Dietary protein intake is associated with better physical function and muscle strength among elderly women

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Abstract

Dietary protein intake might be beneficial to physical function (PF) in elderly. We examined the cross-sectional and prospective associations of protein intake g/kg body weight (BW), fat mass (FM) and lean mass (LM) with PF in 554 women aged 65·3-71·6 year belonging to OSTPRE-FPS study. Participants filled a questionnaire on lifestyle factors and 3-day food record in 2002. Body composition was measured by dual-energy X-ray absorptiometry and PF measures were performed at baseline and at 3 year follow-up. Sarcopenia was defined using European Working Group on Sarcopenia in Older People criteria. At the baseline women with higher protein intake (≥1·2 g/ kg BW) had better performance in hand grip strength/body mass (GS/BM) (*P*=0·001), knee extension/BM (*P*=0·003), one leg stance (*P*=0·047), chair rise (*P*=0·043), squat (*P*=0·019), squat to the ground (*P*=0·001), faster walking speed 10 m (*P*=0·005) and higher short physical performance battery score (*P*=0·004), than those with moderate and lower intakes (0·81-1·19 and ≤ 0·8 g/ kg BW, respectively). In follow-up results, higher protein intake was associated with less decline in GS/BM, one leg stance and tandem walk 6 m over 3years. Overall, results were no longer significant after controlling for FM. Associations were detected between protein intake and PF in non-sarcopenic women, but not in sarcopenic women except for change of GS (*P*=0·037). Further, FM but not LM was negatively associated with PF measures (*P*<0·050). This study suggests that higher protein intake and lower FM might be positively associated with PF in elderly women.

Introduction

The etiology of sarcopenia is multifactorial. The European Working Group on Sarcopenia in Older People (EWGSOP) has provided a working definition of sarcopenia (1,2). They proposed that sarcopenia is diagnosed using the criteria of low lean mass (LM) and low physical performance either low muscle strength (MS) and/or low physical function (PF) in elderly (1,2). It is known that decline in MS and PF are important contributing factors of the quality of life and increase the risk of frailty, fracture and falls in older individuals (2-4). Although the etiology of the decline in physical performance is not fully understood, poor nutrition may contribute to its development and progression (5). Therefore, measurement of MS and PF as indicators of physical performance status as well as nutritional status gained considerable attention in the past years (6).

Indeed, new evidence shows that adequate dietary protein is beneficial to support good health, promote recovery from illness, and maintain LM in older adults (7-11). It also has positive association with MS and PF (12-15). However, the adequacy of current recommended dietary allowance (RDA) (16) for protein 0·8 g/ kg per body weight (BW) has been questioned recently regarding that it might not be enough to maintain the LM and prevent functional decline among elderly (5,17,18). To this end, recent reviews and consensus statements have suggested that a protein intake between 1·0 and 1·5 g/kg/day may confer health benefits beyond those afforded by simply meeting the minimum (5,19). It might be inappropriate also to generalize the protein intake requirements based on healthy young men to older adults (18). PROT-AGE Study Group recommendations for dietary protein intake in healthy older adults is an average in the range of 1·0 to 1·2 g/kg BW (11). Further, Nordic nutrition recommendation 2012 (NNR) for elderly also suggested protein intake in the range of 1·1-1·3 g/kg BW (1·2 g/kg BW for planning purposes on population level) (20-22).

Ageing is accompanied with changes in body composition with a gradual increase in the proportion of fat mass (FM) and decline in LM (23). LM is the main reservoir of protein in human body and it has a significant role in movement and posture, regulation of metabolism, and storage of energy and nitrogen (24). Previous studies supported the correlation between decreased LM and impaired physical performance (25). In a study by Pedrero et al. (26) elderly men and women with sarcopenic obesity showed lower physical fitness levels compared to non-sarcopenic subjects (27). Notably, older individuals have an attenuated muscle protein synthetic response after the ingestion of dietary protein and amino acids. This resistance to the usually anabolic effect of protein on myofibrillar protein synthesis (MPS) may partially contribute to the age-related decline in LM (28). Because of metabolic changes associated with ageing, elderly persons may produce less LM than younger people from the same amount of ingested protein (29). It is recommended, therefore, that in cases of acute illness or psychological stress or sarcopenia higher protein intake is required (30).

Primary aim of present study was to evaluate the differences in MS and PF in elderly women with higher protein intake than current daily allowance as compared to those with lower intake at the baseline and over 3-year follow-up. We hypothesized that a positive association of protein intake with PF measures is more pronounced in non-sarcopenic women as compared to those with diagnosed sarcopenia based on EWGSOP criteria (2). Further, the associations of total body FM and LM with PF and MS measures were examined at the baseline and at 3 year of follow-up.

