Serum albumin in relation to change in muscle mass, muscle strength, and muscle power

A thesis

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CERTIFICATE OF APPROVAL

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Abstract

Background Sarcopenia is a term describing the progressive decline of skeletal muscle mass and muscle function with age and is considered to be an important correlate of disability and loss of independence in elderly adults. Serum albumin is the most ubiquitous plasma protein in human serum and low levels have been associated with an increased risk of adverse health outcomes including disability, cardiovascular disease, and mortality. Serum albumin has also been associated with losses of muscle mass and muscle strength and has been proposed as a risk factor for these age-related muscle changes. The purpose of this study was to add to the limited body of research on this topic by examining serum albumin in relation to change in muscle mass, muscle strength, and muscle power. In addition, this study examined the change in serum albumin concentration in relation to these measures of muscle change.

Methods The Osteoporotic Fractures in Men (MrOS) Study is a prospective cohort of community-dwelling men aged 65 years and older. The association between baseline serum albumin concentration and change in appendicular skeletal muscle (ASM) mass, grip strength, and leg power over an average of 4.6 years of follow-up was examined in 5534 participants. Two serum albumin measures obtained an average of 2 years apart were available for 1267 participants. The change in serum albumin concentration was examined with the simultaneous change in ASM mass, grip strength, and leg power in this sample of the cohort.

Results Baseline serum albumin concentration was not associated with change in ASM mass, grip strength, or leg power after an average of 4.6 years of follow-up in the MrOS study population after adjustment for confounders. These results persisted when evaluating the same outcomes over an average of 2 years of follow-up in a sample of the cohort. The serum albumin change analysis demonstrated no association between serum albumin change and the concurrent change in ASM mass and grip strength over an average of 2 years of follow-up. Participants with a marked decrease ($\geq$ 3 g/L) and mild decrease (1-2 g/L) in serum albumin concentration during follow-up exhibited an average loss of -8.9 (95% CI: -25.6, 7.8) watts and -6.3 (95% CI: -21.2, 8.5) watts of leg power, respectively. This loss was statistically significant compared to participants whose serum albumin concentration remained stable (p=0.02).

Conclusions A single low measure of serum albumin was not associated with loss of muscle mass, muscle strength, or muscle power in a population of community-dwelling elderly men. The serum albumin change analysis demonstrated null results with measures of muscle mass and muscle strength. The statistically significant association with leg power was small in magnitude and may not be clinically meaningful. The results of this study question the utility of albumin serum protein as a risk factor for the age-related loss of muscle mass and muscle function commonly referred to as sarcopenia.

Introduction

Albumin is the most ubiquitous plasma protein in human serum and is a nonspecific protein transport for numerous hormones including aldosterone, thyroid hormones (T3 and T4), and androgens (Rothschild, 1988; Sherwood, 1997). Serum albumin is an acute phase reactant (Rothschild, 1988). Its levels in circulation are reduced from inflammatory cytokines such as tumor necrosis factor (TNF) – α, C-reactive protein, and particularly interleukin (IL)-6 (Johnson, 1999; Gabay & Kushner, 1999; Sullivan et al., 2007). Serum albumin levels can be reduced substantially following major surgery or sepsis (Emerson, 1989) and more modest reductions have been observed from subclinical inflammation (Sullivan et al., 2007). Low serum albumin is associated with an increased risk of cardiovascular disease (Weijenberg et al., 1997; Gillum & Makuc, 1992; Kaysen, 2006), disability (Zuliani et al., 2001), and mortality (Gillum & Makuc, 1992; Fried et al., 1998; Weijenberg et al., 1997).

Sarcopenia is a term used to describe the progressive decline of skeletal muscle mass and muscle function with age. These muscle changes are highly prevalent in the elderly population and have been estimated to affect 13-24% of persons under age 70 and greater than 50% in persons over 80 years of age (Baumgartner et al., 1998; Castillo et al., 2003; Iannuzzi-Sucich, 2002). Loss of muscle mass and muscle function have been associated with a number of deleterious health outcomes including frailty, loss of independence, physical disability (Guralnik et al., 1995; Baumgartner et al., 1998; Morley et al., 2001), falls (Baumgartner et al., 1998; Chan et al., 2007), and all-cause mortality (Rantanen et al., 2003; Newman et al., 2006). These age-related changes in muscle are likely to have multiple causes (Roubenoff, 2000). An imbalance between muscle catabolism and anabolism (Soloman & Bouloux, 2006; Roth et al., 2006; Kaysen, 2006; Roubenoff & Hughes, 2000), acceleration of myocyte loss through apoptosis (Marzetti & Leeeuwenburgh, 2006; Soloman & Bouloux, 2006; Roubenoff & Hughes, 2000), reduction in type II muscle fibers (Karakelides & Nair, 2005; Morley et al., 2001),

inflammation (Roth et al., 2006; Soloman & Bouloux, 2006; Roubenoff & Hughes, 2000), loss of alpha motor neurons (Roubenoff & Hughes, 2000), and age-related anorexia and hormonal changes (Soloman & Bouloux, 2006; Morley et al., 2001; Morley, 2001) have all been proposed as potential physiologic mechanisms.

The involvement of serum albumin in relevant hormone pathways and reaction to systemic inflammation make it a viable biologic risk factor for the age-related decline in skeletal muscle mass and muscle function. In support of this hypothesis, two cross-sectional studies have reported an association between low serum albumin and lower measures of muscle strength (Kwon et al., 2007) and muscle mass (Baumgartner et al., 1996) in elderly persons. In addition, two longitudinal studies in community-dwelling elderly populations have reported a statistically significant association between low baseline measures of serum albumin and declines in appendicular skeletal muscle mass (Visser et al., 2005) and grip strength (Schalk et al., 2005). Nonetheless, the research investigating the role of serum albumin in the progressive decline of muscle mass and muscle function is incomplete. No longitudinal studies have examined serum albumin in relation to multiple measures of muscle change in the same study population. Moreover, no studies have examined change in serum albumin concentration with concurrent change in muscle mass and muscle function.

This prospective study quantified the association of baseline serum albumin with change in muscle mass, muscle strength, and muscle power in a large cohort of older ambulatory men. In addition, the association of serum albumin change with these muscle outcomes was examined in a sample of the cohort for whom the 2 year change in serum albumin concentration could be ascertained.

Methods

Study Setting

The Osteoporotic Fractures in Men (MrOS) Study is a prospective cohort study of 5995 community-dwelling men aged 65 years or older. Men were recruited from March 2000 through April 2002 at six U.S. academic medical centers in Birmingham, Alabama; Minneapolis, Minnesota; Palo Alto, California; Pittsburgh, Pennsylvania; Portland, Oregon; and San Diego, California. Eligible men were at least 65 years of age, able to walk without the assistance of another person, and had at least one native hip. Details regarding the recruitment strategies and study design are described elsewhere (Blank et al., 2005 & Orwoll et al., 2005). All surviving participants were invited to return for a planned second study visit (Visit 2) from March 2005 through April 2006. In addition, participants from the Birmingham, Alabama and Portland, Oregon sites were invited to participate in an ancillary study of oral bone loss. This study visit occurred from September 2002 through May 2003 and included 1353 men. At each study visit measures relevant to the present analysis were obtained and included whole and regional body composition by dual energy x-ray absorptiometry (DXA), grip strength, and leg power. Serum was collected at the baseline MrOS visit and the ancillary study but not at Visit 2. The MrOS study protocol was approved by the Institutional Review Board at each of the participating sites and the same protocol was used at all subsequent visits. All participants gave written informed consent.

Serum Albumin

Main Cohort

Participants provided a fasting morning blood-draw during their baseline visit. All blood samples from this visit were analyzed at the Oregon Veterans Administration Clinical Lab in Portland, Oregon using a Roche COBAS Integra 800 automated analyzer (Roche Diagnostics Corp.,

Indianapolis, IN). The analyzer was calibrated daily in the clinical laboratory and one serum control was included in each assay run. The inter-assay coefficient of variation (CV) was 1.98%.

Figure 1 illustrates the eligibility criteria that yielded the main analytic cohort of 5534 participants. Men had to have at least ten aliquots of stored baseline serum for inclusion. There were no material differences between ineligible participants and the main analytic population with regard to age, body composition, muscle strength, or muscle power (**Appendix C**). A stratified sample of the MrOS cohort had no restriction on the quantity of stored serum aliquots and obtained a baseline serum albumin concentration for 233 of the 461 ineligible participants. The baseline serum albumin concentration obtained from this sample of ineligible participants did not differ from the primary analytic cohort (mean $\pm$ sd: 42.4 $\pm$ 3.0 and 42.7 $\pm$ 2.4, respectively).

Ancillary Study

A sample of 1353 men from the Portland and Birmingham sites returned for an interim clinic visit as part of an ancillary study for oral bone loss. Two serum albumin measures were available for 1267 of these participants. Serum was used from the MrOS baseline visit and the ancillary study visit to obtain measures of albumin an average of two years apart (**Figure 1**). For these assessments, albumin was analyzed at the Oregon Health and Science University Clinical Lab with a Beckman LX 20 analyzer from Beckman-Coulter Instruments (United Kingdom).

