Higher dietary protein intake is associated with sarcopenia in older British twins

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Conflicts of Interest:

Mary Ni Lochlainn, Ruth C. E. Bowyer, Ailsa A. Welch, Kevin Whelan, and Claire J. Steves

report no relevant conflicts of interest.

Ethical approval

TwinsUK main ethics was reviewed and approved by the NHS London – London Bridge Research Ethics Committee (REC reference EC/04/015), and by Guy's and St Thomas' NHS Foundation Trust Research and Development (R&D) in 2012. TwinsUK BioBank was approved by NHS North West - Liverpool East Research Ethics Committee (REC reference 19/NW/0187), IRAS ID 258513. All research therefore carried out in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

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Abstract

Background

Sarcopenia, characterised by an accelerated loss of skeletal muscle mass and function, is associated with negative outcomes. This study aimed to evaluate factors associated with skeletal muscle strength, mass, and sarcopenia, particularly protein intake; and to assess whether shared twin characteristics are important.

Methods

This study utilised cross-sectional data from a study of community dwelling twins aged ≥60 years. Multivariable logistic regression and between- and within- twin pair regression modelling was used.

Results

Participants (n=3302) were 89% female (n=2923), were aged a mean of 72.1 (±7.3) years and comprised of 858 (55%) monozygotic, 709 (45%) dizygotic twin pairs and 168 individual lone twins. Using optimal protein intake as the reference group (1.0-1.3g/kg/day), there was no significant association between protein intake (neither high nor low) and low muscle strength, or between low protein intake and sarcopenia (OR 0.7; 95% CI 0.39-1.25; p=0.229) in unadjusted models. High protein intake (>1,3g/kg/day) was associated with low muscle mass (OR 1.76; 95% CI 1.39-2.2.4; P<0.0001), while low protein intake was protective (OR 0.52; 95% CI 0.40-0.67; P<0.0001). High protein intake was associated with sarcopenia (OR 2.04; 95% CI 1.21-3.44; p=0.008), and this was robust to adjustment for demographic, anthropometric and dietary factors. The association between muscle strength and weight, BMI, healthy eating index, protein intake, and alpha diversity, was not significantly influenced by shared twin factors, indicating greater amenability to interventions.

Conclusions

High protein intake is associated with sarcopenia in a cohort of healthy older twins.

Introduction

Muscle loss with age is a growing problem, particularly as populations around the world are aging. Sarcopenia refers to a progressive and generalised skeletal muscle disorder involving the accelerated loss of skeletal muscle function, and is associated with increased adverse outcomes including falls, functional decline, frailty, reduced quality of life, higher healthcare costs, and mortality (1,2). Data on its prevalence vary widely, ranging from 1% to 31.9% (3–5).

Despite the significant burden of sarcopenia, there are limited therapeutic options available. Much of the literature investigates resistance exercise and protein supplementation as the main treatment approaches, with compelling evidence for resistance exercise and less consistent evidence for protein (6). Beyond resistance exercise and protein intake, many features have been associated with sarcopenia including smoking (7), education (8), income (8), sex (9), diet (1), appetite (10), frailty (1), and physical activity (1).

Anabolic resistance refers to the phenomenon whereby older adults require a higher dose of protein to achieve the same response in muscle protein synthesis as a younger adult (11). This has led to the European Society for Clinical Nutrition and Metabolism (ESPEN) producing guidance recommending higher daily intake of protein (1-1.3 g/kg/day) for older adults, in order to overcome this resistance (12,13).

The gut microbiota and their role in human physiology is a growing field of enquiry, with microbiota diversity typically considered as a marker of overall health. There is an expanding body of evidence linking the gut microbiota to skeletal muscle function, which we have described in full previously (14). The gut microbiota play a key role in many of the postulated mechanisms and aetiologies for anabolic resistance, for example, gut permeability, and inflammation, leading to the suggestion that the microbiota may mediate anabolic resistance to some degree (14). To our knowledge, there have been no previous studies that have investigated the association between the broad range of characteristics investigated in our study, such as healthy eating index, frailty, appetite, indicators of renal function, gut microbiota diversity, and the relevance of shared twin factors with sarcopenia. Therefore, the goals of this study, established a priori, were to: (i) ascertain the prevalence of low muscle strength and sarcopenia, in a large cohort of British twins aged ≥60 years (ii) explore

factors associated with low muscle strength and sarcopenia, in particular dietary protein intake; and (iii) use specialised regression methods to explore whether shared twin factors (e.g., genetics, early environment, etc.) drive the identified associations with muscle strength and/or sarcopenia. While aim (i) and (ii) have been explored in other populations to some degree, aim (iii) has not been done before to our knowledge, and this study represents the first piece of research using twin modelling in this field of enquiry.

Methods

Study population

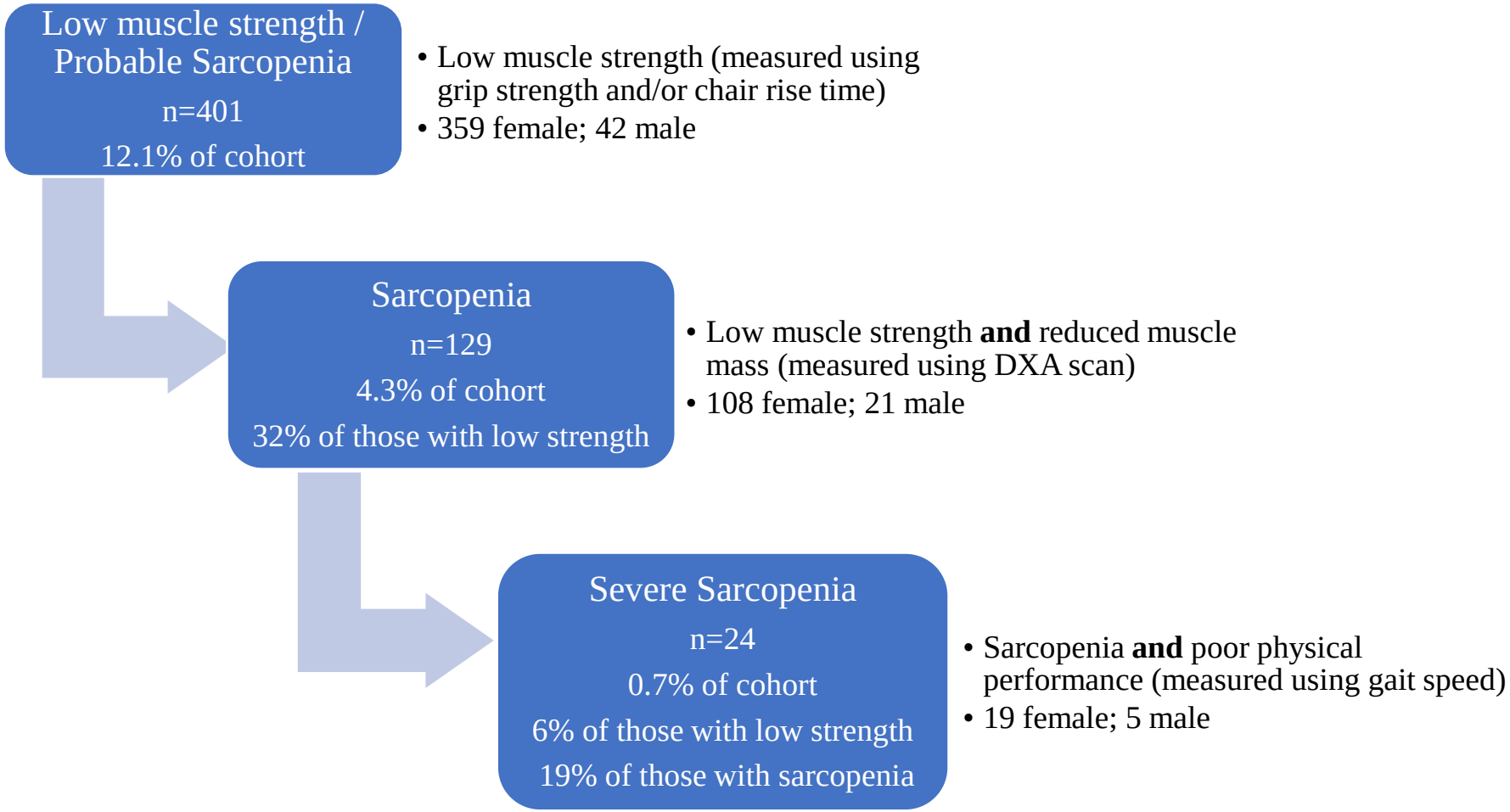
The current study utilises a cross-sectional sample of community dwelling participants in the TwinsUK cohort who had detailed data available on skeletal muscle mass, muscle strength, physical performance, diet, and anthropometry (n = 3302). The TwinsUK cohort has been described in detail elsewhere (15). Eligibility for the analysis was defined by aged ≥60 years and attendance for a visit to the department since 2010 which included detailed physical measures, DXA scans and questionnaire completion. There were no exclusion criteria.

