?	7.2	√			
7.7 Was the period of follow-up long enough for important outcome(s) to occur?	7.3	√			
7.8 Were the observations and measurements based on standard, valid, and reliable data collection instruments / tests / procedures?	7.4	√			

7.9 Was the measurement of effect at an appropriate level of precision?	7.5	√			
7.10Were other factors accounted for (measured) that could affect outcomes?	7.6	√			
7.7 Were the measurements conducted consistently across groups?	7.7	√			
8. Was the <u>statistical analysis</u> appropriate for the study design and type of outcome indicators?		**YES** √	**NO**	**UNCLEAR**	**NA**
8.6 Were statistical analyses adequately described and the results reported appropriately?	8.1	√			
8.7 Were correct statistical tests used and assumptions of test not violated?	8.2	√			
8.8 Were statistics reported with levels of significance and/or confidence intervals?	8.3	√			
8.9 Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	8.4		√		
8.10Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g. multivariate analyses)?	8.5	√			
8.6 Was clinical significance as well as statistical significance reported?	8.6	√			
8.7 If negative findings, was a power calculation reported to address type 2 error?	8.7				√
9. **Are <u>conclusions</u> supported by results with biases and limitations taken into consideration?**	**YES**	**YES**	**NO** √	**UNCLEAR**	**NA**
9.1 Is there a discussion of findings?	9.1	√			
9.2 Are biases and study limitations identified and discussed?	9.2		√		
10. Is bias due to study's <u>funding or sponsorship</u> unlikely?	**YES**	**YES** √	**NO**	**UNCLEAR**	**NA**
10.1 Were sources of funding and investigators' affiliations described?	10.1	√			
10.2 Was there no apparent conflict of interest?	10.2	√			
SYMBOL PLUS/POSITIVE (+)					
PLUS/POSITIVE (+) *If most of the answers to the above validity questions are "Yes" including criteria **2, 3, 6, and 7 and at least one additional "yes"**,(the report should be designated with a plus symbol (+) on the Evidence Quality Worksheet.*					

APPENDIX C

Citation:	Geirdottir, OG, Arnarson, A, Ramel, A, Jonsson, PV, & Thorsdottir, I. Dietary protein intake is associated with lean body mass in community-dwelling older adults. *Nutr Res*. 2013; 33(8): 608-612. doi: 10.1016/j.nutres.2013.05.014
Study design:	Cross-Sectional
Study Class (A,B,C,D)	D
Research Quality Rating	**NEUTRAL (ø)**
Purpose/Population Studied/Practice Studied	
Research purpose:	To determine whether there was a link between dietary protein consumption and leisure-time physical activity by assessing lean body mass in community-dwelling older adults in a previous 12-week intervention resistance training program.
Inclusion criteria:	• ≥ 65 years old • Resident in the Reykjavik area
Exclusion criteria:	• low cognitive function • presence of major orthopedic diseases, musculoskeletal disorders, disorders affecting muscle mass • involvement in pharmacologic interventions that could affect muscle mass or testosterone levels
Recruitment	Participants answered public advertisements in the Reykjavik area
Description of study protocol	**All of the assessments/measurements below were taken at one time period:** **Dietary Assessment:** subjects weighed and reported food intake through use of dietary food recalls consisting of 2 week-days, 1 weekend day. After dietary assessments were analyzed, subjects were placed into different protein take quartile groups. **Anthropometric Measurements:** body composition measures using DXA tool, weight and height for BMI and 2 waist circumference measures **Leisure-time Physical Activity:** subjects recorded hours and types of activities done over the past year **Demographic Characteristics:** consisting of alcohol and smoking behaviors, background on health concerns, and medication usage. **Physical function:** assessed by performance level from the Timed Up and Go and a six-minute walk for distance test results
Statistical analysis:	**Kolmogorov-Smirnov test:** to check for statistical normality **Independent *t*-test and Mann-Whitney *U* test:** to compare subjects who did not complete the 24-hour recalls versus those who did not.

	1-side *t*-test: to assess whether subjects consumed the recommended amount of protein or not. **Linear Models:** to determine if protein intake and/or leisure-time physical activity predicts lean muscle mass. **Significance level:** $p < 0.05$
Timing of measurements:	All measurements were taken at baseline
Dependent variables:	Lean body mass
Independent variables	Protein intake and leisure-time physical activity
Control Variables	For the linear model covariates (age, number of medications used, physical activity level) and fixed factors (sex, protein intake quartiles, BMI categories) were controlled for.
Initial n	Not mentioned.
Final n	n = 237
Age	Age Range: 65-92 years old Mean age: 73.6 + 5.7 years old
Ethnicity	Not mentioned.
Other relevant demographics:	• Community-dwelling older adults • 58% female, 42% male
Anthropometrics:	Participants were no separated into different groups. Mean BMI: 28.8 + 4.8 kg/m2 (men: 29.7 + 4.6 kg/m2 and women: 28.1 + 4.9 kg/m2)
Location:	Reykjavik, Iceland
Summary of Results:	Eighty-two percent reported regular leisure-time physical activity with two-thirds at a recommended level of 30 min/day. The average total energy intake was 1682 + 494 kcal/day and participants consumed quantities of protein significantly above the RDA for protein ($p < 0.001$). Men consumed more protein than women (90.3 + 26.7 g/day compared to 69.6 + 19.1 g/day. Daily protein intake was considered a positive predictor of LBM as was hypothesized.
Author's Conclusions	
Author conclusion:	Dietary protein intake was positively associated with LBM in community-dwelling older adults. There was a significant difference of 2.3 kg between the fourth (1.36 $\pm$ 1.19 g/kg of protein) and first (0.63 $\pm$ 0.08 g/kg of protein) quartile and trending towards significance of 2.0 kg among the fourth (1.36 $\pm$ 1.19 g/kg of protein) and second (0.85 $\pm$ 0.05 g/kg of protein) quartiles. There was no correlation between leisure-time physical activity and LBM, which suggested that endurance exercises may not be connected to LBM for this population regime.
Reviewer comments:	***Strengths:***

- *Many covariates and variables were controlled for during the linear model analysis*
- *Appropriate inclusion and exclusion criteria were implemented*
- *Subjects were evaluated on their routine physical activity instead of adding an intervention so this provides more of a realistic viewpoint for this type of population.*
- *Study population was diverse in male and females*

Limitations:

- *A majority of protein intake was predominately from animal sources, but no further analysis was conducted using this information. Therefore the impact of plant proteins was put into question.*
- *Chosen study design could not reflect a cause-and-effect relationship and could only depict one point in time so may not be representative of subject's usual intake.*
- *Physical activity was self-reported so overestimation and subject bias may have affected the results.*
- *Participants may not be representative of general population since subjects were volunteer and may be more physical active that the average older adult*

RELEVANCE QUESTIONS					
Citation: Geirdottir, OG, Arnarson, A, Ramel, A, Jonsson, PV, & Thorsdottir, I. Dietary protein intake is associated with lean body mass in community-dwelling older adults. *Nutr Res*. 2013; 33(8): 608-612. doi: 10.1016/j.nutres.2013.05.014		YES	NO	UNCLEAR	NA
1. Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	1				√
2. Did the authors study an outcome (dependent variable) or topic that the patients/clients/ population group would care about?	2	√			
3. Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to dietetics practice?	3	√			
4. Is the intervention or procedure feasible (NA for some epidemiological studies)?	4	√			
If the answers to all of the above relevance questions are "yes", the report is eligible for designation with a plus (+) on the Evidence Quality Worksheet, depending on answers to the following validity questions.					

VALIDITY QUESTIONS					
1. Was the <u>research question</u> clearly stated?		YES √	NO	UNCLEAR	NA
1.1 Was the specific intervention(s) or procedure (independent variable(s)) identified?	1.1	√			
1.2 Was the outcome(s) (dependent variable(s)) clearly indicated?	1.2	√			
1.3 Were the target population and setting specified?	1.3	√			

		YES	NO	UNCLEAR	NA
2. Was the selection of study subjects / patients free from bias?		√			
2.3 Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	2.1	√			
2.4 Were criteria applied equally to all study groups?	2.2				√
2.5 Were health, demographics, and other characteristics of subjects described?	2.3	√			
2.4 Were the subjects /patients in a representative sample of the relevant population?	2.4			√	
3. Were study groups comparable?		YES	NO	UNCLEAR	NA √
3.1 Was the method of assigning subjects / patients to groups described and unbiased? (Method of randomization identified if RCT)	3.1				√
3.2 Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	3.2				√
3.3 Were concurrent controls used? (Concurrent preferred over historical controls.)	3.3				√
3.4 If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	3.4			√	
3.5 If case control study, were potential confounding factors comparable for cases and controls? If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.	3.5				√
3.6 If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g. "gold standard")?	3.6				√
4. Was method of handling withdrawals described?		YES	NO	UNCLEAR	NA √
4.1 Were follow up methods described and the same for all groups?	4.1				√
4.4 Was the number, characteristics of withdrawals (i.e. dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80 %.)	4.2				√
4.3 Were all enrolled subjects/patients (in the original sample) accounted for?	4.3	√			
4.6 Were reasons for withdrawals similar across groups?	4.4				√
4.5 If diagnostic test, was decision to perform reference test not dependent on results of test under study?	4.5				√
5. Was blinding used to prevent introduction of bias?		YES	NO	UNCLEAR	NA √
5.8 In intervention study, were subjects, clinicians / practitioners and investigators blinded to treatment group, **as appropriate**?					√

5.9 Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	5.2		√		
5.10In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	5.3		√		
5.11In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	5.4				√
5.5 In diagnostic study, were test results blinded to patient history and other test results?	5.5				√
6. Were intervention / therapeutic regimens / exposure factor or procedure and any comparison(s) described in detail? Were intervening factors described?		**YES**	**NO**	**UNCLEAR**	**NA** √
6.1 In RCT or other intervention trial, were protocols described for all regimens studied?	6.1				√
6.2 In observational study, were interventions, study settings, and clinicians / provider described?	6.2				√
6.3 Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	6.3		√		
6.4Was the amount of exposure and, if relevant, subject / patient compliance measured?	6.4		√		
6.5Were co-interventions (e.g., ancillary treatments other therapies) described?	6.5				√
6.6 Were extra or unplanned treatments described?	6.6				√
6.7 Was the information for 6.4, 6.5, 6.6 and 6.7 assessed the same way for all groups	6.7				√
6.8 In diagnostic study, were details of test administration and replication sufficient?	6.8				√
7. Were <u>outcomes</u> clearly defined and the measurements valid and reliable?		**YES** √	**NO**	**UNCLEAR**	**NA**
7.3 Were primary and secondary endpoints described and relevant to the question?	7.1	√			
7.2 Were nutrition measures appropriate to question and outcomes of concern?	7.2	√			
7.11Was the period of follow-up long enough for important outcome(s) to occur?	7.3				√
7.12Were the observations and measurements based on standard, valid, and reliable data collection instruments / tests / procedures?	7.4	√			
7.13Was the measurement of effect at an appropriate level of precision?	7.5			√	
7.14Were other factors accounted for (measured) that could affect outcomes?	7.6	√			
7.7 Were the measurements conducted consistently across groups?	7.7				√
8. Was the <u>statistical analysis</u> appropriate for the study design and type of outcome indicators?		**YES** √	**NO**	**UNCLEAR**	**NA**
8.11Were statistical analyses adequately described and the results reported appropriately?	8.1	√			
8.12Were correct statistical tests used and assumptions of test not violated?	8.2	√			