The main prospective analysis in this study aimed to quantify the association between a single baseline measure of serum albumin in relation to longitudinal change in measures of muscle mass and muscle function. One concern about this type of study design is the potential for misclassification if there are clinically meaningful changes in serum albumin concentration over time. An interclass correlation coefficient was calculated using the two serum albumin measures

obtained from the sample of men participating in the ancillary study for oral bone loss an average of two years apart. The interclass correlation coefficient was high (0.98) and provided good evidence for the reliability of serum albumin measures over this time period (**Appendix B**).

Body Composition

Body composition was measured using dual energy X-ray absorptiometry (DXA) at all study visits. All enrollment sites used DXA machines of the same model and manufacturer (QDR 4500W, Hologic, Inc.; Waltham, MA) and quality assurance measures were implemented to evaluate and maximize the comparability of machines. Intra-clinic and inter-clinic coefficients of variation were within acceptable limits (Orwoll et al., 2005). Hip, spine, and whole body scans were performed at all visits by certified densitometry technicians. Positioning at visits subsequent to MrOS baseline was matched to the baseline scan to enhance the precision of longitudinal measurements. Appendicular skeletal muscle (ASM) mass was defined as the sum of the nonbone, nonfat lean tissue of the extremities and is highly correlated with skeletal muscle mass (r=0.82) (Heysmfield et al., 1990). The ASM outcome variable was calculated as the absolute change in ASM mass from the MrOS baseline visit to Visit 2 for the main prospective analysis and accounted for an average of 4.6 years of follow-up. The serum albumin change analysis muscle mass outcome variable was the absolute change in ASM mass over an average of two years of follow-up and utilized body composition measures from the MrOS baseline visit and baseline ancillary visit.

Muscle Strength and Power

Grip strength was measured using a JAMAR handheld dynamometer (Sammons Preston, Bolingbrook, Illinois) and two trials were performed for each hand. The average result from all hand measurements was used for the grip strength outcome variable. Leg power was assessed using the Nottingham Power Rig (Bassey & Short, 1990; Bassey et al., 1992). The maximum

result from five trials at each visit was used for the leg power outcome variable. Men who could not perform the grip strength or leg power assessments were classified as "unable" and assigned the first percentile value from the 5-year age category to which they belonged at each study visit (Guralnik et al., 2003). The muscle function outcome variables were calculated as the absolute change in leg power and grip strength from the MrOS baseline visit to Visit 2 for the main prospective analysis and between the MrOS baseline visit and baseline ancillary study visit for the serum albumin change analysis. Leg power was only assessed at the Portland site during the baseline ancillary study visit.

Participant Characteristics

Self-reported information on demographic factors, medication use, alcohol consumption, smoking status, medical history, and diet was ascertained at MrOS baseline, Visit 2, and the baseline ancillary study visit. Participant height (cm) was measured by research staff using a Harpenden stadiometer. Weight (kg) was measured on a balance beam or digital scale while wearing shoes and indoor clothing. Race/ethnicity was categorized into five mutually exclusive categories: White, Black, Asian, Hispanic, and Other (multiracial persons). Smoking status was categorized into "current", "past", and "never". Physical activity was quantified using the Physical Activity Scale for the Elderly (PASE) (Washburn et al., 1993) and was based on exercise, leisure, household, and occupational activities commonly performed by older adults. Total caloric intake and protein intake was obtained from a modified food frequency questionnaire developed specifically for MrOS by Block Dietary Data Systems (Boucher et al., 2006). Protein intake was calculated as a percentage of total caloric intake and was categorized into quintiles. Alcohol consumption was reported as usual number of drinks per day and was categorized into "0 drinks per week", "1-7 drinks per week", and "8+ drinks per week". Medical history was assessed with questions about physician diagnosed myocardial infarction, angina, congestive heart failure, stroke, hypertension, chronic obstructive pulmonary disease,

rheumatoid arthritis, osteoarthritis, diabetes, liver disease, renal disease, cancer, and Parkinson's disease. Medications considered in this analysis were oral corticosteroids, statins, nonsteroidal anti-inflammatory medications (NSAIDS), Cox II inhibitors, aspirin, selective serotonin reuptake inhibitors (SSRIs), and thyroid hormone.

Statistical Analysis

For the main prospective analysis quantifying the association of baseline serum albumin with muscle change outcomes, serum albumin concentration was categorized into 1-unit increments (<40 g/L, 40 g/L, 41 g/L, 42 g/L, 43 g/L, 44 g/L, and >44 g/L). A scatterplot smoothing parameter was utilized to ensure that this categorization captured the nature of the association between serum albumin concentration and baseline muscle mass, muscle strength, and muscle power. Baseline characteristics of the main analytic population by serum albumin category were evaluated using one way analysis of variance (ANOVA) for continuous variables and chi-square tests for categorical variables. Baseline characteristics were also assessed with each outcome using ANOVA and chi-square tests. Multiple linear regression analyses were performed to estimate mean differences and their 95% confidence intervals (CI) for measures of muscle mass and muscle function by baseline serum albumin category in a cross-sectional analysis and over time. Two-sided p-values were reported from the F-test and a value of less than 0.05 was considered statistically significant.

Change in the point estimate was used for the selection of confounders with a backward selection process. The absolute change in ASM mass, grip strength, and leg power were the three outcomes assessed and an initial model included baseline serum albumin category, age, study site, and the baseline value of the outcome measure. The grip strength and leg power absolute change outcome variables were additionally modeled with baseline ASM mass and the absolute change in ASM mass to account for differences in body size. All potential confounders

associated with both serum albumin and the outcome variable ($p<0.25$) were added to the initial model (**Appendix E**). Potential confounding variables that were no longer associated with the outcome in the multivariate model were removed sequentially based on the magnitude of the p-value. A covariate was excluded from the model if the p-value was greater than or equal to 0.25. Further, we eliminated variables whose removal from the model resulted in minimal change to the serum albumin least square mean or model R^2. Multiple linear regression using generalized linear models was used for each model analysis. Graphical assessment of the adjusted association between serum albumin and each outcome was performed using generalized additive models and a Loess smoothing parameter.

In the serum albumin change analysis, the change in serum albumin concentration over an average of two years of follow-up was categorized as "marked decrease" ($\geq$ 3 g/L change), "mild decrease" (1 to 2 g/L change), "stable" (0 g/L change), and "increase" (>0 g/L change). The models developed for the primary analysis were additionally adjusted for baseline serum albumin and were used to examine the association between serum albumin change categories and the simultaneous change in ASM mass, grip strength, and leg power. Multiple linear regression using generalized linear models was performed for each outcome. Stratification by three baseline serum albumin categories (<40 g/L, 40-42 g/L, and $\geq$ 43 g/L) was conducted to assess possible interaction between baseline serum albumin and change in serum albumin.

Statistical analyses were performed using SAS software version 9.1 (SAS Institute Inc., Cary, NC).

Results

Study Population

Between the MrOS baseline visit and Visit 2, 79 (1.4%) participants voluntarily withdrew from the study and 524 (9.5%) participants died. Of the remaining participants, 95 (1.7%) refused to participate in Visit 2, 654 (11.8%) completed the Visit 2 self-administered questionnaire but did not attend the clinic visit, and 4182 (84.8%) completed both the visit and the questionnaire. Men who died or voluntarily withdrew (n=603) or who did not participate in Visit 2 (n=749), respectively, were older at baseline than participants who had Visit 2 clinic measures (mean $\pm$ sd: 77.5 $\pm$ 6.8 and 74.8 $\pm$ 6.0 compared to 72.8 $\pm$ 5.4; $p<0.0001$) and were more likely to have a serum albumin concentration less than 40 g/L at baseline (15% and 9% compared to 7%; $p<0.0001$). These participants also demonstrated lower baseline measures of grip strength (mean $\pm$ sd: 34 $\pm$ 8 and 37 $\pm$ 8 compared to 39 $\pm$ 8; $p<0.0001$) and leg power (mean $\pm$ sd: 174 $\pm$ 63 and 193 $\pm$ 63 compared to 215 $\pm$ 61; $p<0.0001$) (**Appendix D**).

Baseline Serum Albumin

Among the 5534 participants in the main prospective analysis, the mean serum albumin concentration was 42.7 $\pm$ 2.4 g/L. Most men (98.4%) had a serum albumin concentration in the range considered normal ($\geq$ 38 g/L) and 460 (8.3%) had a concentration below 40 g/L. Participants with a serum albumin concentration less than 40 g/L were older (75.4 years and 72.4 years, respectively) and more likely to be African American, nondrinkers, and somewhat less physically active than participants in higher serum albumin categories. They were also more likely to take an oral corticosteroid and less likely to use a statin medication (**Table 1**).