Subjects and methods

Study design and participants

Data of the present study were collected from the Osteoporosis Risk Factor and Prevention study (OSTPRE-FPS), which was a 3-year intervention to investigate the effect of calcium and vitamin D supplementation on the incidence of falls and fractures among elderly women (31). Inclusion criteria were being older than 65 year of age by the end of November 2002, residing in Kuopio region and no previous participation in OSTPRE bone densitometry sample (31). Supplementation group received daily cholecalciferol 800 IU and calcium 1000 mg for 3 years while the control group received neither supplementation nor placebo with the aim to study the effects of vitamin D and calcium supplementation on bone mineral density. In total 750 women were randomly taken into this subsample for participating in detailed examinations including measurement of body composition, physical performance tests and food records (32). Out of those, 554 women returned valid food record and had valid body composition and physical performance measurements for both at the baseline and at the 3-year follow-up. All clinical measurements were performed in Kuopio Musculoskeletal Research Unit of the Clinical research center of the University of Kuopio. All participants provided written permission for participation. The study was approved in October 2001 by the ethical committee of Kuopio University Hospital. The study was registered in Clinical trials.gov by the identification NCT00592917.

Body composition measurements

Height and weight of participants were measured in light indoor clothing without shoes, body mass index (BMI) was calculated by weight (kg) divided in height squared meter. FM and LM were measured by dual-energy X-ray absorptiometry (DXA) by specially trained nurses. The DXA measurements carried out using the same Lunar Prodigy adhering to the imaging and analysis protocols provided by the manufacturer (Lunar Co., Madison, WI, USA) (32). DXA is currently a common tool suitable for estimation of body composition in terms of evaluating the ratio between fat, muscle, and bone in different parts of the body (33). DXA also has been showed to be superior to bioimpedance for estimation of the body composition (34).

Physical performance measurements

Physical performance measures were assessed by trained nurses at the baseline and at year 3, consisting of three main domains: (1) MS: hand grip strength (GS) (kPa), number of chair rises in 30 seconds, ability to squat and ability to squat to the ground and knee extension (kPa). (2) Mobility test: walking speed (WS) 10 m (m/s) and tandem walk for 6 m (m/s); and (3) Balance ability: standing with closed eyes for 10 seconds and one leg stance performance for 30 seconds. GS was measured in a controlled sitting position with a pneumatic hand-held dynamometer (Martin Vigorimeter, Germany) by calculating the mean of three successive measurements from the dominant hand. To standardize, GS and knee extension were further expressed as a ratio to body mass (BM) (FM+LM) which have been suggested to be better predictor of GS and knee extension alone (35,36). The chair rise test was conducted if participant was able to stand at least once without using arms from a straight-backed, non-padded, armless chair. Any measurement errors were excluded from the statistical analysis (37). The follow-up variable of knee extension was excluded from analysis due to unexpected increase in measured extension force and/or possible data entry errors. Further, based on EWGSOP definition short physical performance battery (SPPB) score was calculated using three individual measures of physical performance including WS 10 m (m/s), chair rises in 30 seconds and one leg stance performance categorized in quartiles (38). Each quartile was scored on scale of 1-4 points with the total score ranging to 12; higher scores of SPPB indicates better performance. Further, absolute changes in PF and MS measures were calculated by subtracting the baseline measures from those measured at year 3. The magnitude of meaningful changes in physical performance measures as well as SPPB have been evaluated previously, and these measures are consistently used as preferred indicators of physical performance in older adults (2,38,39).

Diagnosis of sarcopenia

Relative skeletal muscle index (RSMI) was calculated as the sum of the nonfat, nonbone skeletal muscle in arms and legs divided by the square of height (m^2). Women were subdivided into quartiles according to their RSMI values: (1) 5·3–6·3 kg/m^2, (2) 6·3–6·7 kg/m^2, (3) 6·7–7·2 kg/m^2 and (4) 7·2–9·.3 kg/m^2. Baumgartner et al.(23) reported that the sarcopenia cutoff point was 5·45 kg/m^2, which was calculated as two standard deviations below the mean in young reference population. However, in our study there were only six women whose RSMI was less than 5·45