A logistic regression approach was used for the main analyses. For the twin modelling analysis, linear regression was used. A Wald test was used to test the difference between the between-pair and within-pair coefficients. Variable measurement and statistical analysis are described in full in the appendices.

Results

A total of 3302 individual twins were included, with a mean age of 72.1. The overall prevalence of sarcopenia in this cohort was 129 (4.3%) including 21 (6.2%) males and 108 (4.1%) females (Figure 1) (1,33).

Figure 1: Prevalence of Sarcopenia (1)



Factors associated with Muscle Strength and Sarcopenia

When comparing those with low muscle strength to those without, there was no difference between the two groups in protein intake, using both UK RNI, and ESPEN recommended intakes (Table 1). BMI was significantly lower in the participants with sarcopenia, compared to those without sarcopenia. In terms of protein intake, both measures of protein intake (UK RNI, and ESPEN) were significantly different, with those with sarcopenia more likely to have *high* protein intake. Figure 2 presents the logistic regression analysis for the relation between each variable and muscle strength, defined as low or not, and sarcopenia (See also: Supplementary Table 3).

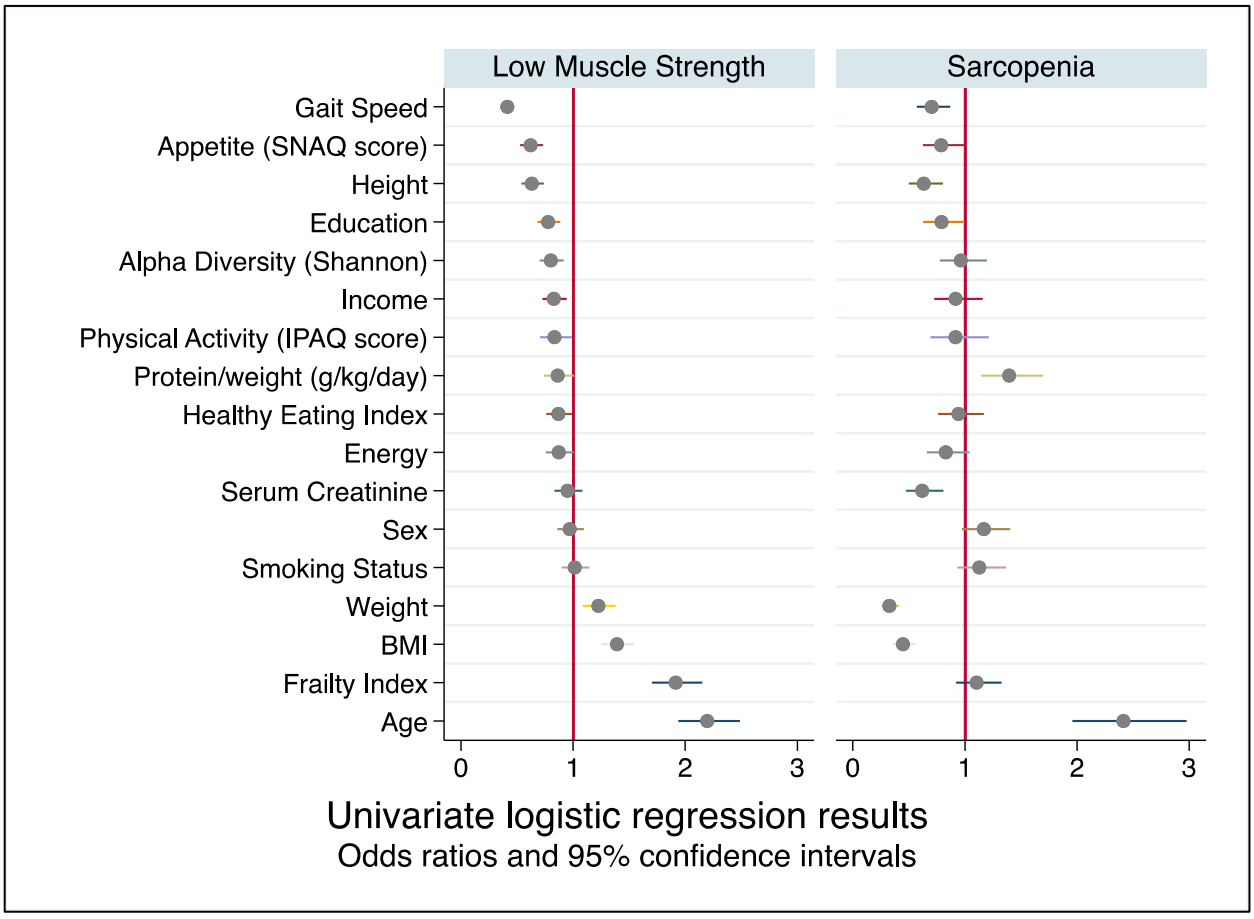


Figure 2: Logistic regression results for covariates of low muscle strength and sarcopenia. All models adjusted for age and sex. All variables standardised therefore each unit of difference refers to one standard deviation of difference for that variable. The prevalence of low muscle strength is 12.1%. The prevalence of sarcopenia is low (4.3% of this cohort) which will impact power in these analyses.

Sarcopenia, but not muscle strength, is associated with protein intake

The results of multivariable logistic regression analyses used to determine adjusted ORs for the relation between protein intake and low muscle strength, low muscle mass, and sarcopenia are presented in Table 2. There was no significant association between protein intake and muscle strength, in any of the models. There was a significant association between protein intake (high and low; when compared to the reference category) and muscle mass, robust to adjustment in all models. Low protein intake was protective of low muscle mass (OR 0.52; 95% CI 0.40-0.67; $p<0.0001$), while high protein intake was associated with an increased odds of having low muscle mass (OR 1.76; 95% CI 1.39-2.24; $p<0.0001$). In terms of sarcopenia, no significant association was noted for low protein intake, however high protein intake was significantly associated with sarcopenia, and this was robust to adjustment in all models (OR 2.04; 95% CI 1.21-3.44; $p=0.008$).

To examine whether the results were consistent when protein was expressed as a proportion of total lean mass (g/kg FFM/d) instead of as a proportion of body weight (g/kg/d), the models were repeated for both low muscle strength and sarcopenia, with no notable differences found (Supplementary Table 4). The missingness of data is shown in Supplementary table 5. To ascertain whether missingness of data had any effect on this result, an analysis was carried out to assess whether variables of interest predicted missingness of protein intake (Supplementary Table 6). Only sex predicted missingness of the protein intake variable. As protein supplementation may have influenced our results, we noted those taking supplements. Four individuals reported taking protein supplements. Two of the four reported a low protein intake from diet, one reported a high intake, and the final one had missing data for protein intake. No subcategory analysis of this group was undertaken due to very low numbers.

Twin Modelling

For income, education, frailty, and gait speed the between-pair coefficients were larger, significantly different from zero, and significantly different from the within-pair coefficients (Wald test $p\leq0.05$),

supporting the inference that the association of these variables with muscle strength (chair-rise time) was confounded by factors that are shared by twins, such as common genes, and early life factors (Table 3).

For weight, BMI, healthy eating index, protein intake and alpha diversity there was minimal difference between the within- and between-pair coefficients, suggesting that shared twin factors were not driving any association between these variables and muscle strength.

Discussion

Older twins with low muscle strength had no difference in protein intake *versus* those without low muscle strength. In contrast, twins with sarcopenia had significantly higher protein intakes than those without. High protein intake (>1.3g/kg/day) was associated with sarcopenia, even after adjustment for a range of relevant potentially confounding variables including biological, socioeconomic, and environmental exposures, muscle, and diet-related variables. These analyses were carried out using protein intake as a proportion of total body weight and were consistent when protein was considered as a proportion of total lean mass.

Considering the definition of sarcopenia is the combination of low skeletal muscle strength and reduced muscle mass, one might expect that the driving force of the association between high protein intake and sarcopenia is the association of protein intake and muscle mass. Indeed, we found protein intake (high and low, versus optimal as the reference category) was associated with low muscle mass (as defined by the EWGSOP2 cut offs for men and women), and this was robust to adjustment in all models. High protein intake was associated with an increased odds of low muscle mass, and low protein intake was associated with a reduced odds (i.e., protective) of having low muscle mass. However, for sarcopenia, the association was only found for *high* protein intake. This suggests that the established relationship between protein intake and muscle mass does not explain all of the relationship seen, and there is a unique relationship between the sarcopenic phenotype, the combination of reduced mass and strength, that is associated with an excessive dietary protein intake, which warrants further exploration.

A recent longitudinal study also reported a negative effect of high protein intake, with higher protein intake from animal sources associated with a deterioration in health-related quality of life scores over 12 years (34).