The cross-sectional analysis demonstrated no association between baseline serum albumin concentration and ASM mass after adjusting for participant age, race, and study site. A positive association was observed between serum albumin concentration and both measures of muscle

function. Men with a serum albumin concentration less than 40 g/L exhibited an average grip strength of 35.3 (95% CI: 34.4, 36.1) kg compared to an average grip strength of 37.8 (95% CI: 37.2, 38.6) kg in men with a baseline serum albumin concentration of 42 g/L. Leg power was also lower in men with a serum albumin concentration less than 40 g/L compared to men with a serum albumin concentration of 42 g/L [mean (95% CI): 190 (184, 197) watts and 201 (95% CI: 196, 206) watts, respectively]. (**Table 2**).

Main Prospective Analysis

Serum albumin concentration at baseline was not associated with the measures of change in muscle mass or muscle function (**Table 3**). Neither the unadjusted nor adjusted mean absolute change in ASM mass, grip strength, or leg power varied according to baseline serum albumin concentration. Serum albumin accounted for a small portion of the variability in each outcome measure (partial R^2: 1%, 0.2% and 0.4%, respectively) (**Appendix F**). Further, the results remained null when serum albumin was entered in each model as a continuous variable or as a dichotomized variable with a cut-point of 40 g/L (data not shown).

Serum Albumin Change Analysis

Of the 1267 participants with two serum albumin measures, 1244 (98.2%) men had two measures of ASM mass and 1152 (90.9%) men provided grip strength measures at both visits. Leg power was only assessed at the Portland site and yielded a sample size of 512 men for this analysis. The average baseline serum albumin concentration in the ancillary study population was 41.7 $\pm$ 3.1 g/L and the average change in this measure over two years of follow-up was -0.79 $\pm$ 2.77 g/L.

The results of the serum albumin change analysis demonstrated no association between serum albumin change categories and measures of change in ASM mass or grip strength (**Table 4**). A

positive association was observed between serum albumin change categories and the simultaneous change in leg power over an average of two years of follow-up in men at the Portland site (**Figure 2**). Participants with a marked decrease (≥ 3 g/L) and mild decrease (1-2 g/L) in serum albumin concentration exhibited an average loss of -8.9 (95% CI: -25.6, 7.8) watts and -6.3 (95% CI: -21.2, 8.5) watts of leg power, respectively. This loss was statistically significant compared to participants whose serum albumin concentration remained stable during follow-up (**Table 4**). Stratification demonstrated that baseline serum albumin concentration did not modify the association between change in serum albumin and change in ASM mass, grip strength, or leg power (data not shown).

When the change in ASM mass, grip strength, and leg power was modeled with baseline serum albumin concentration in the ancillary study population, the results were similar to findings from the prospective analysis in the main analytic population. The unadjusted and adjusted mean change in ASM mass, grip strength, and leg power over an average of two years of follow-up did not vary according to baseline serum albumin category in these participants (**Table 5**).

Discussion

In the MrOS cohort, a single measure of serum albumin was not associated with change in muscle mass, muscle strength, or muscle power. The serum albumin change analysis demonstrated no association between serum albumin change over two years of follow-up and the simultaneous change in muscle mass or muscle strength. A statistically significant association was observed between serum albumin change and concurrent change in leg power in a sample of the cohort. However, the magnitude of change was small and the relevance of a change of this magnitude is unknown.

Two longitudinal studies have reported a statistically significant association between serum albumin concentration and subsequent change in measures of muscle mass (Visser et al., 2005) and muscle strength (Schalk et al., 2005). The current study may appear to contradict past research but there are similarities in the results. In addition, the statistically significant associations have been small in magnitude. The lowest and highest serum albumin categories in the MrOS study population exhibited an adjusted mean loss of -0.97 (-1.11, -0.083) kg and -0.87 (-0.95, -0.79) kg ASM mass, respectively, over an average of 4.6 years of follow-up. The Health, Aging, and Body Composition Study (Health ABC) participants demonstrated comparable loss of ASM mass over 5 years of follow-up in the lowest and highest serum albumin categories after adjusting for a similar set of confounders (-0.93 $\pm$ 0.06 kg and -0.73 $\pm$ 0.05 kg, respectively) (Visser et al., 2005). In addition, the difference in mean ASM mass change between the highest and lowest serum albumin categories was small in both study populations (0.10 kg and 0.21 kg, respectively). It is unknown if a difference of this magnitude would have clinical implications. Schalk and colleagues (2005) reported a statistically significant trend between serum albumin concentration and change in grip strength after 3 years of follow-up in elderly men. The maximum measurement of the right and left hand were summed in the

LASA study to determine the grip strength outcome variable. The lower three serum albumin quartiles exhibited a loss of grip strength that was similar in magnitude (approximately -11.5 kg, -11.0 kg, and -10.0 kg) and only the highest serum albumin quartile differed from the other three categories. The grip strength difference between the highest and lowest serum albumin quartiles was a modest 5 kg in men (Schalk et al., 2005). There was no association between serum albumin quartiles and grip strength among women in this study (one-sided p for trend=0.06) (Schalk et al., 2005). The association between baseline serum albumin and change in grip strength after 6 years of follow-up in the Longitudinal Aging Study Amsterdam (LASA) was not statistically significant (Schalk et al., 2005). The present study found no association between baseline serum albumin concentration and change in grip strength over an average of 4.6 years of follow-up. In addition, the null findings were consistent between upper and lower extremity measures of muscle function in the MrOS study population.

The serum albumin change analysis in the current study used a repeated measure of serum albumin in a sample of the cohort to evaluate whether the change in serum albumin was more predictive of the age-related loss of muscle mass and muscle function than a single measure. The results of this analysis were consistent with the null findings in the main prospective analysis for measures of muscle mass and muscle strength. A statistically significant association between serum albumin change over two years and the simultaneous loss in leg power was observed in this sample of the cohort. However, the difference between men exhibiting a marked change ($\leq$ -3 g/L) in serum albumin concentration and men with stable serum albumin over two years was not large and there was considerable overlap in the confidence intervals (**Figure 2**).

The null results in the current study persisted regardless of the duration of follow-up, use of a single measure versus a change measure of serum albumin concentration, and use of an upper

or lower extremity measure of muscle function. Further, these results do not deviate substantially from past findings when evaluating magnitude of change rather than relying on statistical significance. The apparent differences across study populations may have more to do with analysis and interpretation rather than differences in results. For example, the analysis of data from the LASA study population used a one-sided, trend p-value. This methodology can result in smaller p-values than the two-sided F-test that was reported in the present study. When data was analyzed using a one-sided trend p-value rather than the two-sided F-test p-value that is reported in Table 3, the relation between serum albumin concentration and change in leg power became statistically significant in the current study ($p=0.001$ vs. $p=0.13$; data not shown). In addition, much of the research conducted on this topic has utilized large sample sizes. This can result in associations that are small in magnitude with highly statistically significant p-values.

Albumin is one of the most commonly measured serum proteins and is used to gauge nutritional status or disease severity in elderly patients (Sullivan, 2001). The utility of this serum protein as a marker for the age-related loss of muscle mass and muscle function appears plausible from a biological perspective and promising as a simple tool for early identification of individuals at risk. However, the limited longitudinal research on this topic questions the use of albumin serum protein for this purpose. Serum albumin can decline in response to physiologic stress (Rothschild et al., 1888), renal and liver disease (Rothschild et al., 1988; Doweiko & Nompleggi, 1991), inflammation (Johnson, 1999; Gabay & Kushner, 1999; Sullivan et al., 2007), and major surgery (Emerson, 1989). In addition, serum albumin has been reported to decrease by as much as 5 g/L in ambulatory individuals following eight hours of bed rest (Hyltoft et al., 1980) and can vary as much as 11 g/L from the use of different laboratory techniques for measurement (Brackeen, 1989). It has been argued that the decline of serum albumin in response to non-adverse conditions makes it a questionable prognostic indicator in individual

patients (Sullivan, 2001). More importantly, the results of the available literature are not compelling and do not support the use of serum albumin as a risk factor for the age-related loss of skeletal muscle mass and muscle function.

The potential bias in the current study is unlikely to explain the null results. Participants who died, voluntarily withdrew, or did not participate in the Visit 2 clinic visit were more likely to have a serum albumin concentration below 40 g/L and have lower measures of grip strength and leg power at baseline. However, the MrOS cohort captured a greater proportion of men at the follow-up visit than the Health ABC study (Visser et al., 2005). Misclassification can occur in any study evaluating a baseline serum measure in relation to the development of subsequent disease if the concentration of serum protein fluctuates over time. The high interclass correlation coefficient (r=0.98) determined in a sample of the MrOS cohort a priori made a single measure of serum albumin appear to be relatively stable over two years of follow-up. The duration of follow-up in the main prospective analysis was an average of 4.6 years and it is possible that more change may have occurred with the passage of additional time. However, when the analysis was repeated in men with outcome measures available after two years of follow-up, the results remained the same.