8.13Were statistics reported with levels of significance and/or confidence intervals?	8.3	√			
8.14Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	8.4				√
8.15Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g. multivariate analyses)?	8.5	√			
8.6 Was clinical significance as well as statistical significance reported?	8.6				√
8.7 If negative findings, was a power calculation reported to address type 2 error?	8.7				√
9. Are conclusions supported by results with biases and limitations taken into consideration?	**YES**	**YES** √	**NO**	**UNCLEAR**	**NA**
9.1 Is there a discussion of findings?	9.1	√			
9.2 Are biases and study limitations identified and discussed?	9.2	√			
10. Is bias due to study's funding or sponsorship unlikely?	**YES**	**YES** √	**NO**	**UNCLEAR**	**NA**
10.1 Were sources of funding and investigators' affiliations described?	10.1	√			
10.2 Was there no apparent conflict of interest?	10.2	√			
SYMBOL NEUTRAL (ø)					
NEUTRAL (ø) *If the answers to validity criteria questions **2, 3, 6, and 7** do not indicate that the study is exceptionally strong, the report should be designated with a neutral* (ø) *symbol on the Evidence Quality Worksheet.*					

APPENDIX D

Citation:	Houston, DK, Nicklas, BJ, & Ding, J et al. Dietary protein intake is associated with lean muscle mass change in older, community-dwelling adults: the Health, Aging, and Body Composition (Health ABC) Study. *Am J Clin Nutr.* 2008; 87(1): 150-155. Retrieved from http://ajcn.nutrition.org/content/87/1/150.full.pdf+html
Study design:	Prospective Cohort
Study Class (A,B,C,D)	B
Research Quality Rating	**Positive (+)**
Purpose/Population Studied/Practice Studied	
Research purpose:	To determine if there was an association between dietary protein consumption and alterations in LMM and nonbone aLM in the community-dwelling older adult population.
Inclusion criteria:	• No issues walking 1/4 of a mile, climbing up 10 steps, or performing basic ADLs • Absent of life-threatening illnesses • Willing to stay in the same geographic region throughout the ≥ 3 year study period • Not participating in other lifestyle intervention trials • Completing food frequency questionnaire (FFQ) at year 2 of the study since this is considered baseline for dietary measures
Exclusion criteria	• Missed the FFQ at year 2 or had major errors after completion • Reported caloric intakes either <500 kcals/day or > 3500 kcals/day for women • Reported caloric intakes either < 800 kcals/day or > 4000 kcals/day for men • Missed values for lean muscle either at year 2 or year 5 • Missed other variables significant to the study
Recruitment	Participants were randomly selected from a sample of Medicare-eligible residents within the metropolitan areas of Pittsburg, PA and Memphis, TN. The selection process included those 70 to 79 years old and were black and white men and women.
Description of study protocol:	**All participants were measured and analyzed on these characteristics:** **Body Composition:** • Measured using DXA machine • Included measurements of: total LMM, aLM, and weight **Dietary Assessment:** • FFQ was completed, which was specifically developed for this study • Trained interviewers monitored participants periodically to increase consistency and higher-quality data collection by using visual tools to better estimate portion sizes • Total protein intake, vegetable protein, and animal protein was obtained • After dietary assessments were evaluated, participants were separated into protein quintile intake groups to compare against LMM changes. **Potential Cofounders:** • Interview-administered questionnaire included: demographic characteristics

	(age, sex, race, and study site), smoking status, alcohol consumption, physical activity level • Prevalence of diagnosed health conditions at baseline (ischemic heart disease, diabetes, congestive heart failure, cerebrovascular disease, cancer, chronic obstructive pulmonary disease) • Use of oral steroids are noted • Interim hospitalizations (stay of > 24 hours) was recorded
Intervention:	Not included in this study design.
Statistical analysis:	**Multiple linear regression model:** for associations between protein intake and LM and aLM **Nutrient residual energy adjustment method:** to determine protein intake separate from total caloric intake so it can be used as an independent variable. • Protein intake was calculated as a continuous and categorical variable utilizing sex-specific quintiles • Sex was determine nonsignificant so all analyses are representative of the total population • Additional models adjusted for prevalence the potential cofounders and fat mass **Linear trends**: among the protein intake quintiles by using protein as a continuous variable **Statistical significance:** $P < 0.05$ Associations between protein intake and LM among those at greatest risk for LM losses (> 3% weight loss during study)
Timing of measurements:	**At baseline**: Potential cofounders were obtained, body composition measures **Year 2:** FFQ completion, LMM and aLM **Year 5:** LMM and aLM **Periodically:** FFQ **Annually:** body composition measures
Dependent variables:	LMM and aLM changes
Independent variables	Dietary protein intake (vegetable and animal sources)
Control Variables	**Multiple linear regression model**: initially adjusted for age, sex, race, total energy consumption, study site, baseline height, LMM, and aLM Additional models adjusted for health behaviors (smoking, alcohol consumption, physical activity), health conditions (ischemic heart disease, diabetes, congestive heart failure, cerebrovascular disease, cancer, chronic obstructive pulmonary disease), use of oral steroids, hospital admissions and fat mass.
Initial n	n = 3075
Final n (attrition)	n = 2066 (53.2% women)
Age	**Age range:** 70 to 79 years old, **Mean age**: 74.5 years
Ethnicity	35.4% black and 64.6% white
Other relevant demographics:	Residents of the Pittsburg, PA and Memphis, TN area
Anthropometrics:	BMI ranges depending on protein intake quintile groups ($p < 0.008$) (Q1) 27.2 $\pm$ 4.8 kg/m^2 (Q2) 27.1 $\pm$ 4.4 kg/m^2 (Q3) 27.0 $\pm$ 4.6 kg/m^2 (Q4) 26.9 + 4.3 kg/m^2

	(Q5) 28.0 ± 5.1 kg/m^2
Location:	Pittsburg, PA and Memphis, TN in the USA
Summary of Results:	Participants in the highest protein quintile group significantly lost about 40% less LMM and aLM compared to those in the lowest protein quintile group ($p < 0.01$). Protein intake was associated with body composition changes throughout this study; LMM ($p = 0.004$) and aLM ($p = 0.001$). Men typically consumed more dietary protein (70.8 g/kg/day) compared to the women who consumed 60.9 g/kg/day. In regards to protein intake, total protein and animal protein were both significantly associated with LMM ($p < 0.01$) and aLM ($p < 0.01$) even after adjustments were made. Protein intake was also associated with aLM changes in participants who gained or lost weight, but not for those that remained weight stable.
Author's Conclusions	
Author conclusion:	Protein intake was associated with significant LMM changes, which may have affected overall body composition measures for community-dwelling, older males and females. However, even with the small LMM changes over this 3-years, if a higher amount of protein was consumed a longer period of time, then there may have been even greater significant LMM changes. Dietary protein may be a modifiable factor in preventing sarcopenic losses in the elderly.
Reviewer comments:	***Strengths:*** • *Large sample size of community-dwelling older adults* • *Diverse sample of black and white men and women* • *Using valid and reliable tools such as the DXA scan to determine muscle mass changes* • *Study was conducted over a longer period of time (3 years)* • *Adjusting for multiple cofounders (lifestyle characteristics and presence of chronic diseases)* • *First longitudinal cohort study to evaluate if there is an association between protein intake and body composition changes for older adults* • *The FFQ was modified for the ABC study and its study demographic* • *Dietary assessments were conducted by trained interviewers to increase reliability and quality of data collected* ***Limitations:*** • *Intakes for animal protein was large than plant protein so this may have limited significant association between vegetable protein and lean mass variations* • *Dietary information was provided by a single FFQ so only depicts one point in time* • *Study design was a prospective cohort so a causal association cannot be determined between the dependent and independent variables*

RELEVANCE QUESTIONS					
Citation: Houston, DK, Nicklas, BJ, & Ding, J et al. Dietary protein intake is associated with lean muscle mass change in older, community-dwelling adults: the Health, Aging, and Body Composition (Health ABC) Study. *Am J Clin Nutr.* 2008; 87(1): 150-155. Retrieved from http://ajcn.nutrition.org/content/87/1/150.full.pdf+html		YES	NO	UNCLEAR	NA
1. Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	1				√

2. Did the authors study an outcome (dependent variable) or topic that the patients / clients / population group would care about?	2	√			
3. Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to dietetics practice?	3	√			
4. Is the intervention or procedure feasible (NA for some epidemiological studies)?	4	√			
If the answers to all of the above relevance questions are "yes", the report is eligible for designation with a plus (+) on the Evidence Quality Worksheet, depending on answers to the following validity questions.					

VALIDITY QUESTIONS					
1. Was the research question clearly stated?		YES √	NO	UNCLEAR	NA
1.1 Was the specific intervention(s) or procedure (independent variable(s)) identified?	1.1	√			
1.2 Was the outcome(s) (dependent variable(s)) clearly indicated?	1.2	√			
1.3 Were the target population and setting specified?	1.3	√			
2. Was the selection of study subjects / patients free from bias? As per answers to subquestions below, selection was free from bias, but groups were not comparable (and thus study was biased)		YES √	NO	UNCLEAR	NA
2.4 Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	2.1	√			
2.5 Were criteria applied equally to all study groups?	2.2	√			
2.6 Were health, demographics, and other characteristics of subjects described?	2.3	√			
2.4 Were the subjects /patients in a representative sample of the relevant population?	2.4	√			
3. Were study groups comparable?		YES √	NO	UNCLEAR	NA
3.1 Was the method of assigning subjects / patients to groups described and unbiased? (Method of randomization identified if RCT)	3.1		√		
3.2 Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	3.2	√			
3.3 Were concurrent controls used? (Concurrent preferred over historical controls.)	3.3				√
3.4 If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	3.4	√			
3.5 If case control study, were potential confounding factors comparable for cases and controls? If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.	3.5				√
3.6 If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g. "gold standard")?	3.6				√
4. Was method of handling withdrawals described?		YES √	NO	UNCLEAR	NA