In terms of muscle strength in particular, data from the Hertfordshire Cohort Study found higher grip strength was associated with *lower* meat consumption in men, while those with diets characterised by high consumption of fruit, vegetables, and fatty fish, had higher grip strength, in both men and women (35). Similarly, in the Newcastle 85+ study, dietary patterns high in characteristic British foods, including red meat, and with protein intakes >1g/kg/day were associated with an increased risk of sarcopenia (36). of the available literature focuses on inadequate protein intake (14), as this is more common. Many studies treat protein intake as a binary variable, either below, or meeting the RNI, and thus do not consider those with high intakes. It is plausible that this association is due to those individuals with sarcopenia deliberately consuming more protein, to ameliorate their muscle loss. Considering sarcopenia is not routinely diagnosed in clinical practice (37), one might consider this unlikely, however these individuals may have had another event that led to a dietician referral and so it cannot be ruled out. It is worth highlighting that our cohort have a healthy volunteer bias, with a healthier diet and higher protein intake than average, and therefore are distinct from a clinical inpatient or multi-morbid and/or frail population. Thus, our results indicate that for older adults who are relatively 'healthy', exceeding recommended protein intake may possibly be more detrimental for muscle health, than insufficient protein intake.

While not specific to older adults, there is existing evidence of detrimental effects of high protein intake, including coronary artery disease, cancer, disorders of liver and renal function and disorders of bone and calcium metabolism (38). Furthermore, a growing body of evidence has emerged, linking energy restriction to longevity and healthy aging, as well as a reduced risk of diseases including type 2 diabetes and ischemic heart disease (39). There is evidence that diets with restricted protein and/or specific amino acids are associated with improved health-span, and that protein may be the driving factor behind the benefits of energy restriction, via its effects on the IGF-1/mTOR network (40). This should also be considered in clinical recommendations on protein intake.

Not all sources of protein contain the full range of essential amino acids, and the quantity of leucine varies by protein source (41). The environmental impact of animal sources of protein, particularly red meat, in the context of the global climate crisis, must also be considered. There is an ongoing debate about the ideal protein

source for older adults, and a recent review suggests a mix of sources is likely to be the best approach (42). Using the same method used to calculate the healthy eating index, we estimated a proxy marker of protein from plant-sources, including tofu, meat substitutes, nuts, and beans. None of our participants consumed all their protein from plant sources alone, indeed 98.8% of our participants consumed ≤20% of their protein intake from plant sources (See: Supplementary Figure 1and Table 1). Thus, the results of this study should be considered in the context of a majority animal-source protein intake. This proxy measure does not include protein from other non-animal sources such as bread, though the contributions from these sources tend to be small. Indeed, the proportional contribution in our study compares with another UK study (43). More detailed future work is needed evaluating the impact of animal versus plant sourced protein on muscle health in older adults.

Only sex predicted missingness of the protein intake variable. The literature examining sex differences in self-reported dietary intakes is mixed, with some reporting no sex differences (44,45), and others noting differences by sex (46), however much of the published work in this area is focused on energy intake specifically, rather than protein intake. In our study, men had a higher proportion of missing data for protein intake than women. There is some evidence that women are more likely than men to complete questionnaires (47). This is in keeping with our experiences within the TwinsUK cohort, particularly questionnaires which are longer and/or more laborious, such as the FFQ, and may explain some of this difference.

Renal function should be considered when advising increased protein in diet for older adults, as diets high in protein are more likely to be acidogenic in the context of age-related decline in renal function. Acidogenic diets can lead to mild metabolic acidosis, with detrimental effects on muscle mass (48), unless well balanced by plant-based alkalinogenic foods. In addition, there is some evidence that blood pH does become slightly more acidic with age (49). The association between sarcopenia and high protein intake was robust to adjustment for both HEI, considered a proxy marker of alkalinity of diet ($p<0.001$), and creatinine clearance ($p=0.034$), an indicator of renal function, suggesting that diet alkalinity and renal function do not explain the association reported here. Serum creatinine and muscle mass are known to be correlated. We have used calculated creatinine clearance here, which considers weight and age, and is considered a more accurate

measure of renal function, but this will still be influenced by the participant's muscle mass. Future work examining renal function in the context of sarcopenia and dietary protein should consider other measures of renal function such as Cystatin C (50), which are less associated with lean mass, to explore this relationship further.

In terms of BMI, those with low muscle strength had a higher BMI than those without, however those with sarcopenia had lower BMI than those without. The higher BMI found in the low muscle strength group may be influenced by the presence of sarcopenic obesity. This relatively new concept refers to those with muscle loss typical of sarcopenia, but with a large body mass, although a consensus definition is lacking, which makes diagnosis difficult (51). Perhaps those with low muscle strength represent an earlier point on the pathophysiological pathway of sarcopenia development, and by the time they have reached the criteria for sarcopenia, they have lost body mass, in keeping with the typical image of a person with sarcopenia, with a thin body habitus. Further high quality, longitudinal research is required to explore this further.

Due to a growing body of evidence linking the gut microbiota to skeletal muscle health (14) alpha diversity of the gut microbiota was included as an exposure variable, with a notably less diverse gut microbiota in those with low muscle strength; however, this was not sustained for sarcopenia, perhaps due to our small number of sarcopenia cases lacking power to detect an association. When it comes to muscle health, it may well be that the function of the gut microbiota is likely more important than the diversity, and that diversity alone insufficiently encompasses microbiota composition and function. Ongoing trials are investigating targeting the gut microbiota to improve muscle strength (52) that will provide insights into whether the gut microbiota may represent a future therapeutic target for age-associated muscle loss and muscle strength.

Previous research in this cohort examined the heritability of muscle health, and found a moderate genetic component, with heritability estimates of 0.46 for leg extensor strength, 0.3 for handgrip strength and 0.52 for lean body mass (all $p<0.05$) (53), notably higher for mass than for strength measures. Other research investigating twins discordant for muscle strength found the stronger twins had higher physical activity (54), in keeping with the inference that muscle strength is modifiable by environment and lifestyle, rather than heavily influenced by genetics. However, the evidence linking early birth weight to later sarcopenia

development (55), indicates that sarcopenia's origins are developmental (56), highlighting the importance of twin studies in this field.

The association between muscle strength and each of the variables: weight, BMI, healthy eating index, protein intake, and alpha diversity, does not appear to be significantly influenced by shared twin factors. This tentatively suggests that those variables may be more modifiable in preventing the development of sarcopenia. This is perhaps intuitive when it comes to weight, and diet, however it is promising to see gut microbiota diversity also appears to be modifiable in this way. To our knowledge, this finding has not been shown elsewhere and can guide researchers in this field going forward, where sarcopenia research has struggled to find modifiable treatment targets.

Strengths and Limitations

Due to historical reasons, the TwinsUK cohort is majority female and white (15), as is the case in this study. Despite this, the cohort is largely representative of the UK population (15), however it does have a healthy volunteer bias. This study is cross sectional in nature and therefore definitive conclusions about the direct of associations cannot be made. In addition, while the vast majority of variables were contemporaneously measured at the same visit, occasionally when no data was available for that variable, the most recent previous value was imputed. While DXA scans are a recommended and satisfactory measure of muscle mass, it is worth noting that CT or MRI are the gold standard (1), although whole body measurement can be limited and costly using these methods. The low prevalence of sarcopenia, while not out of keeping with existing literature, means that the number of the individuals with sarcopenia in this study is low, reducing power, and while every effort was made to ensure the conclusions of our analyses are robust, further research with larger number of people living with sarcopenia is warranted to investigate this further. Lastly, while chair-rise time and gait speed are also recognised and accepted by EWGSOP2 as measures of muscle strength, they are not isolated isometric muscle measures and require neurological function, adequate vision etc. which may influence the results of these tests in some participants. Major strengths of our study are our investigation of potential factors that influence sarcopenia, and exploration of shared twin influences on the factors associated with sarcopenia.

Conclusions

We report a sarcopenia prevalence of 4.3% in a cohort of community dwelling volunteer twins, aged ≥60 years. Key factors that influence muscle strength include age, education, income, BMI, healthy diet, physical activity, frailty, appetite, protein intake and gut microbiota diversity. The association between muscle strength and each of the variables: weight, BMI, healthy eating index, protein intake, and alpha diversity, was not significantly influenced by shared twin factors. These potentially modifiable factors may therefore be more amenable to interventions aiming to prevent and/or treat sarcopenia.

High protein intake is associated with sarcopenia, even after adjustment for a range of covariates. This finding should be considered when advising increased protein intake for older adults without assessing baseline consumption. Further analysis is warranted, including longitudinal data, in cohorts with a larger number of individuals living with sarcopenia, to assess this association further.

Appendices

Methods

Variable measurement

Muscle mass was measured using DXA (Hologic Bone Densitometer QDR Horizon W, Serial Number 200884), and appendicular lean mass/height squared was calculated. Two measures of muscle strength were recorded; handgrip strength using Jamar Hydraulic Hand Dynamometer, with the best of 3 attempts recorded, using dominant hand, and chair-rise time (the time taken to rise from a chair 5 times without using hands). Gait speed (metres/second) was estimated from the time it took to walk 4 metres: with the mean of two attempts taken.