Strengths of this study include the availability of a multitude of participant demographic, body composition, serum, dietary, and lifestyle factors. This was the first study to our knowledge that was able to examine the change in serum albumin concentration in relation to measures of both muscle mass and muscle function. In addition, it was the first study that examined upper and lower extremity measures of muscle function in relation to baseline serum albumin concentration. The MrOS study is a cohort of relatively healthy elderly men and a possible limitation is that there was not enough variation in the exposure or outcome to capture the nature of the association. However, the magnitude of change in the outcomes was similar to

other elderly study populations and the LASA study population had a similar distribution of participants with clinically low serum albumin concentration (<38 g/L). Another limitation is the inability to adjust for inflammation. Results from previous literature have found that adjustment for inflammatory cytokines attenuates the association between serum albumin and change in muscle mass (Visser et al., 2005) and muscle strength (Schalk et al., 2005). The direction of confounding is unlikely to explain the null findings in the current study but could partially explain the weak association observed between serum albumin change and change in leg power. Finally, the MrOS study population is a cohort of primarily white men and the results may or may not be generalizable to women or to other racial groups.

Understanding the etiology of the progressive decline of muscle mass and muscle function with age is an important topic in geriatric research. A low concentration of serum albumin has been posited as a potential risk factor due to the biologic plausibility and observation of statistically significant associations that were small in magnitude. The results of this study and the limited body of research on this topic question the utility of albumin serum protein as a prognostic indicator for this process. The investigation of potential risk factors for the age-related loss of muscle mass and muscle function should instead focus on more specific measures of physiologic change such as pro-inflammatory cytokines, growth factor hormones, androgens, and the neuronal and structural changes in skeletal muscle.

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Tables and Figures

Figure 1: Derivation of the main analytic population and serum albumin change analytic population from the MrOS cohort and comparison of baseline serum albumin concentration in participants who were eligible and ineligible for the main analytic population.

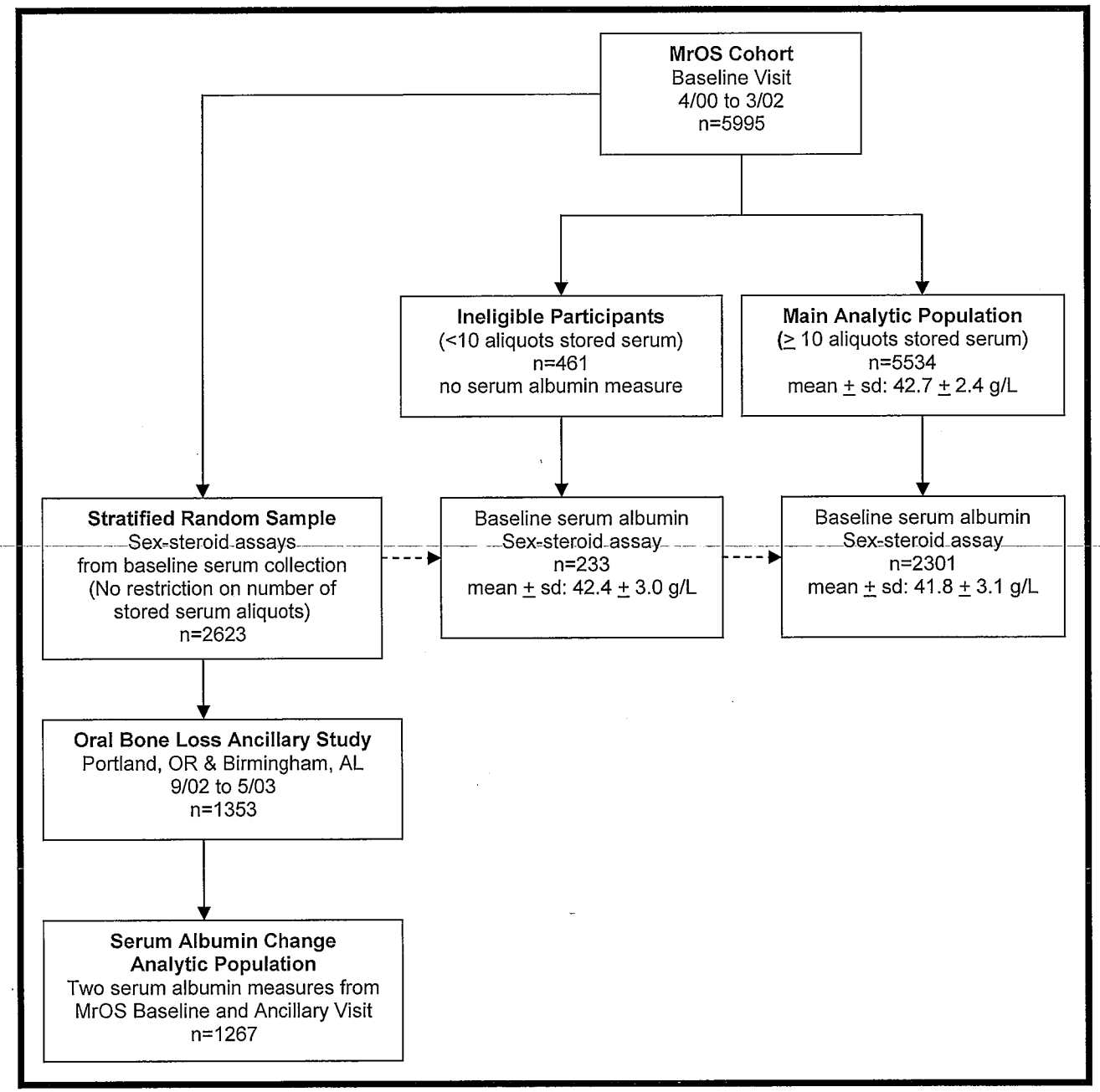


Table 1: Baseline characteristics by serum albumin category in men aged 65 and older; the MrOS study