4.1 Were follow up methods described and the same for all groups?	4.1	√			
4.5 Was the number, characteristics of withdrawals (i.e. dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80 %.)	4.2	√			
4.3 Were all enrolled subjects/patients (in the original sample) accounted for?	4.3	√			
4.7 Were reasons for withdrawals similar across groups?	4.4				√
4.5 If diagnostic test, was decision to perform reference test not dependent on results of test under study?	4.5				√
5. Was <u>blinding</u> used to prevent introduction of bias?		YES	NO √	UNCLEAR	NA
5.12In intervention study, were subjects, clinicians / practitioners and investigators blinded to treatment group, **<u>as appropriate</u>**?					√
5.13Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	5.2		√		
5.14In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	5.3		√		
5.15In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	5.4				√
5.5 In diagnostic study, were test results blinded to patient history and other test results?	5.5				√
6. Were intervention / therapeutic regimens / exposure factor or procedure and any comparison(s) described in detail? Were intervening factors described?		YES √	NO	UNCLEAR	NA
6.1 In RCT or other intervention trial, were protocols described for all regimens studied?	6.1				√
6.7 In observational study, were interventions, study settings, and clinicians / provider described?	6.2				√
6.8 Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	6.3		√		
6.9 Was the amount of exposure and, if relevant, subject / patient compliance measured?	6.4	√			
6.10Were co-interventions (e.g., ancillary treatments other therapies) described?	6.5				√
6.11Were extra or unplanned treatments described?	6.6				√
6.7 Was the information for 6.4, 6.5, 6.6 and 6.7 assessed the same way for all groups?	6.7	√			
6.8 In diagnostic study, were details of test administration and replication sufficient?	6.8				√
7. Were <u>outcomes</u> clearly defined and the measurements valid and reliable?		YES √	NO	UNCLEAR	NA
7.4 Were primary and secondary endpoints described and relevant to the question?	7.1	√			
7.2 Were nutrition measures appropriate to question and outcomes of concern?	7.2	√			

7.15 Was the period of follow-up long enough for important outcome(s) to occur?	7.3	√			
7.16 Were the observations and measurements based on standard, valid, and reliable data collection instruments / tests / procedures?	7.4	√			
7.17 Was the measurement of effect at an appropriate level of precision?	7.5				√
7.18 Were other factors accounted for (measured) that could affect outcomes?	7.6	√			
7.7 Were the measurements conducted consistently across groups?	7.7	√			
8. Was the statistical analysis appropriate for the study design and type of outcome indicators?		YES √	NO	UNCLEAR	NA
8.16 Were statistical analyses adequately described and the results reported appropriately?	8.1	√			
8.17 Were correct statistical tests used and assumptions of test not violated?	8.2	√			
8.18 Were statistics reported with levels of significance and/or confidence intervals?	8.3	√			
8.19 Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	8.4				√
8.20 Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g. multivariate analyses)?	8.5	√			
8.6 Was clinical significance as well as statistical significance reported?	8.6				√
8.7 If negative findings, was a power calculation reported to address type 2 error?	8.7				√
9. Are conclusions supported by results with biases and limitations taken into consideration?	**YES**	YES	NO √	UNCLEAR	NA
9.1 Is there a discussion of findings?	9.1	√			
9.2 Are biases and study limitations identified and discussed?	9.2		√		
10. Is bias due to study's funding or sponsorship unlikely?	**YES**	YES √	NO	UNCLEAR	NA
10.1 Were sources of funding and investigators' affiliations described?	10.1	√			
10.2 Was there no apparent conflict of interest?	10.2	√			
SYMBOL PLUS/POSITIVE (+)					
PLUS/POSITIVE (+) *If most of the answers to the above validity questions are "Yes" including criteria* ***2, 3, 6, and 7 and at least one additional "yes"****, (the report should be designated with a plus symbol (+) on the Evidence Quality Worksheet.*					

APPENDIX E

Citation:	Ruiz Valenzuela RE, Ponce JA, Morales-Figueros GG, Muro KA, Carreon VR, Aleman-Mateo H. Insufficient amounts of inadequate distribution of dietary protein intake in apparently healthy older adults in a developing country: implications for dietary strategies to prevent sarcopenia. *Clin Interv Aging.* 2013;8:1143-1148. doi: 10.2147/CIA.S49810
Study design:	Non-probability Cross-sectional
Study Class (A,B,C,D)	D
Research Quality Rating	**NEUTRAL (ø)**
Purpose/Population Studied/Practice Studied	
Research purpose:	To evaluate dietary protein intake and protein distribution during mealtimes while exploring their association with aLM in, what appears to be, healthy older adults.
Inclusion criteria:	Participants must: • Allow home and retirement home visits during study period. • Participate in telephone calls and posted announcements. • Be physically independent according to the scale of Lawton and Brody. • Be "apparently healthy" through self-reports with results confirmed by biochemical analyses during their clinical examination. • Have intellectual functioning in reference to the scale of Pfeiffer • Be free of major chronic diseases such as heart disease, stroke, cancer, respiratory disease, and diabetes, which were confirmed by evaluating their clinical histories. • Be free of any dietary restrictions, recent weight loss, and physical disabilities.
Exclusion criteria	• Those on protein supplementation. • Body compositions exceeding margins of the DXA scan. • Those who did not complete/report any of the three 24-hour dietary recalls during this study.
Recruitment	No recruitment was mentioned within the article, but stated in a table that the participants were from northwest Mexico.
Description of study protocol	All participants underwent a medical assessment (biochemical analyses, body composition by DXA, anthropometry including body weight, height, waist circumference, BMI). Assessments were conducted from 24-hours recalls to determine average dietary protein intake. The 24-hour recalls were obtained in the participant's homes with a family member who is involved in providing daily resources. In order for volunteers to participate, the blood samples, evaluating body composition and gathering anthropometric measurements required all participants to fast for 8 hours. All these procedures were performed by trained, standardized personnel. Personnel were required to follow corresponding measuring techniques appropriately. After analyzing the dietary recalls, participants were separated into two protein intake groups: Group A (<

	25 g of protein at each meal and Group B (> 25 g of protein during at least one meal time.
Statistical analysis:	**Two-sample *t*-test**: utilized to determine the effects of sex. To determine the significant differences between the estimated average values of daily protein consumed in this sample population. These were tested against the RDA for protein. **One-sample *t*-test:** to look further into the value of protein intake per mealtime against the average recommended value of 30g of protein. **Linear Model Analysis of Variance:** to determine the association between protein intake, protein distribution, and aLM. **Significance level:** P-value of $P \leq 0.05$ **Program to Analyze Statistics:** Number Cruncher Statistical System for Windows 2004 version.
Timing of measurements:	All measurements were taken at baseline only. Appendicular skeletal muscle mass was measured at baseline when the participants were under fasting conditions for the other tests such as obtaining blood samples and anthropometric measures. There is no evidence of when dietary recalls were taken in regards to the muscle mass tests.
Dependent variables:	aLM
Independent variables	Total protein intake and daily protein distribution
Control Variables:	None were mentioned.
Initial n	n = 81
Final n	n = 78 (31 men and 47 women)
Age	**Mean age:** 68.7 $\pm$ 6.3 (men: 68.3 $\pm$ 6.16 years and women: 69.0 $\pm$ 6.5
Ethnicity	Non-Caucasian
Other relevant demographics:	Not mentioned
Anthropometrics:	There were no groups for comparison.
Location:	Northwest Mexico
Summary of Results:	Men consumed 13.4g of protein/day more than women ($p < 0.05$). On average, the amount of daily protein was about 0.9g/kg/day. Twenty-eight percent of subjects reached 100% of the DRI for protein. Breakfast and dinner meals had the least amount of protein when compared to the recommended 25-30g per meal ($p < 0.05$). Appendicular skeletal muscle mass was different between Group A, (those who consumed < 25g of protein at each mealtime) and Group B (who consumed >25g of protein for at least one mealtime).
Author's Conclusions	
Author conclusion:	Even though protein intake was higher than the RDA, this amount still failed to prevent the effects of sarcopenia. There was also less protein consumption at breakfast and dinner meals showing there was a significance between protein consumption per mealtime and loss of muscle mass in older adults.

Reviewer comments:	***Strengths:*** • *This was the first pilot study that focused on non-Caucasian older adults.* • *Protein intake distribution at mealtimes and gender comparisons were analyzed* • *Double-labeled water was used to assess free-living energy expenditure in older adults.* • *Volunteers were trained before interviewing participants, which increased assessment consistency.* ***Limitations:*** • *Small sample size* • *Even though this was a cross-sectional study design and most of these study designs are taken only as baseline, it should have been stated in the methodology section instead of inferred.* • *It would have been informative to know more background demographics on the study population including income and ethnicities since this the first study conducted in a developing country.* • *This was conducted internationally so dietary protein characteristics may be different than in the U.S.* • *Since this study involved sarcopenic older adults, it would have been beneficial to compare protein intake in sarcopenic older adults against those without sarcopenia.*

RELEVANCE QUESTIONS					
Citation: Ruiz Valenzuela RE, Ponce JA, Morales-Figueros GG, Muro KA, Carreon VR, Aleman-Mateo H. Insufficient amounts of inadequate distribution of dietary protein intake in apparently healthy older adults in a developing country: implications for dietary strategies to prevent sarcopenia. Clinical Interventions in Aging. 2013; 8: 1143-1148. http://dx.doi.org/10.2147/CIA.S49810		YES	NO	UNCLEAR	NA
1. Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	1				√
2. Did the authors study an outcome (dependent variable) or topic that the patients / clients / population group would care about?	2	√			
3. Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to dietetics practice?	3	√			
4. Is the intervention or procedure feasible (NA for some epidemiological studies)?	4			√	
If the answers to all of the above relevance questions are "yes", the report is eligible for designation with a plus (+) on the Evidence Quality Worksheet, depending on answers to the following validity questions.					
VALIDITY QUESTIONS					

1. Was the research question clearly stated?		YES √	NO	UNCLEAR	NA
1.1 Was the specific intervention(s) or procedure (independent variable(s)) identified?	1.1	√			
1.2 Was the outcome(s) (dependent variable(s)) clearly indicated?	1.2	√			
1.3 Were the target population and setting specified?	1.3	√			
2. Was the selection of study subjects / patients free from bias? As per answers to subquestions below, selection was free from bias, but groups were not comparable (and thus study was biased)		YES √	NO	UNCLEAR	NA
2.5 Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	2.1	√			
2.6 Were criteria applied equally to all study groups?	2.2	√			
2.7 Were health, demographics, and other characteristics of subjects described?	2.3		√		
2.4 Were the subjects /patients in a representative sample of the relevant population?	2.4			√	
3. Were study groups comparable?		YES	NO √	UNCLEAR	NA
3.1 Was the method of assigning subjects / patients to groups described and unbiased? (Method of randomization identified if RCT)	3.1		√		
3.2 Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	3.2			√	
3.3 Were concurrent controls used? (Concurrent preferred over historical controls.)	3.3				√
3.4 If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	3.4		√		
3.5 If case control study, were potential confounding factors comparable for cases and controls? If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.	3.5				√
3.6 If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g. "gold standard")?	3.6				√
4. Was method of handling withdrawals described?		YES √	NO	UNCLEAR	NA
4.1 Were follow up methods described and the same for all groups?	4.1	√			
4.6 Was the number, characteristics of withdrawals (i.e. dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group?	4.2		√		