Low muscle strength (also known as probable sarcopenia) and sarcopenia were defined based on EWGSOP2 cut-off values, as was low muscle mass (1) . Thus, if a participant met the cut-off for reduced handgrip strength (<27 kg for males; <16 kg for females) and/or chair-rise time (>15 s for 5 chair rises), they were considered to have low muscle strength. The cut offs for muscle mass (appendicular lean mass/height squared) were <7kg/m^2 for men and <5.5kg/m^2 for women (1,16). Muscle strength and mass were then used as binary categorical variables, defined as low or not low. Sarcopenia was also a binary variable, defined as sarcopenia or no sarcopenia. Activity was measured using the International Physical Activity Questionnaire (IPAQ) (17), which computes a score of 1-2-3 representing low-moderate-high physical activity, based on MET minutes and the volume and frequency of physical activity per week (18). MET stands for metabolic equivalent of task, one MET minute is the energy expended at rest in a minute (19). To determine whether appetite was associated with sarcopenia, this was measured using the Simplified Nutritional Appetite Questionnaire (SNAQ) (20).

Dietary intake was measured using self-administered food frequency questionnaires (EPIC-FFQ) which was developed and validated for a UK population (21), from which daily protein intake and energy intake were calculated using the validated FETA (FFQ EPIC Tool for Analysis) tool (22). The FFQ is a valid tool for

estimating protein intake (23,24). To Understand whether diet quality impacted on associations between protein intake and sarcopenia, data from the FFQ was used to calculate the Healthy Eating Index (HEI), as described previously (25). FFQ entries were removed based on the following three criteria: (1) >10 incomplete items from the 130 food items on the FFQ as per recommendations (22) to reduce missing data error; (2) outside of 2 standard deviations (SD) (per batch, of 3) for the ratio of energy intake / basal metabolic rate (calculated using Harris-Benedict equations); (3) >2 SD of mean for macronutrients (protein, fat, and carbohydrate), both of which aim to reduce under-reporting and over-reporting. Protein was presented both as a binary variable, using the UK Reference Nutrient Intake (RNI) for adults which is ≥0.75g/kg body weight/day (26), and as per the ESPEN recommended intakes for older adults, which recommends 1-1.3g/kg/day as optimal intake (12), thus creating a categorical variable of low (<1g/kg body weight/day), optimal (1-1.3g/kg body weight/day) and high (>1.3g/kg body weight/day) intake. Participants were also asked to report use of any dietary supplements.

In all analyses protein intake was expressed and analysed as a factor as grams per kilogram of body weight per day, because it is most easily translated for clinicians and patients alike (who often know their body weight or can easily measure it) and also importantly because the ESPEN guidance for older adults uses this format (12) . In addition, in a supplementary analysis, protein intake was also expressed as grams per kilogram of total fat-free mass (FFM), as it has been suggested that this provides a more accurate representation of individual protein requirements (27).

The gut microbiota was measured from one stool sample and sequenced using Illumina MiSeq as described previously (28). Alpha diversity of the gut microbiota was quantified as observed Shannon diversity index, as described previously (29). Weight (kg) was measured using Marsden MPPS-250 scale, height (cm) was measured using a Leicester Height Measurer and body mass index (BMI) was calculated as weight/height squared. Frailty was quantified through the Rockwood Frailty Index (30), using self-reported data across 36 domains of age-related health deficits (see Supplementary Table 2). Serum creatinine was measured using a standard enzymatic rate (creatinine amidohydrolase) followed by colorimetric assay (Kodak Ektachem dry chemistry analysers, Johnson and Johnson Vitros Ektachem). Creatinine clearance (ml/min) was calculated

using the Cockcroft Gault formula: 1.2 x (140 - age) x body weight[kg]/creatinine[μmol/L], with the answer multiplied by 0.85 if female (31). This was included in the analysis to determine whether renal function may influence the relationship between sarcopenia and protein.

Demographic characteristics were recorded by questionnaires including smoking status, income, and education. Income referred to annual household earnings and was categorised as low (<£30,000), middle (£30,000-50,000) and high (>£50,000 Education level was categorized as low (up to GCSE or equivalent), middle (A levels, diploma) or high (university degree or higher). Available case analysis approach was used to handle missing data. For all data, the most recent collection was used and only data recorded since 2010 was included.

Statistical analysis

Statistical analysis was performed using Stata (Version 15.1). Data distributions were found to be normal, which allowed the use of parametric tests. To characterise the differences between those with low/normal muscle strength, and with/without sarcopenia, continuous variables were compared with two sample t-tests and categorical variables with Pearson's chi-squared tests. To assess for multicollinearity, correlation coefficients were checked for all variables of interest and were found to be <0.7 in all cases.

Univariable logistic regression analysis was used to determine odds ratios (ORs) for the relation between each variable and categories of low muscle strength, low muscle mass, and sarcopenia. All variables were standardised. All univariable analyses were adjusted for age and sex. Multivariable logistic regression analysis was used to determine the adjusted ORs of low muscle strength for dietary protein intake, using optimal intake (1-1.3g/kg/day) as the reference category (12). Variables were selected for the multivariable model based on significance in the univariable model and/or existing evidence for an association with sarcopenia. Values of $P<0.05$ were considered statistically significant. A supplementary analysis was carried out to test whether any exposure variables of interest predicted missingness of the protein intake data, to examine whether missingness influenced the results of the logistic regression analyses.

Twins are naturally matched pairs, with shared genetics, depending on zygosity, and shared early-life experiences. This means that the data has an inherently paired structure, which induces correlation between the pairs. To adjust for this, the data in the regression models were clustered by twin pair. However, a more detailed form of analysis to investigate the relative importance of shared *versus* non-shared factors is the between-pair and within-pair model approach (32). For this twin modelling analysis, the continuous variable of chair-rise time was used as a marker of muscle strength. Linear modelling was used in this analysis, considering the linear relationship between protein intake and muscle strength. The within-pair (variable_within) coefficient predicts the difference in outcome per unit difference within the pair and is free of confounding of shared twin factors. The between-pair (variable_between) coefficient predicts the difference in outcome per unit of the pair average of each predictor variable. A Wald test was used to test the difference between the between-pair and within-pair coefficients. Lone twins were excluded from this analysis.

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Tables

Table 1: Population characteristics and factors associated with low muscle strength, low muscle mass, and sarcopenia

	Total (n=3,302)	Normal muscle strength (n=2,901, 87.9%)	Low muscle strength (n=401, 12.1%)	p-value	Normal muscle mass (n=2024,	Low muscle mass (n=967,	p-value	No Sarcopenia (n=2,862, 95.7%)	Sarcopenia (n=129, 4.3%)	p-value
Age (years), mean (SD)	72.1 (7.3)	71.4 (6.9)	77.3 (8.3)	*<0.001*	71.3 (7.0)	73.0 (7.3)	*<0.001*	71.6 (6.9)	78.2 (8.3)	*<0.001*
Sex, n (%)				0.5			*<0.001*			0.066
Female	2,923 (89%)	2,564 (88%)	359 (90%)		1,754 (87%)	900 (93%)		2,546 (89%)	108 (84%)	
Male	379 (11%)	337 (12%)	42 (10%)		270 (13%)	67 (7%)		316 (11%)	21 (16%)	
Zygosity, n (%)				0.59						0.31

Monozygotic	1,787 (54%)	1,565 (54%)	222 (55%)		1,097 (54%)	535 (55%)	1,556 (54%)	76 (59%)	
Dizygotic	1,515 (46%)	1,336 (46%)	179 (45%)		927 (46%)	432 (45%)	1,306 (46%)	53 (41%)	
Highest Education Level Achieved, n (%)				*<0.001*			0.005		*<0.001*
Low	1,423 (48%)	1,194 (45%)	229 (63%)		822 (45%)	455 (51%)	1,200 (46%)	77 (64%)	
Middle	921 (31%)	830 (32%)	91 (25%)		597 (33%)	245 (28%)	815 (31%)	27 (22%)	
High	651 (22%)	605 (23%)	46 (13%)		406 (22%)	185 (21%)	574 (22%)	17 (14%)	
Annual Household Income, n (%)				*<0.001*			0.022		0.081
Declined to answer	607 (21%)	512 (20%)	95 (26%)		312 (20%)	192 (25%)	522 (21%)	29 (26%)	
Low	1,106 (39%)	928 (37%)	178 (49%)		608 (39%)	309 (40%)	947 (38%)	51 (45%)	
Middle	696 (24%)	637 (25%)	59 (16%)		398 (25%)	168 (21%)	628 (25%)	20 (18%)	