	Baseline Serum Albumin Categories							
Number (%)	< 40 g/L 460 (8.3)	40 g/L 454 (8.2)	41 g/L 722 (13.1)	42 g/L 989 (17.9)	43 g/L 898 (16.2)	44 g/L 885 (16.0)	> 44 g/L 1126 (20.3)	p [1]
	n (%)							
Age (years)								<0.0001
64-69	88 (19.1)	94 (20.7)	195 (27.0)	285 (28.8)	291 (32.4)	276 (31.2)	419 (37.2)	
70-74	127 (27.6)	119 (26.2)	199 (27.6)	252 (25.5)	271 (30.2)	275 (31.1)	332 (29.5)	
75-79	124 (27.0)	125 (27.5)	183 (25.4)	273 (27.6)	196 (21.8)	199 (22.5)	235 (20.9)	
80+	121 (26.3)	116 (25.6)	145 (20.1)	179 (18.1)	140 (15.6)	135 (15.3)	140 (12.4)	
Race								0.0001
Caucasian	400 (87.0)	420 (92.5)	667 (92.4)	891 (90.1)	821 (91.4)	795 (89.8)	1008 (89.5)	
African American	37 (8.0)	20 (4.4)	27 (3.7)	38 (3.8)	28 (3.1)	35 (4.0)	36 (3.2)	
Asian	13 (2.8)	7 (1.5)	13 (1.8)	25 (2.5)	15 (1.7)	20 (2.3)	42 (3.7)	
Hispanic	9 (2.0)	5 (1.1)	11 (1.5)	19 (1.9)	23 (2.6)	17 (1.9)	28 (2.5)	
Smoking Status								0.19
Never	177 (38.5)	178 (39.3)	256 (35.5)	371 (37.5)	336 (37.4)	318 (35.9)	429 (38.1)	
Former	260 (56.5)	260 (57.4)	441 (61.1)	577 (58.3)	528 (58.8)	537 (60.7)	675 (60.0)	
Current	23 (5.0)	15 (3.3)	25 (3.5)	41 (4.2)	34 (3.8)	30 (3.4)	22 (2.0)	
Alcohol Consumption								0.0003
0 drinks/week	194 (42.2)	173 (38.1)	255 (35.3)	330 (33.4)	318 (35.5)	291 (32.9)	392 (34.9)	
1-7 drinks/week	204 (44.4)	211 (46.5)	369 (51.1)	473 (47.9)	434 (48.4)	427 (48.3)	499 (44.4)	
8+ drinks/week	62 (13.5)	70 (15.4)	98 (13.6)	185 (18.7)	144 (16.1)	166 (18.8)	232 (20.7)	
PASE Score [2]	140 ± 72	141 ± 71	146 ± 68	150 ± 71	149 ± 68	147 ± 67	147 ± 65	0.09
Leisure exercise subscore[3]	34 ± 33	33 ± 31	34 ± 33	38 ± 36	35 ± 33	36 ± 33	37 ± 33	0.02
Household activities subscore	89 ± 45	90 ± 45	96 ± 44	95 ± 44	98 ± 42	94 ± 44	95 ± 42	0.009
Anti-inflammatory Use[4]	98 (21.3)	85 (18.7)	140 (19.4)	194 (19.6)	164 (18.3)	176 (19.9)	221 (19.6)	0.91
Cox II Inhibitors	37 (8.0)	33 (7.3)	48 (6.7)	54 (5.5)	43 (4.8)	49 (5.5)	65 (5.8)	0.20
NSAIDS	67 (14.6)	57 (12.6)	98 (13.6)	147 (14.9)	124 (13.8)	133 (15.0)	163 (14.5)	0.89
Oral Corticosteroid Use	33 (7.2)	15 (3.3)	23 (3.2)	16 (1.6)	9 (1.0)	13 (1.5)	7 (0.6)	<0.0001
Aspirin Use	110 (23.9)	113 (24.9)	139 (19.3)	215 (21.7)	200 (22.3)	166 (18.8)	232 (20.6)	0.07
SSRI Use	12 (2.6)	14 (3.1)	21 (2.9)	22 (2.2)	27 (3.0)	28 (3.2)	25 (2.2)	0.77
Thyroid Hormone Use	35 (7.6)	39 (8.6)	52 (7.2)	72 (7.3)	43 (4.8)	70 (7.9)	57 (5.1)	0.01
Statin Use	83 (18.0)	99 (21.8)	172 (23.8)	260 (26.3)	232 (25.8)	230 (26.0)	341 (30.3)	<0.0001
Angina	80 (17.4)	60 (13.2)	99 (13.7)	132 (13.4)	131 (14.6)	122 (13.8)	175 (15.5)	0.37
Myocardial Infarction	67 (14.6)	59 (13.0)	106 (14.7)	137 (13.9)	123 (13.7)	125 (14.1)	166 (14.7)	0.97
Stroke	31 (6.7)	29 (6.4)	37 (5.1)	50 (5.1)	63 (7.0)	43 (4.9)	58 (5.2)	0.30
Congestive Heart Failure	27 (5.9)	25 (5.5)	35 (4.9)	44 (4.5)	36 (4.0)	53 (6.0)	66 (5.9)	0.36
Hypertension	186 (40.4)	183 (40.3)	299 (41.4)	422 (42.7)	380 (42.3)	394 (44.5)	531 (47.2)	0.06
COPD	57 (12.4)	56 (12.3)	96 (13.3)	97 (9.8)	86 (9.6)	99 (11.2)	100 (8.9)	0.03
Diabetes Mellitus	61 (13.3)	48 (10.6)	83 (11.5)	108 (10.9)	94 (10.5)	101 (11.4)	126 (11.2)	0.83
Hypothyroid	33 (7.2)	37 (8.2)	56 (7.8)	68 (6.9)	54 (6.0)	70 (7.9)	69 (6.1)	0.50
Arthritis or Gout	229 (49.8)	228 (50.2)	347 (48.1)	470 (47.5)	434 (48.3)	409 (46.2)	538 (47.8)	0.84
Osteoarthritis	96 (20.9)	83 (18.3)	144 (19.9)	186 (18.8)	175 (19.5)	183 (20.7)	232 (20.6)	0.86
Rheumatoid Arthritis	35 (7.6)	22 (4.9)	44 (6.1)	54 (5.5)	46 (5.1)	42 (4.8)	50 (4.4)	0.22
Cancer	135 (29.4)	134 (29.5)	219 (30.3)	283 (28.6)	267 (29.7)	260 (29.4)	305 (27.1)	0.80
	mean ± sd							
Age (years)	75.4 ± 6.1	75.3 ± 6.1	74.1 ± 6.0	73.8 ± 5.9	73.2 ± 5.8	73.1 ± 5.5	72.4 ± 5.5	<0.0001
Baseline Height (cm)	173.1 ± 7.1	173.8 ± 6.2	174.2 ± 7.0	174.1 ± 7.0	174.2 ± 6.6	174.8 ± 6.7	174.2 ± 6.7	0.005
Baseline Weight (kg)	82.4 ± 14.0	83.5 ± 14.1	83.5 ± 13.6	83.7 ± 13.3	83.9 ± 13.6	83.7 ± 12.8	82.0 ± 12.3	0.01
BMI (kg/m^2)	27.4 ± 4.3	27.6 ± 4.3	27.5 ± 4.0	27.6 ± 3.8	27.6 ± 3.9	27.4 ± 3.6	27.0 ± 3.5	0.005
Total Body Fat (kg)	21.9 ± 8.0	22.1 ± 7.7	22.0 ± 7.3	22.2 ± 7.4	22.1 ± 7.2	21.6 ± 6.7	21.0 ± 6.3	0.003
Daily Caloric Intake (kcal)	1620 ± 665	1614 ± 566	1611 ± 612	1632 ± 661	1654 ± 653	1653 ± 665	1610 ± 655	0.61
Daily Protein Intake (% kcal)	15.8 ± 2.9	16.0 ± 2.7	15.9 ± 2.8	16.1 ± 2.9	16.2 ± 3.0	16.1 ± 3.1	16.2 ± 2.9	0.11

[1] One-way analysis of variance (ANOVA) for continuous variables and chi-square tests for categorical variables [2] PASE: Physical Activity Scale for the Elderly [3] Nonparametric ANOVA to accommodate a skewed distribution [4] Anti-inflammatory drug use is defined as the use of a Cox II inhibitor, ibuprofen, naproxen sodium, or another nonsteroidal anti-inflammatory agent at least 3 times per week

Table 2: Cross-sectional analysis of baseline serum albumin concentration with baseline ASM mass, grip strength, and leg power in men 65 and older; the MrOS study

	Baseline Serum Albumin Category							
	< 40 g/L	40 g/L	41 g/L	42 g/L	43 g/L	44 g/L	> 44g/L	p [1]
Baseline ASM mass (kg)								
Mean (SD)	23.8 ± 3.5	24.1 ± 3.4	24.3 ± 3.6	24.3 ± 3.5	24.5 ± 3.5	24.5 ± 3.4	24.2 ± 3.5	
Adjusted Mean [2]	23.7	24.0	24.0	24.0	24.0	24.0	23.6	0.05
95% CI	(23.3, 24.1)	(23.7, 24.4)	(23.6, 24.3)	(23.7, 24.3)	(23.7, 24.3)	(23.7, 24.3)	(23.3, 23.9)	
p value	0.12	0.71	0.89	Referent	0.99	0.87	0.01	
Baseline Grip Strength (kg)								
Mean (SD)	35.4 ± 8.2	37.0 ± 7.9	37.6 ± 8.2	38.8 ± 8.1	39.0 ± 8.3	39.4 ± 8.0	39.7 ± 7.9	
Adjusted Mean [2]	35.3	36.7	36.7	37.8	37.7	37.9	37.9	<0.0001
95% CI	(34.4, 36.1)	(35.9, 37.6)	(36.0, 37.5)	(37.1, 38.5)	(37.0, 38.4)	(37.2, 38.6)	(37.2, 38.6)	
p value	<0.0001	0.01	0.004	Referent	0.90	0.69	0.73	
Unable/Refused (n, %)	15 (3.3)	11 (2.4)	9 (1.3)	18 (1.8)	20 (2.2)	12 (1.4)	18 (1.6)	
Baseline Leg Power (watts)								
Mean (SD)	190 ± 60	198 ± 60	202 ± 63	210 ± 63	214 ± 66	212 ± 63	213 ± 62	
Adjusted Mean [2]	190	196	196	201	202	201	198	0.004
95% CI	(184, 197)	(189, 203)	(190, 202)	(196, 206)	(197, 208)	(195, 206)	(193, 203)	
p value	0.0007	0.15	0.07	Referent	0.59	0.92	0.19	
Missing/Unable (n, %)	55 (12.0)	42 (9.3)	67 (9.3)	100 (10.1)	89 (9.9)	81 (9.2)	91 (8.1)	

[1] Multiple linear regression using generalized linear models [2] Adjusted for participant age, race, and study site

Table 3: Baseline serum albumin concentration in relation to change in ASM mass, grip strength, and leg power in men aged 65 and older; the MrOS study

Outcome	Serum Albumin Category (n)							p [1]
	< 40 g/L (253-291)	40 g/L (291-328)	41 g/L (459-538)	42 g/L (650-749)	43 g/L (585-664)	44 g/L (586-682)	> 44 g/L (756-862)	
ASM Mass Change [2]								
Mean (SD)	-1.02 (1.47)	-0.98 (1.27)	-0.88 (1.32)	-0.90 (1.22)	-0.85 (1.14)	-0.81 (1.22)	-0.82 (1.27)	
Adjusted Mean [3]	-0.97	-0.92	-0.84	-0.88	-0.85	-0.80	-0.87	0.53
95% CI	-1.11, -0.083	-1.05, -0.78	-0.95, -0.74	-0.97, -0.79	-0.94, -0.75	-0.89, -0.71	-0.95, -0.79	
P value	0.26	0.64	0.61	Referent	0.65	0.22	0.88	
Grip Strength Change [2]								
Mean (SD)	-2.21 (5.89)	-2.70 (5.42)	-2.28 (4.85)	-2.79 (6.02)	-2.58 (5.50)	-2.75 (5.52)	-2.68 (5.81)	
Adjusted Mean [4]	-2.45	-2.81	-2.45	-2.74	-2.51	-2.53	-2.46	0.87
95% CI	-3.05, -1.86	-3.38, -2.25	-2.88, -2.01	-3.10, -2.37	-2.90, -2.12	-2.92, -2.15	-2.80, -2.11	
P value	0.43	0.83	0.32	Referent	0.41	0.45	0.27	
Leg Power Change [2]								
Mean (SD)	-17.6 (49.4)	-24.8 (51.3)	-23.5 (47.2)	-17.6 (52.2)	-20.8 (54.7)	-18.6 (51.2)	-14.4 (50.8)	
Adjusted Mean [5]	-28.5	-35.1	-33.8	-27.8	-31.4	-29.7	-28.8	0.13
95% CI	-35.8, -21.2	-42.3, -27.9	-40.2, -27.3	-33.9, -21.7	-37.6, -25.1	-36.0, -23.5	-34.9, -22.7	
P value	0.84	0.02	0.03	Referent	0.15	0.44	0.67	