4.3 Were all enrolled subjects/patients (in the original sample) accounted for?	4.3	√			
4.8 Were reasons for withdrawals similar across groups?	4.4			√	
4.5 If diagnostic test, was decision to perform reference test not dependent on results of test under study?	4.5				√
5. Was <u>blinding</u> used to prevent introduction of bias?		YES	NO	UNCLEAR	NA
			√		
5.16 In intervention study, were subjects, clinicians / practitioners and investigators blinded to treatment group, **<u>as appropriate</u>**?	5.1				√
5.17 Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	5.2		√		
5.18 In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	5.3		√		
5.19 In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	5.4				√
5.5 In diagnostic study, were test results blinded to patient history and other test results?	5.5				√
6. Were intervention / therapeutic regimens / exposure factor or procedure and any comparison(s) described in detail? Were intervening factors described?		YES	NO	UNCLEAR	NA
					√
6.1 In RCT or other intervention trial, were protocols described for all regimens studied?	6.1				√
6.12 In observational study, were interventions, study settings, and clinicians / provider described?	6.2				√
6.13 Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	6.3		√		
6.14 Was the amount of exposure and, if relevant, subject / patient compliance measured?	6.4	√			
6.15 Were co-interventions (e.g., ancillary treatments other therapies) described?	6.5				√
6.16 Were extra or unplanned treatments described?	6.6				√
6.7 Was the information for 6.4, 6.5, 6.6 and 6.7 assessed the same way for all groups?	6.7				√
6.8 In diagnostic study, were details of test administration and replication sufficient?	6.8				√
7. Were <u>outcomes</u> clearly defined and the measurements valid and reliable?		YES	NO	UNCLEAR	NA
		√			
7.5 Were primary and secondary endpoints described and relevant to the question?	7.1	√			
7.2 Were nutrition measures appropriate to question and outcomes of concern?	7.2	√			
7.19 Was the period of follow-up long enough for important outcome(s) to occur?	7.3				√
7.20 Were the observations and measurements based on standard, valid, and reliable data collection instruments / tests / procedures?	7.4	√			

7.21Was the measurement of effect at an appropriate level of precision?	7.5				√
7.22Were other factors accounted for (measured) that could affect outcomes?	7.6	√			
7.7 Were the measurements conducted consistently across groups?	7.7				√
8. Was the statistical analysis appropriate for the study design and type of outcome indicators?		YES √	NO	UNCLEAR	NA
8.21Were statistical analyses adequately described and the results reported appropriately?	8.1	√			
8.22Were correct statistical tests used and assumptions of test not violated?	8.2	√			
8.23Were statistics reported with levels of significance and/or confidence intervals?	8.3	√			
8.24Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	8.4				√
8.25Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g. multivariate analyses)?	8.5		√		
8.6 Was clinical significance as well as statistical significance reported?	8.6				√
8.7 If negative findings, was a power calculation reported to address type 2 error?	8.7				√
9. Are conclusions supported by results with biases and limitations taken into consideration?	**YES**	YES √	NO	UNCLEAR	NA
9.1 Is there a discussion of findings?	9.1	√			
9.2 Are biases and study limitations identified and discussed?	9.2			√	
10. Is bias due to study's funding or sponsorship unlikely?	**YES**	YES √	NO	UNCLEAR	NA
10.1 Were sources of funding and investigators' affiliations described?	10.1	√			
10.2 Was there no apparent conflict of interest?	10.2	√			
SYMBOL NEUTRAL (ø)					
NEUTRAL (ø) *If the answers to validity criteria questions **2, 3, 6, and 7** do not indicate that the study is exceptionally strong, the report should be designated with a neutral (ø) symbol on the Evidence Quality Worksheet.*					

APPENDIX F

Citation:	Meng X, Zhu K, Devine A, Kerr DA, Binns CW, Prince RL. A 5-Year Cohort Study of the Effects of High Protein Intake on Lean Mass and BMC in Elderly Postmenopausal Women. *J Bone Miner Res*. 2009; 24: 1827-1834. doi: 10.1359/JBMR.090513
Study design:	Prospective randomized controlled cohort
Study Class (A,B,C,D)	B
Research Quality Rating	NEUTRAL (ø)
Purpose/Population Studied/Practice Studied	
Research purpose:	To determine the association between baseline protein intake and bone-free lean mass, muscle size, and bone mass in community-dwelling postmenopausal older women after 5-years.
Inclusion criteria:	• Women between the ages of 70 to 85 years old • Those living in Western Australia • Those who fully completed FFQ at baseline • Those who had an evaluation of whole body composition and BMC at 5-years
Exclusion criteria:	• Presence of any medical condition that could affect the 5-yr survival period • Taking bone active medications (calcium supplements, estrogen, bisphosphonates, and vitamin D)
Recruitment:	From the Western Australian area by sending letters to those that fit within the inclusion criteria after being randomly selected from the electoral roll. A total of 24,800 letters were sent out with 5586 responses.
Description of study protocol	**Subjects were randomized into 2 groups:** 1) consumption of 1.2g of calcium carbonate daily intervention group 2) a matched placebo control group However, these groups were no compared against one another. **All participants were analyzed on the following measurements throughout this study:** **Dietary intakes:** a self-administered FFQ was implemented from over the past year. After the dietary assessment, subjects were separated into 3 groups depending on protein intake consumption (1st tertile was <66g/day, 2nd tertile was 66-87g/day, and 3rd tertile was >87g/day). **Anthropometrics:** included height, weight, BMI, and upper arm girth and triceps skin fold tests to determine upper arm girth and triceps skin fold. **Body Composition:** included DXA scan to determine lean mass and BMC measurements. **Other assessments:** included demographic and lifestyle factors through

	the use of questionnaires to classify subjects as sedentary or active. Activities were recorded along with the duration of each.
Intervention:	Not Applicable.
Statistical analysis:	**Levene and Kolmogorov-Smirnov tests:** to check homogeneity of variance and normality assumptions. **Spearman's correlation test:** examine correlation between protein intake and lean mass, upper arm muscle area, and BMC. **One-way ANOVA**: the effects of protein on lean mass upper arm muscle area, and BMC by grouping subjects by protein intake quantities; post hoc comparisons were made using Tukey's statistical test. **ANCOVA:** further analyses were conducted by analysis of covariance by adjusting for potential cofounders; post hoc comparisons were made using Bonferroni test. **Statistical significance:** P-value of $p < 0.05$
Timing of measurements:	**Baseline:** FFQ, height/weight for BMI, demographic and lifestyle factors questionnaire and a physical activity questionnaire **Year 5:** height/weight for BMI, upper arm girth and triceps skin folds, whole body DXA scan
Dependent variables:	Lean mass and BMC
Independent variables	Dietary protein intake
Control Variables	In the ANOVA test, adjustments were made for the potential covarties
Initial n	n = 1500
Final n	n = 862 (450 in calcium treatment group and 412 in placebo group)
Age	**Mean age**: 73+5years old
Ethnicity	Caucasian
Other relevant demographics:	The total sample size favored those of higher economic status however, it did not make a difference from the whole population's health resources.
Anthropometrics:	Those in the 3rd tertile group had a significantly greater BMI than the moderate and low protein intake groups along with being more physically active than the lowest protein tertile group. **Mean BMI for total sample size:** 26.8 $\pm$4.4kg/m^2 (representing 23% obese, 40% overweight, 35% normal weight and 2% underweight)
Location:	Western Australia
Summary of Results:	Subjects consumed 80.6 $\pm$ 27.6 g of protein/day, which represented 19 $\pm$ 3% of their total energy intake. The majority of subjects (89%) consumed > 0.75 g/kg/day of protein daily while 71% consumed > 0.94 g/kg/day. Subjects in the highest protein quartile group (> 87g/day) had significantly higher weights, BMIs, and physical activity levels than those in the lowest quartile group. At 5-year, LMM represented approximately 55 $\pm$ 5% total body mass while aLM and fat mass represented 34 $\pm$ 6%. Upper arm muscle area was 45.5 $\pm$ 12.4 cm^2. Total body and aLM were positively correlated to baseline protein intake,

	weight, height, energy intake, and physical activity at 5-yr. Whole body LMM had the strongest correlation with protein intake than any other macronutrient. Baseline protein intakes were positively associated with whole body fat mass at the 5-year. Subjects in the highest quartile group had significantly higher whole body LMM and aLM in comparison to the lowers quartile groups. The strongest correlation was between whole body BMC and protein intake, but after adjusting more for 5-y lean body mass this did not remain significant.
Author's Conclusions	
Author conclusion:	Protein intake could have a positive impact on bone health due to maintenance of LMM. The highest protein intake quartile of > 87 g/day (1.6 g/kg/day) showed favorable affects on LMM and BMC for women >70 years old. Researchers stated the RDA for protein should be between 1.0 to 1.25 g/kg/day to offset the aging metabolism.
Reviewer comments:	***Strengths:*** • *Studied the long-term effects of protein intake and changes in muscle mass in a free-living environment.* • *Study took into account other influences that affect muscle mass like protein intakes, energy balance, physical activity, age and body size.* • *Study was conducted over an extensive 5-years, which helps create stronger associations between the independent and dependent variables.* • *One of few studies that focused on association between protein intake and bone health.* • *Large sample size of randomly selected elderly women.* • *Many body composition and anthropometric measures considered for analysis (upper arm muscle area and triceps skin fold) other than solely BMI, lean muscle mass or fat mass.* ***Limitations:*** • *The resources to measure body composition at baseline are not available for study analyses so unable to determine the amount of time it takes for high protein to take effect on muscle mass.* • *May not be representative of the general older population since the subjects were Caucasian, majority with high incomes, and were living independently within the community.* • *FFQ and physical activity questionnaires were measured only at baseline so may not be representative over the whole 5 year period to develop a stronger association.* • *A protein-rich food intervention was not included, which would have shown a more direct cause-and-effect conclusion.*

RELEVANCE QUESTIONS					
Citation: Meng X, Zhu K, Devine A, Kerr DA, Binns CW, Prince RL. A 5-Year Cohort Study of the Effects of High Protein Intake on Lean Mass and BMC in Elderly Postmenopausal Women. *J Bone Miner Res*. 2009; 24: 1827-1834. doi: 10.1359/JBMR.090513		YES	NO	UNCLEAR	NA
1. Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	1	√			
2. Did the authors study an outcome (dependent variable) or topic that the patients/clients/	2	√			

population group would care about?						
3. Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to dietetics practice?	3	√				
4. Is the intervention or procedure feasible (NA for some epidemiological studies)?	4					√
If the answers to all of the above relevance questions are "yes", the report is eligible for designation with a plus (+) on the Evidence Quality Worksheet, depending on answers to the following validity questions.						
VALIDITY QUESTIONS						
1. Was the research question clearly stated?		YES √	NO	UNCLEAR	NA	
1.1 Was the specific intervention(s) or procedure (independent variable(s)) identified?	1.1	√				
1.2 Was the outcome(s) (dependent variable(s)) clearly indicated?	1.2	√				
1.3 Were the target population and setting specified?	1.3	√				
2. Was the selection of study subjects / patients free from bias? As per answers to subquestions below, selection was free from bias, but groups were not comparable (and thus study was biased)		YES √	NO	UNCLEAR	NA	
2.1 Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	2.1	√				
2.2 Were criteria applied equally to all study groups?	2.2	√				
2.3 Were health, demographics, and other characteristics of subjects described?	2.3	√				
2.4 Were the subjects /patients in a representative sample of the relevant population?	2.4		√			
3. Were study groups comparable?		YES √	NO	UNCLEAR	NA	
3.1 Was the method of assigning subjects / patients to groups described and unbiased? (Method of randomization identified if RCT)	3.1		√			
3.2 Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	3.2	√				
3.3 Were concurrent controls used? (Concurrent preferred over historical controls.)	3.3				√	
3.4 If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	3.4	√				
3.5 If case control study, were potential confounding factors comparable for cases and controls? If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.	3.5				√	
3.6 If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g. "gold standard")?	3.6				√	
4. Was method of handling withdrawals described?		YES √	NO	UNCLEAR	NA	
4.1 Were follow up methods described and the same for all groups?	4.1	√				