High	451 (16%)	423 (17%)	28 (8%)		253 (16%)	113 (14%)		412 (16%)	13 (12%)	
Smoking Status, n (%)				0.69						0.49
Never Smoked	1,886 (58%)	1,649 (57%)	237 (60%)		1,095 (57%)	566 (61%)		1,644 (58%)	68 (53%)	
Ex-smoker	1,215 (37%)	1,075 (37%)	140 (35%)		745 (39%)	323 (35%)		1,046 (37%)	54 (42%)	
Current smoker	165 (5%)	145 (5%)	20 (5%)		87 (5%)	43 (5%)		139 (5%)	6 (5%)	
Weight (kg), mean (SD)	70 (14)	70 (14)	71 (15)	0.23	74.5 (13.4)	60.2 (7.9)	*<0.001*	70 (14)	61 (8)	*<0.001*
Height (m), mean (SD)	1.63 (0.08)	1.63 (0.07)	1.60 (0.08)	*<0.001*	1.63 (0.08)	1.62 (0.07)	*0.009*	1.63 (0.07)	1.60 (0.08)	*<0.001*
BMI (kg/m2), mean (SD)	27 (5)	26 (5)	28 (5)	*<0.001*	28.1 (4.8)	22.9 (2.6)	*<0.001*	27 (5)	24 (3)	*<0.001*

Serum creatinine (µmol/L), mean (SD)	74.4 (15.5)	74.3 (14.8)	75.5 (20.3)	0.14	76.0 (15.8)	70.9 (13.7)	<0.001	74.5 (15.2)	72.1 (18.1)	0.094
Creatinine Clearance (ml/min), mean (SD)	68.7 (19.2)	69.2 (18.4)	65.2 (23.7)	*<0.001*	72.7 (19.5)	60.7 (14.7)	*<0.001*	69.3 (18.8)	58.4 (19.7)	*<0.001*
Healthy Eating Index, mean (SD)	61 (10)	61 (10)	59 (9)	*0.002*	60.7 (9.1)	61.4 (10.0)	0.18	61 (10)	59 (10)	0.069
Protein Intake adequacy (RNI), n (%)				0.27				<0.001		*0.009*
Optimal (≥0.75g/kg/day)	1,720 (87%)	1,526 (88%)	194 (85%)		1,041 (84.5%)	595 (93.4%)		1,552 (87%)	84 (97%)	
Low (<0.75g/kg/day)	249 (13%)	215 (12%)	34 (15%)		191 (15.5%)	42 (6.6%)		320 (13%)	3 (3%)	
Protein Intake (ESPEN), n (%)				0.60				<0.001		*<0.001*

Low (<1g/kg/day)	733 (37%)	644 (37%)	89 (39%)		386 (31%)	204 (32%)		671 (38%)	19 (22%)	
Optimal (1-1.3g/kg/day)	619 (31%)	554 (32%)	65 (29%)		541 (44%)	149 (23%)		567 (32%)	23 (26%)	
High (>1.3g/kg/day)	617 (31%)	543 (31%)	74 (32%)		305 (25%)	284 (45%)		544 (31%)	45 (52%)	
Energy intake (kcal/day), mean (SD)	1757.7 (445.9)	1761.8 (449.7)	1726.5 (415.9)	0.26	1770.0 (447.2)	1743.9 (450.4)	0.23	1762.7 (449.3)	1727.9 (429.2)	0.48
Muscle Mass (appendicular lean mass/height^2), mean (SD)	6.1 (1.0)	6.1 (1.0)	6.0 (0.9)	0.15	6.5 (0.9)	5.2 (0.5)	*<0.001*	6.1 (1.0)	5.3 (0.6)	*<0.001*
Gait speed (m/sec), mean (SD)	1.1 (0.2)	1.2 (0.2)	0.9 (0.3)	*<0.001*	1.1 (0.2)	1.1 (0.2)	0.49	1.1 (0.2)	1.0 (0.3)	*<0.001*
Physical Activity (IPAQ), mean (SD)	2.1 (0.8)	2.1 (0.8)	1.9 (0.8)	*0.002*	2.1 (0.8)	2.1 (0.8)	0.5	2.1 (0.8)	2.0 (0.8)	0.21

Frailty Index, mean (SD)	0.2 (0.1)	0.2 (0.1)	0.3 (0.2)	*<0.001*	0.2 (0.1)	0.2 (0.1)	*0.025*	0.2 (0.1)	0.3 (0.1)	*<0.001*
Appetite (SNAQ), mean (SD)	15.4 (1.8)	15.5 (1.7)	14.4 (2.4)	*<0.001*	15.5 (1.7)	15.2 (1.8)	0.003	15.4 (1.7)	14.9 (1.9)	*0.017*
Alpha Diversity (Shannon), mean (SD)	5.2 (0.7)	5.2 (0.7)	5.1 (0.7)	*0.007*	5.16 (0.72)	5.23 (0.70)	0.046	5.2 (0.7)	5.2 (0.7)	0.87

Italics = statistically significant; BMI: Body Mass Index; RNI: Reference Nutrient Intake; ESPEN: European Society for Clinical Nutrition and Metabolism; IPAQ: International Physical Activity Questionnaire; SNAQ: Simplified Nutritional Appetite Questionnaire

Table 2: ORs and 95% CIs for low muscle strength, low muscle mass, and sarcopenia according to protein intake comparing low and high protein intakes to optimal protein intakes (reference)

	Low Muscle Strength		**Low Muscle Mass**		**Sarcopenia**	
Protein Intake (g/kg/day)	Low (<1g/kg/d)	High (>1.3g/kg/d)	Low (<1g/kg/d)	High (>1.3g/kg/d)	Low (<1g/kg/d)	High (>1.3g/kg/d)
Unadjusted	1.18 (0.84-1.65)	1.16 (0.81-1.66)	0.52 (0.40-0.67)	1.76 (1.39-2.24)	0.70 (0.39-1.25)	2.04 (1.21-3.44)
	P=0.340	P=0.414	*P<0.0001*	*P<0.0001*	P=0.229	*P=0.008*
Model 1	1.31 (0.92-1.86)	1.10 (0.75-1.60)	0.54 (0.42-0.70)	1.72 (1.35-2.19)	0.77 (0.42-1.42)	2.21 (1.26-3.87)
age, sex	P=0.131	P=0.640	*P<0.0001*	*P<0.0001*	P=0.401	*P=0.006*
Model 2	1.20 (0.82-1.76)	1.02 (0.68-1.53)	0.53 (0.40-0.70)	1.80 (1.39-2.34)	0.75 (0.37-1.53)	2.48 (1.36-4.52)
1 + smoking, income, education	P=0.349	P=0.921	*P<0.0001*	*P<0.0001*	P=0.434	*P=0.003*
Model 3	1.23 (0.84-1.81)	0.92 (0.61-1.40)	0.53 (0.40-0.70)	1.86 (1.43-2.41)	0.78 (0.39-1.57)	2.36 (1.28-4.36)
2 + height	P=0.281	P=0.702	*P<0.0001*	*P<0.0001*	P=0.489	*P=0.006*
Model 4: frailty/activity	0.98 (0.60-1.58)	0.99 (0.57-1.71)	0.55 (0.38-0.79)	1.71 (1.20-2.43)	0.73 (0.28-1.92)	2.46 (1.08-5.60)

2 + frailty index + activity level (IPAQ)	P=0.921	P=0.967	*P=0.001*	*P=0.003*	P=0.528	*P=0.031*
Model 5: muscle	0.92 (0.55-1.55)	1.04 (0.59-1.82)	Not done (outcome)		Not done (part of sarcopenia definition)	
4 + lean mass/height2	P=0.767	P=0.889				
Model 6: renal function	0.92 (0.57-1.51)	1.13 (0.65-1.97)	0.56 (0.39-0.81)	1.37 (0.95-1.97)	0.76 (0.30-1.95)	2.46 (1.07-5.69)
4 + creatinine clearance	P=0.750	P=0.662	*P=0.002*	P=0.096	P=0.569	*P=0.034*
Model 7: diet	1.09 (0.71-1.68)	1.33 (0.71-1.77)	0.30 (0.22-0.41)	3.01 (2.23-4.06)	0.40 (0.19-0.86)	4.58 (2.37-8.87)
2 + energy intake (kcal/day), healthy eating index	P=0.689	P=0.639	*P<0.0001*	*P<0.0001*	*P=0.019*	*P<0.001*
Model 8: diet	1.28 (0.69-2.39)	1.70 (0.91-3.15)	0.31 (0.21-0.44)	3.16 (2.17-4.59)	0.49 (0.17-1.40)	5.97 (2.26-15.81)
7 + SNAQ score	P=0.433	P=0.095	*P<0.0001*	*P<0.0001*	P=0.182	*P<0.0001*
Model 9: Muscle & Gut Microbiota	1.38 (0.86-2.22)	1.39 (0.84-2.28)	0.56 (0.37-0.86)	1.56 (1.04-2.35)	1.25 (0.50-3.15)	2.67 (1.20-5.95)
4 + shannon diversity	P=0.188	P=0.199	*P=0.008*	*P=0.033*	P=0.628	*P=0.016*

Italics = statistically significant; IPAQ: International Physical Activity Questionnaire; SNAQ: Simplified Nutritional Appetite Questionnaire

Table 3: Univariable linear regression results for muscle strength: Between-Within Model