[1] Multiple linear regression using generalized linear models [2] Absolute change over an average of 4.6 years of follow-up [3] Adjusted for participant age, study site, and baseline ASM mass [4] Adjusted for participant age, study site, baseline grip strength, baseline ASM mass, and ASM mass change [5] Adjusted for participant age, study site, baseline leg power, baseline ASM mass, ASM mass change, Cox II Inhibitor Use, rheumatoid arthritis, and smoking status

Table 4: Change in serum albumin concentration in relation to change in ASM mass, grip strength, and leg power in men aged 65 and older; the MrOS study

Outcome	Serum Albumin Change Category (n)				p [1]
	Marked Decrease ≤ -3 g/L (84-296)	Mild Decrease -1 to -2 g/L (161-383)	Stable 0 g/L (116-200)	Increase >0 g/L (223-365)	
ASM Mass Change (kg) [2]					
Mean (SD)	-0.10 (0.97)	-0.12 (0.90)	-0.13 (1.11)	-0.22 (0.89)	
Adjusted Mean [3]	-0.06	-0.12	-0.15	-0.24	0.17
95% CI	(-0.17, 0.06)	(-0.21, -0.03)	(-0.28, -0.02)	(-0.34, -0.14)	
p value	0.33	0.74	Referent	0.28	
Grip Strength Change (kg) [2]					
Mean (SD)	0.47 (4.50)	0.77 (4.04)	1.03 (4.70)	0.95 (4.56)	
Adjusted Mean [4]	0.51	0.75	0.87	1.08	0.53
95% CI	(-0.03, 1.05)	(0.31, 1.19)	(0.25, 1.49)	(0.61, 1.54)	
p value	0.40	0.76	Referent	0.59	
Leg Power Change (watts) [2,5]					
Mean (SD)	-7.86 (34.7)	-2.44 (35.0)	8.46 (48.5)	3.75 (36.9)	
Adjusted Mean [6]	-8.9	-6.3	5.4	2.1	0.02
95% CI	(-25.6, 7.8)	(-21.2, 8.5)	(-10.5, 21.3)	(-12.1, 16.4)	
p value	0.02	0.01	Referent	0.48	

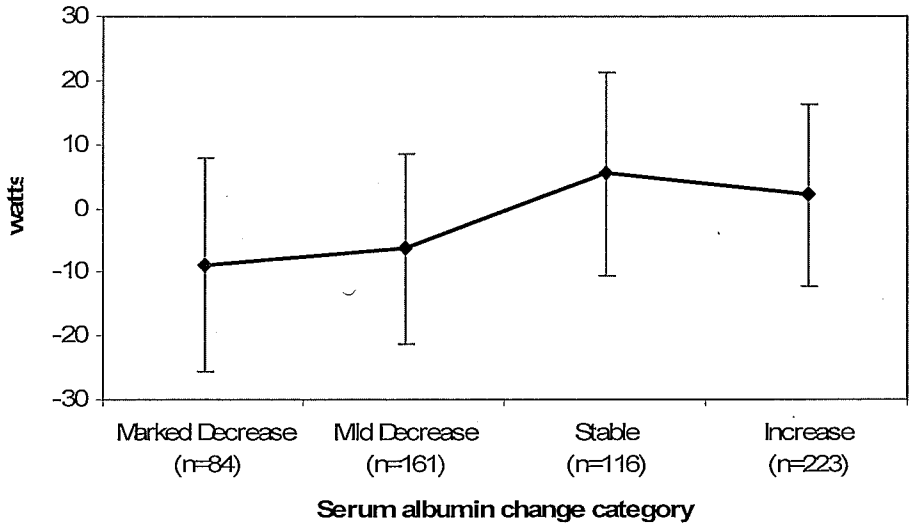
[1] Multiple linear regression using generalized linear models [2] Absolute change over an average of 2 years of follow-up [3] Adjusted for participant age, study site, and baseline ASM mass [4] Adjusted for participant age, study site, baseline grip strength, baseline ASM mass, and ASM mass change [5] Leg power assessments were only performed at the Portland site (n=512) [6] Adjusted for participant age, study site, baseline leg power, baseline ASM mass, ASM mass change, Cox II Inhibitor Use, rheumatoid arthritis, and smoking status

Table 5: Baseline serum albumin concentration in relation to change in ASM mass, grip strength, and leg power in participants with two serum albumin measures; the MrOS study

Outcome	Serum Albumin Category (n)							p [1]
	≤ 39 g/L (102-263)	40 g/L (65-161)	41 g/L (79-173)	42 g/L (84-162)	43 g/L (74-154)	44 g/L (78-135)	≥ 45 g/L (102-196)	
ASM Mass Change (kg) [2]								
Mean (SD)	-0.27 (1.08)	-0.17 (0.86)	-0.08 (0.88)	-0.13 (1.05)	-0.13 (0.89)	-0.09 (0.86)	-0.08 (0.93)	
Adjusted Mean [3]	-0.25	-0.15	-0.06	-0.15	-0.12	-0.10	-0.12	0.49
95% CI	(-0.37, -0.14)	(-0.29, -0.002)	(-0.20, 0.08)	(-0.29, -0.004)	(-0.26, 0.03)	(-0.26, 0.06)	(-0.25, 0.01)	
P value	0.27	0.99	0.39	Referent	0.76	0.67	0.79	
Grip Strength Change (kg) [2]								
Mean (SD)	0.66 (4.34)	1.22 (4.13)	0.71 (4.52)	0.86 (4.67)	1.15 (4.14)	0.97 (3.74)	0.20 (5.00)	
Adjusted Mean [4]	0.71	1.23	0.75	0.86	1.14	1.07	0.18	0.31
95% CI	(0.18, 1.25)	(0.56, 1.91)	(0.10, 1.40)	(0.20, 1.53)	(0.45, 1.83)	(0.32, 1.82)	(-0.44, 0.79)	
P value	0.74	0.44	0.81	Referent	0.57	0.69	0.14	
Leg Power Change (watts) [2]								
Mean (SD)	1.21 (35.1)	7.05 (39.1)	2.77 (33.4)	2.45 (37.4)	0.57 (38.6)	-2.61 (51.4)	-0.78 (37.8)	
Adjusted Mean [5]	-3.38	7.83	-2.00	-2.38	-1.11	-5.87	-7.17	0.36
95% CI	(-19.4, 12.6)	(-8.3, 23.9)	(-18.3, 14.3)	(-18.1, 13.4)	(-17.6, 15.4)	(-22.4, 10.7)	(-22.9, 8.6)	
P value	0.86	0.11	0.95	Referent	0.84	0.57	0.41	

[1] Multiple linear regression using generalized linear models [2] Absolute change over an average of 2 years of follow-up [3] Adjusted for participant age, study site, and baseline ASM mass [4] Adjusted for participant age, study site, baseline ASM mass, ASM mass change, and baseline grip strength [5] Adjusted for participant age, study site, baseline ASM mass, ASM mass change, baseline leg power, Cox II inhibitor use, rheumatoid arthritis, and smoking status

Figure 2: Adjusted mean change in leg power by serum albumin change category in men aged 65 and older at the Portland, Oregon study site; the MrOS study [1]



Appendices

Appendix A:
Description of Variables

Table 5: Description of variables

Variable	Measurement	Description
Serum Albumin	Morning fasting phlebotomy at baseline. Performed at the interim study visit for a sample of the cohort.	1= < 40 g/L 2= 40 g/L 3= 41 g/L 4= 42 g/L 5= 43 g/L 6= 44 g/L 7= > 44 g/L
Participant Characteristics		
Participant Age	Verified age at enrollment	Continuous variable in years and 5-year age categories 1=64-69 years 2=70-74 years 3=75-79 years 4=80+ years
Study Site	Site of participant enrollment	1= Birmingham, Alabama 2= Minneapolis, Minnesota 3= Palo Alto, California 4= Pittsburgh, Pennsylvania 5= Portland, Oregon 6= San Diego, California
Race/ethnicity	Self-report	1= Caucasian 2= African American 3= Asian 4= Hispanic
Height	Harpenden stadiometer	Centimeters (cm)
Weight	Balance beam or digital scale wearing shoes and indoor clothing	Kilograms (kg)
Body Mass Index (BMI)	Calculated using height and weight measurements	kg/m^2
Smoking Status	Self-report	1=Never 2=Former 3=Current
Alcohol Consumption	Self reported usual number of drinks per day	1=0 drinks/week 2=1-7 drinks/week 3=8+ drinks/week
Physical Activity	Physical Activity Scale for the Elderly (PASE) Self-report questionnaire	Continuous scoring system Total PASE questionnaire score Leisure exercise subscore Household activities subscore.
Daily Caloric Intake	Self-report questionnaire developed by Block Dietary Systems.	Kilocalories (kcal)
Daily Protein Intake	Self-report questionnaire developed by Block Dietary	Percentage of protein intake derived from total caloric intake. Categorized into