4.7 Was the number, characteristics of withdrawals (i.e. dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80 %.)	4.2	√			
4.3 Were all enrolled subjects/patients (in the original sample) accounted for?	4.3	√			
4.9 Were reasons for withdrawals similar across groups?	4.4			√	
4.5 If diagnostic test, was decision to perform reference test not dependent on results of test under study?	4.5				√
5. Was <u>blinding</u> used to prevent introduction of bias?		**YES**	**NO** √	**UNCLEAR**	**NA**
5.20In intervention study, were subjects, clinicians / practitioners and investigators blinded to treatment group, **<u>as appropriate</u>**?					√
5.21Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	5.2		√		
5.22In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	5.3		√		
5.23In case control study, was case definition explicit and case ascertainment not influenced by exposure status?.	5.4				√
5.5 In diagnostic study, were test results blinded to patient history and other test results?	5.5				√
6. Were intervention / therapeutic regimens / exposure factor or procedure and any comparison(s) described in detail? Were intervening factors described?		**YES** √	**NO**	**UNCLEAR**	**NA**
6.1 In RCT or other intervention trial, were protocols described for all regimens studied?	6.1				√
6.2 In observational study, were interventions, study settings, and clinicians / provider described?	6.2				√
6.3 Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	6.3	√			
6.4Was the amount of exposure and, if relevant, subject / patient compliance measured?	6.4	√			
6.5Were co-interventions (e.g., ancillary treatments other therapies) described?	6.5				√
6.6 Were extra or unplanned treatments described?	6.6				√
6.7 Was the information for 6.4, 6.5, 6.6 and 6.7 assessed the same way for all groups?	6.7	√			
6.8 In diagnostic study, were details of test administration and replication sufficient?	6.8				√
7. Were <u>outcomes</u> clearly defined and the measurements valid and reliable?		**YES** √	**NO**	**UNCLEAR**	**NA**
7.1 Were primary and secondary endpoints described and relevant to the question?	7.1	√			
7.2 Were nutrition measures appropriate to question and outcomes of concern?	7.2	√			

7.3 Was the period of follow-up long enough for important outcome(s) to occur?	7.3	√			
7.4 Were the observations and measurements based on standard, valid, and reliable data collection instruments / tests / procedures?	7.4	√			
7.5 Was the measurement of effect at an appropriate level of precision?	7.5	√			
7.6 Were other factors accounted for (measured) that could affect outcomes?	7.6	√			
7.7 Were the measurements conducted consistently across groups?	7.7	√			
8. Was the <u>statistical analysis</u> appropriate for the study design and type of outcome indicators?		YES √	NO	UNCLEAR	NA
8.1 Were statistical analyses adequately described and the results reported appropriately?	8.1	√			
8.2 Were correct statistical tests used and assumptions of test not violated?	8.2	√			
8.3 Were statistics reported with levels of significance and/or confidence intervals?	8.3	√			
8.4 Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	8.4				√
8.5 Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g. multivariate analyses)?	8.5	√			
8.6 Was clinical significance as well as statistical significance reported?	8.6				√
8.7 If negative findings, was a power calculation reported to address type 2 error?	8.7				√
9. Are <u>conclusions</u> supported by results with biases and limitations taken into consideration?	**YES**	YES	NO √	UNCLEAR	NA
9.1 Is there a discussion of findings?	9.1	√			
9.2 Are biases and study limitations identified and discussed?	9.2		√		
10. Is bias due to study's <u>funding or sponsorship</u> unlikely?	**YES**	YES √	NO	UNCLEAR	NA
10.1 Were sources of funding and investigators' affiliations described?	10.1	√			
10.2 Was there no apparent conflict of interest?	10.2	√			
SYMBOL NEUTRAL (ø)					
NEUTRAL (ø) *If the answers to validity criteria questions **2, 3, 6, and 7** do not indicate that the study is exceptionally strong, the report should be designated with a neutral* (ø) *symbol on the Evidence Quality Worksheet.*					

APPENDIX G

Citation:	Scott D, Blizzard L, Fell J, Giles G, Jones G. Associations Between Dietary Nutrient Intake and Muscle Mass and Strength in Community-Dwelling Older Adults: The Tasmanian Older Adult Cohort Study. *J Am Geriatr Soc.* 2010; 58(11): 2129-2134. doi: 10.1111/j.1532-5415.2010.03147.x Reference Article: Scott D, Blizzard L, Fell J, Jones G. Ambulatory Activity, Body Composition, and Lower-Limb Muscle Strength in Older Adults. *The American College of Sports Medicine*. 2009; 383-389. doi: 10.1249/MSS.0b013e3181882c85
Study design:	Prospective Cohort
Study Class (A,B,C,D)	B
Research Quality Rating	**NEUTRAL (ø)**
Purpose/Population Studied/Practice Studied	
Research purpose:	To figure out if there are any associations between dietary nutrient intake and the development of sarcopenia by evaluting muscle strength and aLM changes.
Inclusion criteria:	• 50-79 years old • Community-dwelling older adults • South Tasmanian men and women
Exclusion criteria	• Institutionalized older adults • Contraindication for magnetic resonance imaging
Recruitment	Participants were selected from electoral rolls using sex-stratified random sampling without replacement; if responded then considered for the study.
Description of study protocol	**Participants underwent the following measurements below so these measurements could be compared against protein intake groups:** **Anthropometrics:** • Height, weight, BMI • DXA whole body scan to assess soft body tissue • Absolute body fat, body fat percentage, and aLM obtained **Leg Strength:** • To determine muscle strength of dominant leg **Physical Activity:** • At baseline: Pedometers were worn for 1 week • At follow-up: Determined if participants still wore them at the end of the study • (Reference Article) Participants were given a pedometer and asked to record in a diary their daily steps to determine the average; the duration, type of physical activity and start and stop times were

	recorded daily. **Dietary Assessment:** • FFQ specific to Australian adults and was self-administered • FFQ was reviewed by interviewer to make sure all questionnaire elements were finished • 28 dietary nutrients were analyzed along with total energy intake • Participants were split into two protein intake groups indicative of failing to meet the RDI for protein versus 2) meeting or exceeding the RDI) after their dietary assessments were analyzed. • The RDI values in Australia are 64 and 81g/day for men aged 51-70 and > 70, 46 and 57g/day for women in the same age groups.
Intervention:	Not applicable.
Statistical analysis:	**Independent-samples-*t*-test:** used to evaluate mean differences between descriptive characteristics at baseline verses follow-up data **Multivariable Regressions:** used within the stepwise process to determine which nutrients showed positive association with aLM at baseline and follow-up for regression analyses; statistical significance of $p < 0.10$. This was also used to compare cross-sectional differences between baseline and follow-up measures of aLM and muscle strength. It was additionally utilized with the longitudinal analyses to determine associations between energy-adjust nutrient intakes and aLM alterations from baseline to follow-up **Statistically significance:** $p < 0.05$
Timing of measurements:	Anthropometrics, leg strength, physical activity (baseline measurement-calculated over 7 days wearing the pedometer) and assessment of dietary nutrient intakes were all obtained at baseline and at year 2 and year 3 follow-up. For physical activity, the length of time between baseline and follow-up measures were on average 2.6 ± 0.4 years.
Dependent variables:	aLM and muscle strength
Independent variables	Dietary nutrient intake (specifically protein intake)
Control Variables:	**Multivariable Regressions:** • Adjusted for sex, energy intake and physical activity to determine muscle mass and strength differences • Adjusted for age, sex and physical activity to determine cross-sectional differences between dietary nutrient intake and aLM differences • Examined cross-sectional (adjusted for age, sex, baseline muscle strength, and change in physical activity) and longitudinal correlations between energy-adjusted nutrient intake and muscle strength only in regards to nutrients that are seen as positively linked to aLM in the longitudinal analyses **Stepwise multivariable linear regression model:** • Adjusted for age, sex, aLM at baseline, change in physical activity and change in body fat

	Longitudinal analyses: • First adjusted for age, sex, change in physical activity and body fat when examining a possible correlation between energy-adjusted nutrient intake and aLM • Then further adjustments were made for baseline aLM values • Further analysis controlled for protein intake over 2.6 years were conducted
Initial n	n = 1,099 (98% Caucasian)
Final n (attrition)	n = 740 (370 or 50% female)
Age	**Mean age:** 62 + 7 years old
Ethnicity (if given)	A majority of participants were Caucasian, but there is no table recording this information.
Other relevant demographics:	Not mentioned.
Anthropometrics:	Similar across protein intake groups, except for the dependent variables.
Location:	Tasmania, Australia
Summary of Results:	Eighty-nine (12%) older adults failed to meet the RDI for protein, at baseline, while 106 (14%) consumed inadequate protein at follow-up. Additionally, those in lower protein intake category also had significantly lower aLM at baseline and follow-up (-0.81 kg, 95% CI (-1.54 to -0.08); p = 0.03 and -0.79 kg, 95% CI (-1.42 to -0.17); p = 0.01, respectively). Muscle strength did not differ between these two groups. The nutrients that showed a positive correlation to aLM were included in a forward stepwise regression model; a significance level of $p < 0.10$ was required for inclusion. As a result, protein was the only macronutrient shown to be a significant independent predictor of aLM changes (p = 0.007), indicating it's strong correlation with sarcopenia. After further adjustments were implemented, protein, iron, magnesium, phosphorus, and zinc remained significantly associated with positive changes in aLM over the course of the study.
Author's Conclusions	
Author conclusion:	Several nutrients including protein intake were associated with changes in muscle mass and losses in older adults however, not for muscle strength. As a result, there may be many different nutrients that could potentially delay the progression of sarcopenia as a person ages.
Reviewer comments:	***Strengths:*** • *28 nutrients were statistically analyzed, which is high in comparison to other studies* • *Large study population representative of community-dwelling older adults* • *The study was conducted over a prolonged period of time, 2 to 3 years* • *Evaluates the nutrients with stronger associations by breaking each nutrient into further quartiles* • *Stratified protein intakes to create a greater understanding of which interventions should be implemented and the amounts older adults are actually consuming in comparison to the RDI for protein*