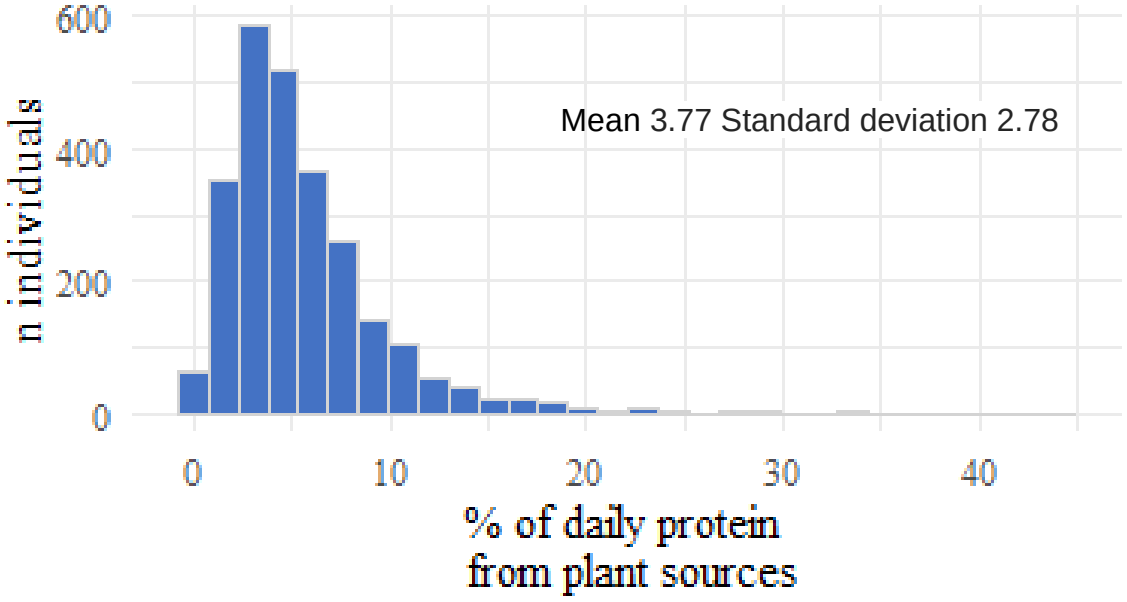
Variable	coefficient; 95% CI	P value	Wald Test coefficient P value
Income	-0.62; [-0.81, -0.43]	P<0.001	
income_between	-0.73; [-0.97, -0.50]	P<0.001	-0.41P=0.036
income_within	-0.33; [-0.61, -0.04]	P=0.025	
Education	-0.58; [-0.81, -0.36]	P<0.001	
education_between	-0.69; [-0.96, -0.42]	P<0.001	-0.52 P=0.020
education_within	-0.17; [-0.53, 0.18]	P=0.345	
Weight	0.06; [0.04, 0.73]	P<0.001	
weight_between	0.05; [0.04, 0.07]	P<0.001	-0.02 P=0.242
weight_within	0.07; [0.04, 0.10]	P<0.001	
BMI	0.16; [0.12, 0.21]	P<0.001	
bmi_between	0.16; [0.12, 0.21]	P<0.001	-0.004 P=0.928
bmi_within	0.17; [0.09, 0.24]	P<0.001	
Frailty index	10.89; [9.14, 12.64]	P<0.001	
frailty_between	11.97; [10.13, 13.81]	P<0.001	4.23 P=0.008
frailty_within	7.74; [4.64, 10.84]	P<0.001	
Gait speed	-8.27; [-9.35, -7.18]	P<0.001	

gaitspeed_between	-8.72; [-9.80, -7.64]	P<0.001	-1.64 P=0.050
gaitspeed_within	-7.07; [-8.87, -5.28]	P<0.001	
Physical activity (IPAQ)	-0.55; [-0.84, -0.26]	P<0.001	
ipaq_between	-0.67; [-1.07, -0.28]	P=0.001	-0.40 P=0.116
ipaq_within	-0.28; [-0.60, 0.04]	P=0.090	
Health eating index	-0.31; [-0.05, -0.01]	P=0.006	
hei_between	-0.04; [-0.07, -0.01]	P=0.009	-0.03 P=0.229
hei_within	-0.01; [-0.04, 0.03]	P=0.679	
Protein Intake (g/kg)	-1.46; [-2.26, -0.67]	P<0.001	
protein_between	-1.38; [-2.31, -0.44]	P=0.004	0.36 P=0.557
protein_within	-1.74; [-2.71, -0.76]	P<0.001	
Appetite (SNAQ)	-0.19; [- 0.33, -0.10]	P=0.006	
snaq_between	-0.72; [-1.55, 0.10]	P=0.087	-0.59 P=0.210
snaq_within	-0.13; [-0.31, 0.04]	P=0.129	
Alpha diversity	-0.46; [-0.81, -0.12]	P=0.011	
shannon_between	-0.41; [-0.89, 0.08]	P=0.104	0.18 P=0.630
shannon_within	-0.58; [-1.05, -0.11]	P=0.016	

BMI: Body Mass Index; IPAQ: International Physical Activity Questionnaire; SNAQ: Simplified Nutritional Appetite Questionnaire

Online supplementary materials

Supplementary Figure 1: Proportion of protein intake that comes from plant sources



*Please note this proxy measure of plant-sourced protein includes tofu, meat substitutes, nuts, and beans, and does not include all non-animal sourced protein – for example the protein in bread.

Supplementary Table 1: Conversion factors used to ascertain proportion of dietary protein from plant sources

Variable Name	Description	Food Code (6th)	Food Code (5th)	Full Food Name	Proportion	Portion size	Protein (g) per portion
Tofu	Meat substitutes e.g. tofu, soyameat, textured vegetable protein, vegeburger	13-119	50-723	Tofu, soya bean, steamed	0.4	120	9.4656
		15-331	15-331	Vegeburger, retail, grilled	0.6	56	9.4656
PeanutButter	Peanut butter (teaspoon)	14-876	14-876	Peanut butter, smooth	1	20	4.56
NutsSalted	Salted nuts e.g. peanuts, cashews (handful)	14-812	14-812	Cashew nuts, roasted and salted	0.2	25	5.965
		14-834	14-834	Peanuts, roasted and salted	0.8	25	5.965
NutsUnsalted	Unsalted nuts, e.g. brazil, walnuts (handful)	14-871	14-871	Brazil nuts	0.4	10	2.336
		14-879	14-879	Walnuts	0.6	20	2.336
Seeds	Seeds e.g. Sunflower, pumpkin (tablespoon)	14-845	14-845	Sunflower seeds	0.5	16	3.592
		14-842	14-842	Pumpkin seeds	0.5	16	3.592
Peas	Peas	13-440	13-440	Peas, frozen, boiled in unsalted water	1	70	4.2
GreenBeans	Green beans, broad beans, runner beans	13-432	13-432	Green beans/French beans, frozen, boiled in unsalted water	1	90	1.62
BakedBeans	Baked beans	13-044	13-044	Baked beans, canned in tomato sauce, re-heated	1	135	7.02
Beansprouts	Beansprouts	13-426	13-426	Beansprouts, mung, raw	1	20	0.58
DriedLentils	Pulses e.g. lentils, beans, peas	13-434	13-434	Lentils, red, split, dried, boiled in unsalted water	1	70	0.27

Supplementary Table 2: Domains included to quantify the Frailty Index

	Domain name	Question(s)
1	**Sleep problem**	How would you describe your sleep quality over the last month?
2	**Low physical activity**	In the past year, how frequently have you typically engaged in physical exercises that raise your heart rate and last for 20 minutes at a time? (Note: You would know if an activity raised your heart rate since you would probably feel your heart beating faster, you would sweat, and/or feel out of breath)
3	**Disability**	Do you currently have a long-term disability that seriously restricts your activities?
		Please, specify number of years you have had this disability for \ years
		Please, specify number of years you have had this disability for \ months
4	**Dizziness**	Over the last year, have you had episodes of "dizziness" or "funny turns"? (Tick only one box)
5	**Chronic lung disease**	Has a doctor ever told you that you have/had any of the following conditions? \ Chronic bronchitis, chronic obstructive pulmonary disease (COPD) or emphysema
		Is this an ongoing condition? \ Chronic bronchitis, chronic obstructive pulmonary disease (COPD) or emphysema
6	**Arthritis**	Has a doctor ever told you that you have/had any of the following conditions? \ Osteoarthritis (ordinary age-related arthritis)
		Is this an ongoing condition? \ Osteoarthritis (ordinary age-related arthritis)
		Has a doctor ever told you that you have/had any of the following conditions? \ Rheumatoid arthritis
		Is this an ongoing condition? \ Rheumatoid arthritis
		Has a doctor ever told you that you have/had any of the following conditions? \ Gout
		Is this an ongoing condition? \ Gout
		Has a doctor ever told you that you have/had any of the following conditions? \ Lupus (SLE)
		Is this an ongoing condition? \ Lupus (SLE)
		Has a doctor ever told you that you have/had any of the following conditions? \ Other arthritis (psoriatic arthritis, seronegative arthritis)
		Is this an ongoing condition? \ Other arthritis (psoriatic arthritis, seronegative arthritis)
		Has a doctor ever told you that you have/had any of the following conditions? \ Polymyalgia rheumatica
		Is this an ongoing condition? \ Polymyalgia rheumatica
7	**Osteoporosis**	Has a doctor ever told you that you have/had any of the following conditions? \ Osteoporosis
		Is this an ongoing condition? \ Osteoporosis
8	**Diabetes**	Has a doctor ever told you that you have/had any of the following conditions? \ Type 2 diabetes (or 'adult onset')