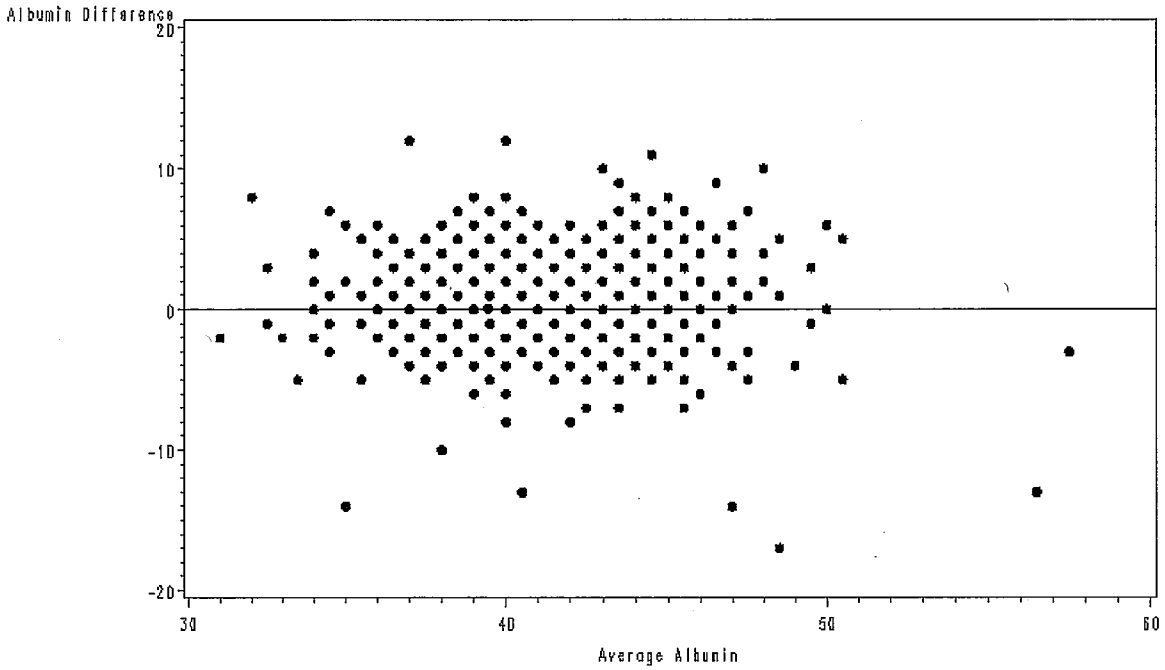
	Systems.	quintiles.
Medication Use		
Anti-inflammatory drug Use	Clinic interview at baseline	Defined as the use of a Cox II Inhibitor, ibuprofen, naproxen sodium, or another nonsteroidal anti-inflammatory agent at least three times per week 0=no 1=yes
Cox II Inhibitor	Clinic Interview at baseline	0=no 1=yes
Nonsteroidal Anti-inflammatory agents (NSAIDS)	Clinic Interview at baseline	0=no 1=yes
Oral Corticosteroid	Clinic Interview at baseline	0=no 1=yes
Aspirin Use	Clinic Interview at baseline	0=no 1=yes
Selective Serotonin Reuptake Inhibitor (SSRI)	Clinic Interview at baseline	0=no 1=yes
Medical History		
Thyroid Hormone Use	Clinic Interview at baseline	0=no 1=yes
Statin Use	Clinic Interview at baseline	0=no 1=yes
Angina	Self-report at baseline	0=no 1=yes
Myocardial Infarction	Self-report at baseline	0=no 1=yes
Stroke	Self-report at baseline	0=no 1=yes
Congestive Heart Failure	Self-report at baseline	0=no 1=yes
Hypertension	Self-report at baseline	0=no 1=yes
Chronic Obstructive Pulmonary Disease (COPD)	Self-report at baseline	0=no 1=yes
Diabetes Mellitus	Self-report at baseline	0=no 1=yes
Hypothyroid	Self-report at baseline	0=no 1=yes
Cancer	Self-report at baseline Any type other than skin	0=no 1=yes
Renal Disease	Self-report at Visit 2	0=no 1=yes
Liver Disease	Self-report at Visit 2	0=no 1=yes
Parkinson's Disease	Self-report at baseline	0=no 1=yes
Arthritis or Gout	Self-report at baseline	0=no 1=yes
Rheumatoid Arthritis	Self-report at baseline	0=no 1=yes
Osteoarthritis	Self-report at baseline	0=no 1=yes

Outcome Variables		
Appendicular Skeletal Muscle Mass (ASM)	Dual energy x-ray absorptiometry (DXA) at baseline and Visit 2. Performed at the interim study visit for a sample of the cohort.	Kilograms (kg) Defined as the sum of the nonbone, nonfat lean tissue of the extremities
Grip Strength	JAMAR Handheld dynamometer at baseline and Visit 2. Two trials per hand. Average of all hand measurements. Performed at the interim study visit in a sample of the cohort.	Kilograms (kg)
Leg power	Nottingham Power Rig at baseline and Visit 2. Five trials at baseline and Visit 2. Maximum result from all leg trials. Performed at the interim study visit for a sample of the cohort.	Watts

Appendix B:

Bland-Altman plot of repeated serum albumin measures

Figure 4: Bland-Altman plot of two serum albumin measurements obtained an average of two years apart in men participating in the sex steroid ancillary study (n=1267); the MrOS study.



Appendix C:
Characteristics of eligible and ineligible participants

Table 6: Baseline characteristics of the analytic population versus men who were missing a baseline serum albumin concentration and were ineligible for inclusion; the MrOS study

	Ineligible Participants (n=461)	**Analytic Population (n=5534)**	**p**
Age (mean, s.d.)	74.2 ± 5.9	73.6 ± 5.9	0.05
Age (n, %)			0.27
64-69	121 (6.8)	1648 (93.2)	
70-74	134 (7.9)	1574 (92.2)	
75-79	113 (7.8)	1334 (92.2)	
80+	95 (8.9)	976 (91.1)	
Race (n, %) [1]			<0.0001
Caucasian	364 (6.8)	5021 (93.2)	
African American	23 (9.4)	221 (90.6)	
Asian	56 (29.2)	136 (70.8)	
Study Site (n, %) [1]			<0.0001
Birmingham	40 (4.1)	929 (95.9)	
Minneapolis	11 (1.1)	994 (98.9)	
Palo Alto	191 (19.2)	805 (80.8)	
Pittsburgh	4 (0.4)	1001 (99.6)	
Portland	155 (15.4)	852 (84.6)	
San Diego	62 (6.1)	951 (93.9)	
Smoking Status (n, %)			0.52
Never	185 (8.2)	2064 (91.8)	
Former	262 (7.4)	3277 (92.6)	
Current	16 (7.8)	190 (92.2)	
Alcohol Consumption (n, %)			0.85
0 drinks/week	169 (8.0)	1952 (92.0)	
1-7 drinks/week	216 (7.6)	2616 (92.4)	
8+ drinks/week	77 (7.5)	957 (92.6)	
Weight Change, V1 to V2	-1.7 ± 5.4	-1.6 ± 5.5	0.64
Protein Intake (% kcal)	16.4 ± 2.8	16.1 ± 2.9	0.03
Daily Caloric Intake (kcal)	1527 ± 543	1630 ± 646	0.001
PASE Score	145 ± 67	147 ± 68	0.65
Leisure Exercise Subscore	39.9 ± 37.1	35.8 ± 33.5	0.02
Household Subscore	91.7 ± 44.0	94.5 ± 43.6	0.19
Anti-inflammatory Drug Use (n, %)			0.56
No	378 (7.8)	4454 (92.2)	
Yes	85 (7.3)	1078 (92.7)	
Oral Corticosteroid Use (n, %)			0.31
No	450 (7.7)	5416 (92.3)	
Yes	13 (10.1)	116 (89.9)	
Aspirin Use (n, %)			<0.0001
No	302 (6.5)	4358 (93.5)	
Yes	161 (12.1)	1174 (87.9)	
SSRI Use (n, %)			0.68
No	452 (7.8)	5383 (92.3)	
Yes	11 (6.9)	149 (93.1)	
Thyroid Hormone Use (n, %)			0.89
No	433 (7.7)	5164 (92.3)	
Yes	30 (7.5)	368 (92.5)	
Androgen Use (n, %)			n/a
No	458 (7.7)	5511 (92.3)	
Yes	5 (19.2)	21 (80.8)	
Testosterone Injections (n, %)			n/a
No	455 (7.7)	5482 (92.3)	