Limitations:

- *FFQ might have some participant bias since it was self-reported*
- *During recruitment the response rate was low (57%) even though retention rate at follow-up was high (82%)*
- *There was lack of nutrient intake or muscle mass differences between those participants included and those who failed to continue on through follow-up*
- *No adjustment was made for the multiple comparisons completed during the study so every conducted analysis was reported and emphasis was placed only on nutrients that were continuously significant with aLM in cross-sectional and longitudinal analyses*
- *Study results are not generalizable since the majority of participants were white, community-dwelling older adults aged 70 to 79 years old*
- *Since the majority of participants were younger than 75 years old, these results may not be applicable to those aged 80 years and older*
- *Other carotenoids, beyond beta-carotenoids, or polyphenols were not assessed to this may weaken the impact antioxidants can have on muscle mass and strength*

RELEVANCE QUESTIONS					
Citation: Aleman-Mateo H, Macias L, Esparza-Romero J, Astiazaran-Garcia H, Blancas AL. Physiological effects beyond the significant gain in muscle mass in sarcopenic elderly men: evidence from a randomized clinical trial using a protein-rich food. Clinical Interventions in Aging. 2012; 7: 225-234. http://dx.doi.org/10.2147/CIA.S32356		YES	NO	UNCLEAR	NA
1. Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	1			√	
2. Did the authors study an outcome (dependent variable) or topic that the patients / clients / population group would care about?	2	√			
3. Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to dietetics practice?	3	√			
4. Is the intervention or procedure feasible (NA for some epidemiological studies)?	4	√			
If the answers to all of the above relevance questions are "yes", the report is eligible for designation with a plus (+) on the Evidence Quality Worksheet, depending on answers to the following validity questions.					

VALIDITY QUESTIONS					
1. Was the <u>research question</u> clearly stated?		YES √	NO	UNCLEAR	NA
1.1 Was the specific intervention(s) or procedure (independent variable(s)) identified?	1.1	√			
1.2 Was the outcome(s) (dependent variable(s)) clearly indicated?	1.2	√			
1.3 Were the target population and setting specified?	1.3	√			
2. Was the <u>selection</u> of study subjects / patients free from bias?		YES	NO √	UNCLEA	NA

				R	
2.1 Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	2.1		√		
2.2 Were criteria applied equally to all study groups?	2.2	√			
2.3 Were health, demographics, and other characteristics of subjects described?	2.3	√			
2.4 Were the subjects /patients in a representative sample of the relevant population?	2.4		√		
3. Were study groups comparable?		YES √	NO	UNCLEAR	NA
3.1 Was the method of assigning subjects / patients to groups described and unbiased? (Method of randomization identified if RCT)	3.1		√		
3.2 Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	3.2				√
3.3 Were concurrent controls used? (Concurrent preferred over historical controls.)	3.3				√
3.4 If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	3.4	√			
3.5 If case control study, were potential confounding factors comparable for cases and controls? If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.	3.5				√
3.6 If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g. "gold standard")?	3.6				√
4. Was method of handling withdrawals described?		YES √	NO	UNCLEAR	NA
4.1 Were follow up methods described and the same for all groups?	4.1	√			
4.2 Was the number, characteristics of withdrawals (i.e. dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80 %.)	4.2	√			
4.3 Were all enrolled subjects/patients (in the original sample) accounted for?	4.3	√			
4.4 Were reasons for withdrawals similar across groups?	4.4	√			
4.5 If diagnostic test, was decision to perform reference test not dependent on results of test under study?	4.5				√
5. Was blinding used to prevent introduction of bias?		YES	NO √	UNCLEAR	NA
5.1 In intervention study, were subjects, clinicians / practitioners and investigators blinded to treatment group, **as appropriate**?	5.1				√
5.2 Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	5.2		√		
5.3 In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	5.3		√		

5.4 In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	5.4				√
5.5 In diagnostic study, were test results blinded to patient history and other test results?	5.5				√
6. Were intervention / therapeutic regimens / exposure factor or procedure and any comparison(s) described in detail? Were intervening factors described?		YES √	NO	UNCLEAR	NA
6.1 In RCT or other intervention trial, were protocols described for all regimens studied?	6.1				√
6.2 In observational study, were interventions, study settings, and clinicians / provider described?	6.2				√
6.3 Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	6.3	√			
6.4 Was the amount of exposure and, if relevant, subject / patient compliance measured?	6.4	√			
6.5 Were co-interventions (e.g., ancillary treatments other therapies) described?	6.5				√
6.6 Were extra or unplanned treatments described?	6.6				√
6.7 Was the information for 6.4, 6.5, 6.6 and 6.7 assessed the same way for all groups?	6.7	√			
6.8 In diagnostic study, were details of test administration and replication sufficient?	6.8				√
7. Were outcomes clearly defined and the measurements valid and reliable?		YES √	NO	UNCLEAR	NA
7.1 Were primary and secondary endpoints described and relevant to the question?	7.1	√			
7.2 Were nutrition measures appropriate to question and outcomes of concern?	7.2	√			
7.3 Was the period of follow-up long enough for important outcome(s) to occur?	7.3	√			
7.4 Were the observations and measurements based on standard, valid, and reliable data collection instruments / tests / procedures?	7.4	√			
7.5 Was the measurement of effect at an appropriate level of precision?	7.5			√	
7.6 Were other factors accounted for (measured) that could affect outcomes?	7.6	√			
7.7 Were the measurements conducted consistently across groups?	7.7	√			
8. Was the statistical analysis appropriate for the study design and type of outcome indicators?		YES √	NO	UNCLEAR	NA
8.1 Were statistical analyses adequately described and the results reported appropriately?	8.1	√			
8.2 Were correct statistical tests used and assumptions of test not violated?	8.2	√			
8.3 Were statistics reported with levels of significance and/or confidence intervals?	8.3	√			

8.4 Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	8.4				√
8.5 Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g. multivariate analyses)?	8.5	√			
8.6 Was clinical significance as well as statistical significance reported?	8.6				√
8.7 If negative findings, was a power calculation reported to address type 2 error?	8.7				√
9. Are <u>conclusions</u> supported by results with biases and limitations taken into consideration?	**YES**	**YES**	**NO** √	**UNCLEAR**	**NA**
9.1 Is there a discussion of findings?	9.1	√			
9.2 Are biases and study limitations identified and discussed?	9.2		√		
10. Is bias due to study's <u>funding or sponsorship</u> unlikely?	**YES**	**YES** √	**NO**	**UNCLEAR**	**NA**
10.1 Were sources of funding and investigators' affiliations described?	10.1	√			
10.2 Was there no apparent conflict of interest?	10.2	√			

SYMBOL NEUTRAL (ø)

NEUTRAL (ø)

*If the answers to validity criteria questions **2, 3, 6, and 7** do not indicate that the study is exceptionally strong, the report should be designated with a neutral (ø) symbol on the Evidence Quality Worksheet.*

APPENDIX H

Citation:	Gregorio L, Brindisi J, Kleppinger R, et al. Adequate Dietary Protein is Associated with Better Physical Performance Among Post-Menopausal Women 60-90 Years. *J Nutr Health Aging*. 2013; 18(2):155-160. doi: 10.1007/s12603-013-0391-2.
Study design:	Cross-sectional observational
Study Class (A,B,C,D)	D
Research Quality Rating	**NEUTRAL (ø)**
Purpose/Population Studied/Practice Studied	
Research purpose:	The purpose of this study was to further evaluate the relationship between protein intake, body composition, and physical performance in community-dwelling, independent post-menopausal females.
Inclusion criteria:	Three previous studies were combined for this current study to increase subject size so more diverse women were included with greater variability in protein intake and exercise. Criteria from the 3 studies, using the primary journals from each study, were as followed: **1st Study (evaluated dehydroepiandosterone (DHEA) in combination with light aerobic/yoga exercise):** • Women ≥65years old • DHEA levels <550ng/dL • BMD more than 1 SD below normal for a young adult • Present of at least one of the five frailty criteria • Underwent a mammogram within the prior year with normal result **2nd Study (evaluated how a 1.2g of fish oil verses a placebo affected physical performance and frailty):** • Postmenopausal women >65 years old • Free of bone, cancer, or liver disease • No use of bisphosphonates, hormonal therapies, or long-term corticosteroids • Without record of a hip or vertebral fracture within the last year • No medical or herbal supplementation including anticoagulation or anti-platelet activity • No seafood allergies **3rd Study (was undergoing recruitment so background information was limited in this study's methodology)** • Selection was based on reports of lower protein intake of older women **Current Study:** • Community-dwelling, independent, post-menopausal women • Between the ages of 60-90 years old
Exclusion criteria:	**Current Study:** • Being treated for osteoporosis or diseases that affect bone metabolism • Taking medications known to affect bone health • If life expectancy is less than 2 years (timeline of study)
Recruitment:	All participants from the 3 studies were recruited from the central Connecticut

	community
Description of study protocol:	**Body Composition** • using DXA • Lunar Prodigy used at UCHC (University of Connecticut Health Center) • Hologic 4500W used at Yale University • Measurements of: lean body mass (including total lean mass and appendicular skeletal mass (ASM) and fat mass) **Strength and Function** • Physical function assessed using Physical Performance test (PPT) and Short Physical Performance Battery (SPPB) • PPT measures upper extremity strength fine and course motor function, mobility and coordination by performing simple tasks; higher scores equals higher performance • SPPB measures balance, walking speed and strength; higher score represents a better physical function • Handgrip strength **Questionnaires** • Studies 1 and 3, the Physical Activity Scale in the Elderly (PASE) was used to evaluate physical activity; higher score represents more activity • Medical Outcomes Survey Short-form 8 (MOS SF-8) assessed participant's quality of life; higher score indicates greater well-being • Participants reported history of falls, fractures and medical diagnoses • 4-day food record was obtained by a registered dietitian
Statistical analysis:	• PRO intakes were divided into 2 groups (below or above the reference amount of 0.8g PRO/kg of body weight/day) • **Independent *t*-test:** mean group differences between the 2 protein intake groups • **Analysis of covariance:** to further evaluate differences in physical performance between groups • **Linear regression:** to analysis physical function tests (SPPB and PPT) and protein intake along with carbohydrate and fat intakes • Statistical significance is a P-value of $P<0.05$
Timing of measurements:	All measurements taken from baseline data
Dependent variables:	Physical performance levels and body composition measures. Additionally, in the linear regression analyses, SPPB/PPT were representative of dependent variables to confirm findings from the analysis of covariance test.
Independent variables	Protein intake
Control Variables	Analysis of covariance controlled for BMI differences
Initial n	Not mentioned
Final n	n = 387
Age	72.7 + 7.0 years old
Ethnicity	95.5% Caucasian, 3.4% African-American, 1.1% Asian
Other relevant demographics:	No other demographics were mentioned
Anthropometrics:	The 2 groups are referred to as low protein intake group and high protein intake group. In comparison to the lower protein group, women in the high protein group weighed less and had lower BMIs, P-value of $P<0.001$ respectively. In addition, those in the high protein group had lower fat, lean mass, and ratio of fat/lean mass. Even after adjusting for BMI measures, the women in the low