9	**Fragility fractures**	Have you had any of the following fractures since the age of 16? \ Hip
		Have you had any of the following fractures since the age of 16? \ Spine
		Have you had any of the following fractures since the age of 16? \ Wrist
10	**Falls**	How many times have you fallen in the past 6 months? A `fall' is defined as any event that led to an unplanned, unexpected contact with a supporting surface
11	**Fatigue**	Over the past 3 months, have you often felt tired or fatigued?
		Does tiredness or fatigue significantly limit your activities?
12	**Cardiac disease**	Has a doctor ever told you that you have/had any of the following conditions? \ Congestive heart failure
		Is this an ongoing condition? \ Congestive heart failure
		Has a doctor ever told you that you have/had any of the following conditions? \ Angina
		Is this an ongoing condition? \ Angina
		Has a doctor ever told you that you have/had any of the following conditions? \ Atrial fibrillation
		Is this an ongoing condition? \ Atrial fibrillation
		Has a doctor ever told you that you have/had any of the following conditions? \ Coronary heart disease
		Is this an ongoing condition? \ Coronary heart disease
		Has a doctor ever told you that you have/had any of the following conditions? \ Congenital heart disease (Heart valve problems)
		Is this an ongoing condition? \ Congenital heart disease (Heart valve problems)
13	**Cardiac risk factors**	Has a doctor ever told you that you have/had any of the following conditions? \ Hypertension (high blood pressure)
		Is this an ongoing condition? \ Hypertension (high blood pressure)
		Has a doctor ever told you that you have/had any of the following conditions? \ High cholesterol
		Is this an ongoing condition? \ High cholesterol
		Has a doctor ever told you that you have/had any of the following conditions? \ A heart murmur
		Is this an ongoing condition? \ A heart murmur
		Has a doctor ever told you that you have/had any of the following conditions? \ A heart attack (Myocardial infarction)
14	**Venous disease**	Has a doctor ever told you that you have/had any of the following conditions? \ Deep vein thrombosis (DVT)
		Is this an ongoing condition? \ Deep vein thrombosis (DVT)
		Has a doctor ever told you that you have/had any of the following conditions? \ Varicose veins
		Is this an ongoing condition? \ Varicose veins

		Has a doctor ever told you that you have/had any of the following conditions? \ Pulmonary embolism (PE)
		Is this an ongoing condition? \ Pulmonary embolism (PE)
15	**Gastrointestinal disease**	Has a doctor ever told you that you have/had any of the following conditions? \ Stomach or Duodenal ulcer (diagnosed with an Endoscopy or Barium Test)
		Is this an ongoing condition? \ Stomach or Duodenal ulcer (diagnosed with an Endoscopy or Barium Test)
		Has a doctor ever told you that you have/had any of the following conditions? \ Polyps in the colon or rectum
		Is this an ongoing condition? \ Polyps in the colon or rectum
		Has a doctor ever told you that you have/had any of the following conditions? \ Diverticular disease
		Is this an ongoing condition? \ Diverticular disease
		Has a doctor ever told you that you have/had any of the following conditions? \ Gallstones/cholelithiasis
		Is this an ongoing condition? \ Gallstones/cholelithiasis
16	**Cancer**	What kind(s) of cancer have you been diagnosed with? \ Bladder
		Is this an ongoing condition? \ Bladder
		What kind(s) of cancer have you been diagnosed with? \ Brain
		Is this an ongoing condition? \ Brain
		What kind(s) of cancer have you been diagnosed with? \ Breast
		Is this an ongoing condition? \ Breast
		What kind(s) of cancer have you been diagnosed with? \ Cervix
		Is this an ongoing condition? \ Cervix
		What kind(s) of cancer have you been diagnosed with? \ Colon
		Is this an ongoing condition? \ Colon
		What kind(s) of cancer have you been diagnosed with? \ Kidney
		Is this an ongoing condition? \ Kidney
		What kind(s) of cancer have you been diagnosed with? \ Leukaemia
		Is this an ongoing condition? \ Leukaemia
		What kind(s) of cancer have you been diagnosed with? \ Lung
		Is this an ongoing condition? \ Lung
		What kind(s) of cancer have you been diagnosed with? \ Lymphoma
		Is this an ongoing condition? \ Lymphoma
		What kind(s) of cancer have you been diagnosed with? \ Oesophagus

		Is this an ongoing condition? \ Oesophagus
		What kind(s) of cancer have you been diagnosed with? \ Ovary
		Is this an ongoing condition? \ Ovary
		What kind(s) of cancer have you been diagnosed with? \ Prostate
		Is this an ongoing condition? \ Prostate
		What kind(s) of cancer have you been diagnosed with? \ Skin (non-melanoma)
		Is this an ongoing condition? \ Skin (non-melanoma)
		What kind(s) of cancer have you been diagnosed with? \ Skin (melanoma)
		Is this an ongoing condition? \ Skin (melanoma)
		What kind(s) of cancer have you been diagnosed with? \ Uterus
		Is this an ongoing condition? \ Uterus
		What kind(s) of cancer have you been diagnosed with? \ Other kind of cancer
		Is this an ongoing condition? \ Other kind of cancer
17	**Incontinence**	Have you EVER regularly leaked urine, also known as suffering from incontinence?
		Do you currently leak urine regularly?
18	**Neurological disease**	Has a doctor ever told you that you have/had any of the following conditions? \ Bipolar disorder (manic depression)
		Is this an ongoing condition? \ Bipolar disorder (manic depression)
		Has a doctor ever told you that you have/had any of the following conditions? \ Anxiety or stress disorder
		Is this an ongoing condition? \ Anxiety or stress disorder
		Has a doctor ever told you that you have/had any of the following conditions? \ Clinical depression
		Is this an ongoing condition? \ Clinical depression
		Has a doctor ever told you that you have/had any of the following conditions? \ Stroke or Transient ischemic attack (TIA)
		Is this an ongoing condition? \ Stroke or Transient ischemic attack (TIA)
		Has a doctor ever told you that you have/had any of the following conditions? \ Parkinson's disease
19	**Subjective memory impairment**	Has a doctor ever told you that you have/had any of the following conditions? \ Alzheimer's disease
		Has a doctor ever told you that you have/had any of the following conditions? \ Memory loss
		Is this an ongoing condition? \ Memory loss
		During the past 12 months, have you experienced confusion or memory loss that is happening more often or is getting worse?

		To what extent does memory loss currently affect your day-to-day life?
20	**Eye disease**	Has a doctor or an optician ever told you that you have/had any of the following conditions? \ Glaucoma
		Is this an ongoing condition? \ Glaucoma
		Has a doctor or an optician ever told you that you have/had any of the following conditions? \ Age-related macular degeneration (AMD)
		Has a doctor or an optician ever told you that you have/had any of the following conditions? \ Cataract
21	**Glasses**	Do you wear spectacles/contact lenses?
22	**Hearing loss**	Do you suffer from hearing loss?
23	**Thyroid disease**	Has a doctor ever told you that you have/had any of the following conditions? \ Hyperthyroidism (overactive thyroid, characterized by weight loss)
		Is this an ongoing condition? \ Hyperthyroidism (overactive thyroid, characterized by weight loss)
		Has a doctor ever told you that you have/had any of the following conditions? \ Hypothyroidism (underactive thyroid, characterized by weight gain)
		Is this an ongoing condition? \ Hypothyroidism (underactive thyroid, characterized by weight gain)
24	**Overweight**	What is your current height? (Only one measurement type is required) \ feet
		What is your current height? (Only one measurement type is required) \ inches
		What is your current height? (Only one measurement type is required) \ centimetres
		What is your current height? (Only one measurement type is required) \ Don't know
		What is your current weight? (Only one measurement type is required) \ stones
		What is your current weight? (Only one measurement type is required) \ pounds
		What is your current weight? (Only one measurement type is required) \ kilograms
		What is your current weight? (Only one measurement type is required) \ Don't know
		Is this an ongoing condition? \ Cataract
25	**Weight loss**	Over the past 6 months have you LOST more than 10 lbs (4 kg) in weight without trying to?
26	**Poor General health**	In general, would you say your health is: excellent \ very good \ good \ fair \ poor
27	**Physical function limitation**	The following items are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much? \ Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf
28	**ADL limitation**	The following items are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much? \ Climbing several flights of stairs
29	**Occupational limitation**	During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical health? \ Accomplished less than you would like

		During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical health? \ Were limited in the kind of work or other activities
30	**Emotional limitation**	During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)? \ Accomplished less than you would like
		During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)? \ Didn't do work or other activities as carefully as usual
31	**Pain**	During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?
		In the past 3 months, have you had pain in your muscles, bones, or joints lasting at least 1 week?
		Has this pain actually lasted more than 3 months?
32	**Mental health problem**	These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the past 4 weeks… \ Have you felt calm and peaceful?
33	**Low Energy**	These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the past 4 weeks… \ Did you have a lot of energy?
34	**Mood disorder**	These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the past 4 weeks… \ Have you felt downhearted and blue?
35	**Social activity limitation**	During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting friends, relatives, etc.)?
36	**Polypharmacy**	Names of your currently prescribed medication(s) including hormone treatments (1). 'Currently prescribed' means medications/supplements/hormones that are currently taken on an intermittent or continued basis.