Yes	8 (13.8)	50 (86.2)	
Angina (n, %)			0.21
No	406 (7.9)	4733 (92.1)	
Yes	57 (6.7)	799 (93.3)	
Myocardial Infarction (n, %)			0.08
No	411 (8.0)	4749 (92.0)	
Yes	52 (6.2)	783 (93.8)	
Stroke (n, %)			0.18
No	430 (7.6)	5221 (92.4)	
Yes	33 (9.6)	311 (90.4)	
Congestive Heart Failure (n, %)			0.16
No	432 (7.6)	5246 (92.4)	
Yes	31 (9.8)	286 (90.2)	
Hypertension (n, %)			0.23
No	276 (8.1)	3138 (91.9)	
Yes	187 (7.3)	2394 (92.8)	
COPD (n, %)			0.95
No	414 (7.7)	4941 (92.3)	
Yes	49 (7.7)	591 (92.3)	
Arthritis or Gout (n, %)			0.007
No	271 (8.6)	2877 (91.4)	
Yes	192 (6.7)	2655 (93.3)	
Osteoarthritis (n, %)			0.007
No	395 (8.2)	4433 (91.8)	
Yes	68 (5.8)	1099 (94.2)	
Rheumatoid Arthritis (n, %)			0.61
No	441 (7.8)	5239 (92.2)	
Yes	22 (7.0)	293 (93.0)	
Liver Disease (n, %) [2]			0.09
No	382 (7.5)	4703 (92.5)	
Yes	13 (11.9)	96 (88.1)	
Renal disease (n, %) [2]			0.16
No	384 (7.5)	4713 (92.5)	
Yes	11 (11.3)	86 (88.7)	
Cancer (n, %)			0.39
No	320 (7.5)	3929 (92.5)	
Yes	143 (8.2)	1603 (91.8)	
Parkinson's (n, %)			n/a
No	459 (7.7)	5484 (92.3)	
Yes	4 (7.7)	48 (92.3)	
Total body fat index (kg/m^2)	7.0 ± 2.2	7.2 ± 2.3	0.29
Total body lean mass index (kg/m^2)	18.6 ± 1.8	18.8 ± 1.9	0.08
ASM mass index (kg/m^2)	7.9 ± 0.9	8.0 ± 0.9	0.002
Leg Power (watts)	213 ± 64	208 ± 63	0.12
Grip Strength (kg)	38.9 ± 8.0	38.5 ± 8.2	0.40

[1] Palo Alto has the highest proportion of Asian participants and can account for the higher proportion of ineligible Asian participants

Appendix D:
Participation and nonparticipation in the Visit 2 clinic visit

Table 7: Characteristics of men who completed the Visit 2 clinic visit versus men who did not participate; the MrOS study

	Completed Visit 2 (n=4182)	No participation in the Visit 2 clinic visit (n=749)	Voluntary withdrawal or deceased (n=603)	P-value
Age (mean, s.d.)	72.8 ± 5.4	74.8 ± 6.0	77.5 ± 6.8	<0.0001
Age (n, %)				<0.0001
64-69	1378 (33.0)	184 (24.6)	86 (14.3)	
70-74	1280 (30.6)	181 (24.2)	114 (18.9)	
75-79	976 (23.3)	207 (27.6)	152 (25.2)	
80+	548 (13.1)	177 (23.6)	251 (41.6)	
Race (n, %) [1]				<0.0001
Caucasian	3822 (91.4)	636 (84.9)	544 (90.2)	
African American	132 (3.2)	53 (7.1)	36 (6.0)	
Asian	104 (2.5)	23 (3.1)	8 (1.3)	
Hispanic	74 (1.8)	28 (3.7)	10 (1.7)	
Smoking Status (n, %)				0.0002
Never	1619 (38.7)	252 (33.6)	194 (32.2)	
Former	2436 (58.3)	465 (62.1)	377 (62.5)	
Current	126 (3.0)	32 (4.3)	32 (5.3)	
Height (cm)	174.5 ± 6.7	173.3 ± 6.6	172.8 ± 6.9	<0.0001
Weight (kg)	83.6 ± 13.0	83.2 ± 13.9	80.5 ± 13.6	<0.0001
BMI (kg/m^2)	27.4 ± 3.8	27.6 ± 4.0	26.9 ± 4.0	0.002
PASE Score	152 ± 67	137 ± 69	123 ± 68	<0.0001
Leisure Exercise Subscore	37.1 ± 33.8	33.2 ± 32.8	29.9 ± 31.8	<0.0001
Household Subscore	97.0 ± 42.8	90.2 ± 44.8	82.4 ± 45.5	<0.0001
Total body fat index (kg/m^2)	7.2 ± 2.3	7.3 ± 2.4	7.1 ± 2.3	0.06
Total body lean mass index (kg/m^2)	19.1 ± 1.9	19.1 ± 2.0	18.8 ± 2.1	<0.0001
ASM mass index (kg/m^2)	8.0 ± 0.9	8.0 ± 1.0	7.7 ± 1.0	<0.0001
Leg Power (watts)	215 ± 61	193 ± 63	174 ± 63	<0.0001
Grip Strength (kg)	39.4 ± 7.9	37.2 ± 8.2	33.7 ± 8.2	<0.0001
Serum albumin (g/L)	42.8 ± 2.3	42.6 ± 2.4	42.1 ± 2.7	<0.0001
Serum albumin categories (n, %)				<0.0001
<40 g/L	300 (7.2)	69 (9.2)	91 (15.1)	
40 g/L	332 (7.9)	63 (8.4)	59 (9.8)	
41 g/L	541 (12.9)	98 (13.1)	83 (13.8)	
42 g/L	763 (18.2)	127 (17.0)	99 (16.4)	
43 g/L	672 (16.1)	124 (16.6)	102 (16.9)	
44 g/L	694 (16.6)	115 (15.4)	76 (12.6)	
>44 g/L	880 (21.0)	153 (20.4)	93 (15.4)	

Appendix E:
List of potential confounders

Table 8: List of all variables associated with serum albumin and each outcome (p<0.25). All potential confounders were included in model building; the MrOS study

Percent Change ASM (p-value albumin, outcome)	**Percent Change Grip Strength** (p-value albumin, outcome)	**Percent Change Leg Power** (p-value albumin, outcome)
Age (<0.0001, <0.0001)	Age (<0.0001, <0.0001)	Age (<0.0001, <0.0001)
Race (0.0001, 0.25)	Race (0.0001, 0.34)	Race (0.0001, 0.002)
Study Site (<0.0001, <0.0001)	Study Site (<0.0001, <0.0001)	Study Site (<0.0001, <0.0001)
Smoking Status (0.19, 0.11)	Protein Intake (0.11, 0.12)	Smoking Status (0.19, 0.006)
Protein Intake (0.11, 0.26)*	PASE (0.09, 0.0001)	Alcohol Consumption (0.0003, <0.0001)
PASE (0.09, 0.02)	PASE Household Subscore (0.01, 0.005)	Protein Intake (0.11, 0.0002)
PASE Household Subscore (0.009, 0.02)	Aspirin Use (0.07, 0.13)	PASE (0.09, 0.02)
Thyroid Hormone Use (0.01, 0.10)	Thyroid Hormone Use (0.01, 0.09)	PASE Leisure Subscore (0.02, 0.003)
Statin Use (<0.0001, 0.12)	Statin Use (<0.0001, 0.23)	Hypertension (0.06, 0.01)
Hypertension (0.06, 0.006)	Hypertension (0.06, 0.07)	Rheumatoid Arthritis (0.22, 0.06)
COPD (0.03, 0.003)	Cox II Inhibitor (0.20, 0.04)	Cox II Inhibitor (0.20, 0.003)

Appendix F:
Total and partial R^2

Table 9: The total and partial R^2 of the final models quantifying the association between baseline serum albumin and the 4.6 year change in ASM mass, grip strength, and leg power ; the MrOS study

Absolute Change ASM Lean Mass			**Absolute Change Average Grip Strength**			**Absolute Change Maximum Leg Power**		
Ind. Variables	**p-value**	**R^2**	**Ind. Variables**	**p-value**	**R^2**	**Ind. Variables**	**p-value**	**R^2**
Albumin Categories	0.73	0.001	Albumin Categories	0.84	0.002	Albumin Categories	0.10	0.004
Age	<0.0001	0.024	Age	<0.0001	0.023	Age	<0.0001	0.046
Study Site	<0.0001	0.019	Study Site	<0.0001	0.026	Study Site	<0.0001	0.141
Baseline ASM	<0.0001	0.034	Baseline Grip Strength	<0.0001	0.110	Baseline Leg Power	<0.0001	0.132
			Baseline ASM	<0.0001	0.015	Baseline ASM	<0.0001	0.017
			Absolute change ASM	<0.0001	0.034	Absolute change ASM	<0.0001	0.045
						Cox II Inhibitor Use	0.07	0.003
						Smoking Status	0.0007	0.005
						Rheumatoid Arthritis	0.08	0.003
Overall Model	<0.0001	0.07	Overall Model	<0.0001	0.1914	Overall Model	<0.0001	0.2852