	protein group reported more fractures. For physical performance, there were many significant differences between low and high protein intake groups.
Location:	Central Connecticut clinical research centers **Two sites for body composition measures:** Farmington, Connecticut- University of Connecticut Health Center or New Haven, Connecticut- Yale University
Summary of Results:	Seven percent of women reported heart disease, 39% had hypertension, 15% had osteoporosis, 27% had osteoarthritis. Those in the lower protein intake group (< 0.8 g/kg/day) reported higher rates of hypertension, osteoarthritis, and fractures. For body mass index (BMI) 43% of women were normal, 33% were overweight, and 23.5% were considered obese. The range of protein intakes reported were 0.31g/kg/day to 3.16 g/kg/day. Ninety-seven (25%) of subjects consumed < 0.8 g/kg/day (considered the low protein intake group), while 290 (75%) reported > 0.8 g/kg/day (considered the high protein intake group). Those in the high protein group tended to weighed less and lower BMIs along with lower fat and lean mass (ratio of fat/lean mass) in comparison to the low protein intake group. Total calories, protein, fat and carbohydrates were more consumed in the high protein group. There were significant differences between the high and low protein groups in regards to physical function; SPPB and PPT scores were significantly different. However, after controlling for lean mass only SPPB remained significant while the chair rise time became significant. Women in the low protein group performed lower in the one leg stance when compared to the other group ($p = 0.002$). Hand grip strength was similar between groups.
Author's Conclusions	
Author conclusion:	On average, healthy, older post-menopausal women consumed approximately 1.1g/kg/day of protein with 25% consuming below the RDA (<0.8g/kg/day). Those who consumed less than the RDA, experienced impaired walking speed and single leg stance. Higher fat and fat-to-lean ratios were more prevalent in those with lower protein consumption when compared to subjects who had higher protein intake. In conclusion, there was an association between protein intake and physical function so protein intake should be evaluated when older women are experience physical performance declines.
Reviewer comments:	***Strengths:*** • *Evaluated dietary intake and body composition using valid and reliable instruments* • *Dietary information was obtained by registered dietitians* • *Used a range of self-reported and observational data to assess physical function* • *Methods included multiple strength and physical function measurements* • *Used diverse women population from three other studies to increase sample size, which could make the results more generalizable* ***Limitations:*** • *Specific protein sources were not evaluated to determine which protein foods are most consumed by older women for a more appropriate nutrition intervention* • *Study was primarily compromised of community-dwelling, independent, affluent, Caucasian women so the results may not reflect the general older population (i.e. protein intake and physical activity levels).* • *Unable to determine cause and effect due to the nature of the study, only strong/weak associations, and only depicted one point in time* • *Food records and some physical function aspects were self-reported so*

there may have been subject bias • *Only separated protein intake into 2 groups so detail is limited on establishing a more appropriate RDA in preventing, delaying, or slowing progression of sarcopenia* • *Total caloric intake was not controlled for when analyzing protein intake, which could have reduced validity of results.*

RELEVANCE QUESTIONS					
Citation: Gregorio L, Brindisi J, Kleppinger R, et al. Adequate Dietary Protein is Associated with Better Physical Performance Among Post-Menopausal Women 60-90 Years. *J Nutr Health Aging*. 2013; 18(2):155-160. doi: 10.1007/s12603-013-0391-2.		**YES**	**NO**	**UNCLEAR**	**NA**
1. Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	1				√
2. Did the authors study an outcome (dependent variable) or topic that the patients / clients / population group would care about?	2	√			
3. Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to dietetics practice?	3	√			
4. Is the intervention or procedure feasible (NA for some epidemiological studies)?	4	√			
If the answers to all of the above relevance questions are "yes", the report is eligible for designation with a plus (+) on the Evidence Quality Worksheet, depending on answers to the following validity questions.					

VALIDITY QUESTIONS					
1. Was the <u>research question</u> clearly stated?		**YES** √	**NO**	**UNCLEAR**	**NA**
1.1 Was the specific intervention(s) or procedure (independent variable(s)) identified?	1.1	√			
1.2 Was the outcome(s) (dependent variable(s)) clearly indicated?	1.2	√			
1.3 Were the target population and setting specified?	1.3	√			
2. Was the <u>selection</u> of study subjects / patients free from bias?		**YES**	**NO** √	**UNCLEAR**	**NA**
2.1 Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	2.1		√		
2.2 Were criteria applied equally to all study groups?	2.2	√			
2.3 Were health, demographics, and other characteristics of subjects described?	2.3	√			
2.4 Were the subjects /patients in a representative sample of the relevant population?	2.4		√		
3. Were <u>study groups comparable</u>?		**YES** √	**NO**	**UNCLEAR**	**NA**
3.1 Was the method of assigning subjects / patients to groups described and unbiased? (Method of randomization identified if RCT)	3.1		√		

3.2 Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	3.2	√				
3.3 Were concurrent controls used? (Concurrent preferred over historical controls.)	3.3					√
3.4 If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	3.4	√				
3.5 If case control study, were potential confounding factors comparable for cases and controls? If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.	3.5					√
3.6 If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g. "gold standard")?	3.6					√
4. Was method of handling <u>withdrawals</u> described?		YES	NO √	UNCLEAR	NA
4.1 Were follow up methods described and the same for all groups?	4.1	√				
4.2 Was the number, characteristics of withdrawals (i.e. dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80 %.)	4.2		√			
4.3 Were all enrolled subjects/patients (in the original sample) accounted for?	4.3		√			
4.4 Were reasons for withdrawals similar across groups?	4.4					√
4.5 If diagnostic test, was decision to perform reference test not dependent on results of test under study?	4.5					√
5. Was <u>blinding</u> used to prevent introduction of bias?		YES	NO √	UNCLEAR	NA
5.1 In intervention study, were subjects, clinicians / practitioners and investigators blinded to treatment group, **<u>as appropriate</u>**?	5.1					√
5.2 Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	5.2		√			
5.3 In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	5.3		√			
5.4 In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	5.4					√
5.5 In diagnostic study, were test results blinded to patient history and other test results?	5.5					√
6. Were intervention / therapeutic regimens / exposure factor or procedure and any comparison(s) described in detail? Were intervening factors described?		YES √	NO	UNCLEAR	NA
6.1 In RCT or other intervention trial, were protocols described for all regimens studied?	6.1					√
6.2 In observational study, were interventions, study settings, and clinicians / provider described?	6.2					√
6.3 Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	6.3		√			

6.4 Was the amount of exposure and, if relevant, subject / patient compliance measured?	6.4	√			
6.5 Were co-interventions (e.g., ancillary treatments other therapies) described?	6.5				√
6.6 Were extra or unplanned treatments described?	6.6		√		
6.7 Was the information for 6.4, 6.5, 6.6 and 6.7 assessed the same way for all groups?	6.7	√			
6.8 In diagnostic study, were details of test administration and replication sufficient?	6.8				√
7. Were outcomes clearly defined and the measurements valid and reliable?		YES √	NO	UNCLEAR	NA
7.1 Were primary and secondary endpoints described and relevant to the question?	7.1	√			
7.2 Were nutrition measures appropriate to question and outcomes of concern?	7.2	√			
7.3 Was the period of follow-up long enough for important outcome(s) to occur?	7.3		√		
7.4 Were the observations and measurements based on standard, valid, and reliable data collection instruments / tests / procedures?	7.4	√			
7.5 Was the measurement of effect at an appropriate level of precision?	7.5	√			
7.6 Were other factors accounted for (measured) that could affect outcomes?	7.6		√		
7.7 Were the measurements conducted consistently across groups?	7.7	√			
8. Was the statistical analysis appropriate for the study design and type of outcome indicators?		YES √	NO	UNCLEAR	NA
8.1 Were statistical analyses adequately described and the results reported appropriately?	8.1	√			
8.2 Were correct statistical tests used and assumptions of test not violated?	8.2	√			
8.3 Were statistics reported with levels of significance and/or confidence intervals?	8.3	√			
8.4 Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	8.4				√
8.5 Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g. multivariate analyses)?	8.5	√			
8.6 Was clinical significance as well as statistical significance reported?	8.6	√			
8.7 If negative findings, was a power calculation reported to address type 2 error?	8.7				√
9. Are conclusions supported by results with biases and limitations taken into consideration?	**YES**	YES √	NO	UNCLEAR	NA
9.1 Is there a discussion of findings?	9.1	√			
9.2 Are biases and study limitations identified and discussed?	9.2	√			
10. Is bias due to study's funding or sponsorship unlikely?	**YES**	YE	NO	UN	NA

		S		CLEAR	
		√			
10.1 Were sources of funding and investigators' affiliations described?	10.1	√			
10.2 Was there no apparent conflict of interest?	10.2	√			
SYMBOL NEUTRAL (ø)					
NEUTRAL (ø) *If the answers to validity criteria questions **2, 3, 6, and 7** do not indicate that the study is exceptionally strong, the report should be designated with a neutral* (ø) *symbol on the Evidence Quality Worksheet.*					

APPENDIX I

Citation:	Aleman-Mateo H, Macias L, Esparza-Romero J, Astiazaran-Garcia H, Blancas AL. Physiological effects beyond the significant gain in muscle mass in sarcopenic elderly men: evidence from a randomized clinical trial using a protein-rich food. *Clin Interv Aging*. 2012; 7: 225-234. http://dx.doi.org/10.2147/CIA.S32356
Study design:	RCT
Study Class (A,B,C,D)	A
Research Quality Rating	**PLUS/POSITIVE (+)**
Purpose/Population Studied/Practice Studied	
Research purpose:	The purpose was to test whether or not adding a protein-rich food, like ricotta cheese, to the habitual dietary pattern would positively affect aLM and muscle strength in elderly with sarcopenia.
Inclusion criteria:	• Participants ≥ 60 years old • **Competed the 1st eligibility phase:** participants had to undergo a DXA scan and the medical screening to determine physical independency, free of type-2 DM and have no kidney or liver disease • **2nd eligibility phase:** participants only diagnosed with sarcopenia after results were determined by 1st eligibility phase were chosen for this study. • **3rd eligibility phase:** participants who to undergo a medical exam. Those without any evidence of health issues were included.
Exclusion criteria	• After being diagnosed with sarcopenia, any participants showed evidence of impaired kidney function, glucose >126 g/dL, and microalbuminuria during the medical exam. • Refused to eat ricotta cheese • Those who had gastrointestinal problems related to consuming dairy products or refusal to participate were excluded.
Recruitment	Though home visits and phone calls within the study area.
Blinding used:	Personnel were blinded to participants assigned to conditions (ricotta cheese + habitual diet group vs. habitual diet group).
Description of study protocol	The medical exam was conducted at the beginning and end of the study. This included labs for: hemoglobin, fasting glucose, lipid profile, hepatic profile, kidney functioning test such as creatinine, uric acid, urea blood levels, and GFR. The reason to re-test at the end was to ensure the extra protein intake did not affect the kidney function of the participants. To assess the anabolic effects of adding ricotta cheese, the insulin-like growth factor and fasting insulin were measured by enzyme-linked immunosorbent assay. In addition, insulin resistance was also measured. To determine body weight, participants were on a standing scale barefoot and lightly dressed while standing height was obtained, which would be used to calculate BMI. The aLM, total body mass, and other components were measured using DXA. For muscle strength, participants used a handgrip dynamometer. The experimental design consisted of 40 total participants who were randomly assigned (1:1) to the intervention group