Supplementary Table 3: Univariable logistic regression results for covariates of low muscle strength and sarcopenia

	Low Muscle Strength		Sarcopenia	
Variable	**OR (95% CI)**	**p value**	**OR (95% CI)**	**p value**
Age	2.20 (1.94-2.49)	*P<0.001*	2.41 (1.96-2.98)	P<0.001
Sex	0.97 (0.86-1.10)	P=0.624	1.17 (0.97-1.40)	P=0.096
Smoking	1.01 (0.90-1.14)	P=0.812	1.13 (0.93-1.38)	P=0.218
Income	0.83 (0.73-0.94)	*P=0.004*	0.92 (0.73-1.16)	P=0.465
Education	0.78 (0.68-0.89)	*P<0.001*	0.79 (0.63-1.00)	*P=0.047*
Height	0.63 (0.54-0.74)	*P<0.001*	0.63 (0.50-0.80)	*P<0.001*
Weight	1.23 (1.09-1.38)	*P=0.001*	0.33 (0.26-0.41)	*P<0.001*
BMI	1.39 (1.25-1.54)	*P<0.001*	0.45 (0.36-0.55)	*P<0.001*
Serum creatinine	0.95 (0.83-1.08)	P=0.451	0.62 (0.47-0.81)	*P<0.001*
Frailty index	1.92 (1.70-2.15)	*P<0.001*	1.10 (0.92-1.33)	P=0.286
Muscle mass	1.02 (0.86-1.21)	P=0.797	0.70 (0.57-0.87)	*P=0.001*
Gait speed	0.41 (0.36-0.47)	*P<0.001*	0.92 (0.69-1.21)	P=0.541
Physical activity (IPAQ)	0.83 (0.70-0.99)	*P=0.036*	0.94 (0.76-1.17)	P=0.590

Health eating index	0.87 (0.76-0.99)	*P=0.037*	1.39 (1.15-1.70)	*P=0.001*
Protein/body weight (g/kg/day)	0.86 (0.74-1.01)	P=0.059	0.83 (0.66-1.04)	P=0.106
Protein/total lean mass (g/kg/day)	0.97 (0.75-1.26)	P=0.828	2.04 (1.45-2.88)	*P<0.001*
Energy intake (kcal/day)	0.87 (0.76-1.01)	P=0.062	0.79 (0.62-0.99)	*P=0.045*
Appetite (SNAQ)	0.62 (0.53-0.73)	*P<0.001*	0.96 (0.78-1.20)	P=0.735
Alpha diversity (Shannon)	0.80 (0.70-0.92)	*P=0.001*	OR (95% CI)	p value

BMI: Body Mass Index; IPAQ: International Physical Activity Questionnaire; SNAQ: Simplified Nutritional Appetite Questionnaire. All results are adjusted for age and sex.

Supplementary Table 4: ORs and 95% CIs for low muscle strength according to protein intake (measured as g/total lean mass). Reference category is middle tertile

	Low Muscle Strength		**Sarcopenia**	
Protein/total lean mass (g/kg/day)	**Low tertile**	**High tertile**	**Low tertile**	**High tertile**
Unadjusted	0.82 (0.57-1.16)	1.04 (0.74-1.47)	0.59 (0.30-1.15)	1.91 (1.14-3.20)
	P=0.261	P=0.828	P=0.121	*P=0.014*
Model 1 ***age, sex***	0.93 (0.64-1.36)	0.99 (0.69-1.42)	0.55 (0.26-1.16)	2.17 (1.27-3.71)
	P=0.708	P=0.954	P=0.116	*P=0.005*
Model 2 ***1 + smoking, income, education***	0.86 (0.57-1.29)	1.00 (0.68-1.47)	0.48 (0.21-1.12)	2.37 (1.35-4.17)
	P=0.459	P=0.993	P=0.089	*P=0.003*
Model 3 ***2 + height***	0.89 (0.59-1.34)	0.87 (0.59-1.28)	0.50 (0.21-1.15)	2.20 (1.26-3.85)
	P=0.572	P=0.480	P=0.104	*P=0.006*
Model 4: frailty/activity ***2 + frailty index + activity level (IPAQ)***	1.01 (0.60-1.69)	1.04 (0.63-1.74)	0.40 (0.13-1.25)	2.15 (1.01-4.57)
	P=0.965	P=0.869	P=0.115	*P=0.047*

Model 5: muscle	0.93 (0.54-1.58)	1.06 (0.63-1.79)	0.44 (0.14-1.38)	1.26 (0.53-2.99)
4 + lean				
mass/height2	P=0.777	P=0.836	P=0.161	P=0.593
Model 6: renal	0.98 (0.58 – 1.65)	1.01 (0.60-1.69)	0.42 (0.13-1.36)	2.05 (0.97-4.37)
function				
4 + serum	P=0.935	P=0.977	P=0.149	P=0.061
creatinine				
Model 7: diet	0.69 (0.43-1.09)	1.21 (0.71-1.91)	0.22 (0.08-0.55)	4.53 (2.29-8.96)
2 + energy intake				
(kcal/day), healthy	P=0.111	P=0.424	*P=0.001*	*P<0.001*
eating index				
Model 8: diet	0.61 (0.32-1.17)	1.51 (0.82-2.77)	0.12 (0.03-0.47)	5.22 (2.08-13.09)
6 + SNAQ score				
	P= 0.137	P=0.184	*P=0.003*	*P<0.0001*
Model 9: Muscle &	1.29 (0.69-2.43)	1.50 (0.80-2.79)	0.58 (0.14-2.47)	3.86 (1.34-11.07)
Gut Microbiome				
4 + shannon	P=0.422	P=0.206	P=0.465	*P=0.012*
diversity				

IPAQ: International Physical Activity Questionnaire; SNAQ: Simplified Nutritional Appetite Questionnaire.

Supplementary Table 5: Missingness of data

Age	0	3,302	0
Sex	0	3,302	0
Muscle strength	0	3,302	0
Height	1	3,302	0.03
Weight	4	3,302	0.12
Body Mass Index (BMI)	5	3,302	0.15
Smoking status	36	3,302	1.09
Serum creatinine	55	3,302	1.67
Muscle mass (appendicular lean mass/height^2)	124	3,302	3.76
Frailty index	261	3,302	7.9
Education	307	3,302	9.3
Gait speed	340	3,302	10.3
Income	442	3,302	13.39
Protein intake (g/d)	705	3,302	21.35
Energy intake (kcal/d)	705	3,302	21.35
Shannon diversity of the gut microbiome	1,306	3,302	39.55
Appetite (SNAQ score)	1,510	3,302	45.73
Physical activity (IPAQ score)	1,909	3,302	57.81

SNAQ: Simplified Nutritional Assessment Questionnaire. IPAQ: International Physical Activity Questionnaire.

Supplementary Table 6: Multivariable logistic regression analysis for missingness of protein intake

Variable	OR (95% CI)	p value
Age	1.19 (0.75-1.89)	P=0.452
Sex	1.86 (1.06-3.24)	P=0.029
Zygosity	1.07 (0.54-2.14)	P=0.840
Income	0.99 (0.69-1.43)	P=0.961
Education	0.71 (0.49-1.03)	P=0.069
Height	0.86 (0.05-14.07)	P=0.914
Weight	0.72 (0.001-304.96)	P=0.914
BMI	2.04 (0.01-487.41)	P=0.799
Serum creatinine	1.22 (0.84-1.78)	P=0.305
Frailty index	1.36 (0.86-2.14)	P=0.183
Muscle mass	0.62 (0.28-1.41)	P=0.256
Gait speed	1.10 (0.70-1.72)	P=0.676
Chair rise time	1.01 (0.59-1.73)	P=0.966
Sarcopenia	0.23 (0.02-2.43)	P=0.220
Physical activity (IPAQ)	1.35 (0.94-1.95)	P=0.103

Health eating index	0.75 (0.52-1.10)	P=0.141
Appetite (SNAQ)	1.10 (0.72-1.67)	P=0.674
Alpha Diversity (shannon)	0.88 (0.61-1.27)	P=0.493

BMI: Body Mass Index; IPAQ: International Physical Activity Questionnaire; SNAQ: Simplified Nutritional Appetite Questionnaire