	(ricotta cheese + habitual diet) or control group (habitual diet). Each group was followed during the 3 month intervention and to ensure participants followed protocol both groups were visited 3 times per week at their homes. Participants were asked to maintain their normal daily physical activities and habitual diet throughout this study. This study intervention lasted for 3 months.
Intervention:	Ricotta cheese supplementation was added to the participants habitual eating pattern during breakfast, lunch and dinner. This consisted of 210g/day equaling 15.7g of protein, 18.4g of fat and 10.4g of carbohydrates. Portions were previously divided, weighed and packed in 70g/serving. Participants recorded the amount of ricotta cheese left in their plastic containers while continuing to consume the typical amount of food or meals daily. The food was personally transported, followed safe food handling techniques and temperatures. Deliveries were made three times per week to the subject's homes. The amount of ricotta cheese given was determined by the recommended 15 g intake needed to have an anabolic effect on skeletal muscle in healthy older adults subjects.
Statistical analysis:	**Two-tailed *t*-test**: used to determine sample size (alpha level was set at 0.05). A mean different of 1.8 kg and standard deviation of 2.8kg of lean body mass was configured. **Statistical Power Analysis:** an 80% statistical power was determined if a total of 40 participants (20 participants in each group) were involved in this study. This would allow researchers to find differences in kg of lean body mass between the two randomized groups. **An independent *t*-test:** confirmed if differences were found between lean body mass and the two randomized groups. Also used under the intent-to-treat strategy.
Timing of measurements:	Measurements were obtained at baseline, 2 weeks later starting the intervention, and at 3 month follow-up after the intervention was finished.
Dependent variables:	The percentage of relative change in aLM and muscle strength
Independent variables:	Consuming 210g/day of ricotta cheese (high-protein food source) in addition to the habitual eating patterns patients typically ingested.
Control Variables	The participants who followed a habitual diet pattern with no additional dietary interventions (the control group).
Initial n	n = 302
Final n (attrition)	n = 40 for 20 in the intervention group and 20 in the control group (17 men and 23 women) n = 29 (fully completed the study)
Age	**Mean age:** 76 + 5.4 years
Ethnicity	Not mentioned.
Other relevant demographics:	Not mentioned.
Anthropometrics:	There were no significant differences between groups. The intervention group showed some improvement with a positive percentage change, but only showed a trend towards significance for muscle strength.

Location:	Hermosillo Sonora, Mexico
Summary of Results:	The percentage of aLM changes were not significantly between groups after the intervention, but participants did show improvement in muscle strength within the intervention group. Overall, men in the intervention group had the most improvements in weight gain, totaling 270g in TASM, compared to men in the control group and all the women participants. In addition, these male individuals also improved their fasting insulin levels ($p = 0.05$) along with other factors such as muscle strength, lean body mass specifically in the arms and other body weight variables.
Author's Conclusions	
Author conclusion:	Adding the ricotta cheese to the subject's habitual diet, but was no shown to be effective in reversing sarcopenia. However, muscle strength showed a positive tendency in both men and women. Men in the intervention groups also experienced increased aLM, decreased insulin resistance, muscle strength, increased LMM in arms, and body weight improvements. Overall, the amount of ricotta cheese may have been too large and difficult for some participants to fully consume, but this could be an option for some elderly individuals. There still needs to be more research on the gender affects of protein, in particular, elderly male subjects with sarcopenia since these individuals showed the most benefit.
Reviewer comments:	***Strengths****:* • *Provided detailed information on methods and study design.* • *A randomized control trial with blinding prevented bias and was able to compare data between sexes.* • *This provided more descriptive characteristics.* • *Involving individuals with sarcopenia was unique to this study and is the main target population for increasing protein rich foods.* • *Appropriate exclusion/inclusion criteria* ***Weaknesses****:* • *Small sample size* • *A high amount of ricotta cheese was consumed so this may have favored men's normal intake instead of women.* • *This sample size was calculated based on gains in lean body mass in women and not TASM for reasons unknown.* • *Not all protocols had to be met for the participant to be involved in the intervention.* • *Involved only one high-protein source* • *A high number of drop-out rates limited the final sample size* • *This study was conducted internationally so their dietary patterns may have been different than in the United States so results may have been different if done nationally.*

RELEVANCE QUESTIONS					
Citation:		**YE**	**NO**	**UN**	**NA**

Aleman-Mateo H, Macias L, Esparza-Romero J, Astiazaran-Garcia H, Blancas AL. Physiological effects beyond the significant gain in muscle mass in sarcopenic elderly men: evidence from a randomized clinical trial using a protein-rich food. Clinical Interventions in Aging. 2012; 7: 225-234. http://dx.doi.org/10.2147/CIA.S32356		YES		CLEAR	
1. Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	1	√			
2. Did the authors study an outcome (dependent variable) or topic that the patients / clients / population group would care about?	2	√			
3. Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to dietetics practice?	3	√			
4. Is the intervention or procedure feasible (NA for some epidemiological studies)?	4	√			
If the answers to all of the above relevance questions are "yes", the report is eligible for designation with a plus (+) on the Evidence Quality Worksheet, depending on answers to the following validity questions.					
VALIDITY QUESTIONS					
1. Was the research question clearly stated?		YES √	NO	UNCLEAR	NA
1.1 Was the specific intervention(s) or procedure (independent variable(s)) identified?	1.1	√			
1.2 Was the outcome(s) (dependent variable(s)) clearly indicated?	1.2	√			
1.3 Were the target population and setting specified?	1.3	√			
2. Was the selection of study subjects / patients free from bias? As per answers to subquestions below, selection was free from bias, but groups were not comparable (and thus study was biased)		YES √	NO	UNCLEAR	NA
2.1 Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	2.1	√			
2.2 Were criteria applied equally to all study groups?	2.2	√			
2.3 Were health, demographics, and other characteristics of subjects described?	2.3	√			
2.4 Were the subjects /patients in a representative sample of the relevant population?	2.4		√		
3. Were study groups comparable?		YES √	NO	UNCLEAR	NA
3.1 Was the method of assigning subjects / patients to groups described and unbiased? (Method of randomization identified if RCT)	3.1	√			
3.2 Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	3.2	√			
3.3 Were concurrent controls used? (Concurrent preferred over historical controls.)	3.3	√			

3.4 If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	3.4				√
3.5 If case control study, were potential confounding factors comparable for cases and controls? If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.	3.5				√
3.6 If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g. "gold standard")?	3.6				√
4. Was method of handling <u>withdrawals</u> described?		YES √	NO	UNCLEAR	NA
4.1 Were follow up methods described and the same for all groups?	4.1	√			
4.2 Was the number, characteristics of withdrawals (i.e. dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group?	4.2	√			
4.3 Were all enrolled subjects/patients (in the original sample) accounted for?	4.3	√			
4.4 Were reasons for withdrawals similar across groups?	4.4	√			
4.5 If diagnostic test, was decision to perform reference test not dependent on results of test under study?	4.5				√
5. Was <u>blinding</u> used to prevent introduction of bias?		YES √	NO	UNCLEAR	NA
5.1 In intervention study, were subjects, clinicians / practitioners and investigators blinded to treatment group, **<u>as appropriate</u>**?	5.1	√			
5.2 Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	5.2	√			
5.3 In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	5.3				√
5.4 In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	5.4				√
5.5 In diagnostic study, were test results blinded to patient history and other test results?	5.5				√
6. Were intervention / therapeutic regimens / exposure factor or procedure and any comparison(s) described in detail? Were intervening factors described?		YES √	NO	UNCLEAR	NA
6.1 In RCT or other intervention trial, were protocols described for all regimens studied?	6.1	√			
6.2 In observational study, were interventions, study settings, and clinicians / provider described?	6.2				√
6.3 Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	6.3	√			
6.4 Was the amount of exposure and, if relevant, subject / patient compliance measured?	6.4	√			
6.5 Were co-interventions (e.g., ancillary treatments other therapies) described?	6.5		√		

6.6 Were extra or unplanned treatments described?	6.6		√		
6.7 Was the information for 6.4, 6.5, 6.6 and 6.7 assessed the same way for all groups?	6.7	√			
6.8 In diagnostic study, were details of test administration and replication sufficient?	6.8				√
7. Were outcomes clearly defined and the measurements valid and reliable?		YES √	NO	UNCLEAR	NA
7.1 Were primary and secondary endpoints described and relevant to the question?	7.1	√			
7.2 Were nutrition measures appropriate to question and outcomes of concern?	7.2	√			
7.3 Was the period of follow-up long enough for important outcome(s) to occur?	7.3	√			
7.4 Were the observations and measurements based on standard, valid, and reliable data collection instruments / tests / procedures?	7.4	√			
7.5 Was the measurement of effect at an appropriate level of precision?	7.5	√			
7.6 Were other factors accounted for (measured) that could affect outcomes?	7.6	√			
7.7 Were the measurements conducted consistently across groups?	7.7	√			
8. Was the statistical analysis appropriate for the study design and type of outcome indicators?		YES √	NO	UNCLEAR	NA
8.1 Were statistical analyses adequately described and the results reported appropriately?	8.1	√			
8.2 Were correct statistical tests used and assumptions of test not violated?	8.2	√			
8.3 Were statistics reported with levels of significance and/or confidence intervals?	8.3	√			
8.4 Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	8.4	√			
8.5 Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g. multivariate analyses)?	8.5			√	
8.6 Was clinical significance as well as statistical significance reported?	8.6	√			
8.7 If negative findings, was a power calculation reported to address type 2 error?	8.7				√
9. Are conclusions supported by results with biases and limitations taken into consideration?	**YES**	YES √	NO	UNCLEAR	NA
9.1 Is there a discussion of findings?	9.1	√			
9.2 Are biases and study limitations identified and discussed?	9.2	√			
10. Is bias due to study's funding or sponsorship unlikely?	**YES**	YES √	NO	UNCLEAR	NA
10.1 Were sources of funding and investigators' affiliations described?	10.1	√			

10.2 Was there no apparent conflict of interest?	10.2	√			
SYMBOL PLUS/POSITIVE (+)					
PLUS/POSITIVE (+) *If most of the answers to the above validity questions are "Yes" including criteria* ***2, 3, 6, and 7 and at least one additional "yes",*** *(the report should be designated with a plus symbol (+) on the Evidence Quality Worksheet.*					