kg/m^2. Accordingly, we decided to use the lowest quartile below 6·3 kg/m^2 as cutoff in the present study (37) . The study population was divided into quartiles also for their GS: (1) < 22·3 kPa, (2) 22·3–25·7 kPa, (3) 25·7–28·7 kPa and (4) 28·7–40 kPa. Physical performance test was assessed by measuring WS by a 10-meter-WS test in a controlled situation and the WS was divided into quartiles: (1) < 0·51 m/s, (2) 1·42–1·63 m/s, (3) 1·64–1·85 m/s and (4) >1·85 m/s. The women who were not able to walk were allocated into the group of the lowest quartile. A woman was classified as sarcopenic if she belonged to the lowest quartile of RSMI and the lowest quartile of either GS or WS or both. A non-sarcopenic woman did not belong to the lowest quartile of any measurement (RSMI, GS or WS), whereas pre-sarcopenic women were in the lowest quartile of RSMI but not in the lowest quartile of any other outcome measure. Non-classified women belonged to the lowest quartile of either GS or WS or both, but not to that of RSMI.

Dietary intakes

Dietary intake was collected by using 3-day food record at the baseline. A questionnaire and instructions were sent to the participants beforehand, and they were returned on the visiting day. Participants were advised to fill the questionnaire for 3 consecutive days, including 2 days during the week and one day in the weekend (Saturday or Sunday). Participants were instructed to write down everything they ate and drank and to evaluate the amount of food consumed using household measures. In case of uncertainties in the food record, a nutritionist called the participant for additional information (40). To assess the underreporting the ratio of energy intake to estimated basal metabolic rate was calculated based on BW according to equations given by Department of Health in the UK(41). The ratio of energy intake to basal metabolic rate cutoff value for under-reporting was chosen to be 1·49, as derived from Goldberg et al.(42) and Black (43) and none of the participants was excluded from the analyses. Nutritional intake from food was calculated using Nutrica program (version 2·5, Finnish social insurance institute, Turku, Finland). Collected data provided calculations of animal and plant sources of protein in addition to total protein intake.

Potential confounders

All lifestyle related information was gathered by the self-administered questionnaire. The questionnaire included questions on age, hormone therapy use (never used or used), time since menopause (year), smoking status (never, former and current), self-reported calcium and vitamin D supplementation and alcohol consumption (portions/week). Total physical activity was based on self-reported amounts of sports, recreation and miscellaneous activities, including walking, jogging, skiing, cycling, swimming, aerobic exercise, ball sports and other more strenuous activities. Women were asked how many days they performed each activity per month. The sum of each activity days during all twelve months were summed and divided by 12 in order to obtain the mean activity level per month. Furthermore, the mean activity level was multiplied by self-reported of strenuousness of the exercise (the scale was 1 (low) to 4 (strenuous)) (37).

Statistical analysis

Protein intake was reported as crude protein intake per BW (g/kg BW). Protein intake was categorized based on three different nutrition recommendations, RDA (16) (≤ 0·8 g/ kg BW), PROT-AGE Study Group recommendation (11) (0·81-1·19· g/ kg BW), and NNR recommendation (≥ 1·2 g/ kg BW) (20). For the purpose of this study, these three categories were referred to as lower, moderate and higher intake, respectively. Continuous variables were compared across the protein intake categories using ANOVA and ANCOVA and categorical variables using chi-square tests.

Mean and SD of PF and MS measures at the baseline and absolute changes in them were tested in the ANCOVA across the categories of protein intake. Multiple linear regression or logistic regression models were used to calculate (β) and 95% CI of PF and MS measures at the baseline and changes in them across categories of protein intake. Tests for a linear trend across categories of protein intake were conducted by using the median value in each category as a continuous variable in the linear and logistic regression models. Pair wise comparisons of the group means were performed with Tukey's post hoc test. Linear and logistic regression analyses evaluated the association of FM and LM with PF and MS measures at the baseline and over 3-year follow-up. We examined further the association of protein intake g/kg BW with PF measures at baseline and over 3-year follow-up according to sarcopenia status. To achieve balanced numbers of participants in the stratified analysis and to evaluate our secondary hypothesis, women were classified as sarcopenic if they belonged to pre-sarcopenia, sarcopenia and severe sarcopenia (lowest quartile of RSMI) and non-sarcopenic group was compiled from normal and non-classified groups (normal RSMI).

We initially assessed known covariates of frailty, including age, total energy intake, smoking status, alcohol consumption (portions/week), physical activity (hours/ week), hormone therapy use, osteoporosis and self-reported history of medical conditions (fall in last 12 months, depression, diabetes mellitus, hypertension and rheumatoid arthritis) and also for baseline height, FM and LM. Further, covariates were selected based on their multicollinearity and their predictive values alone, which lead to selection of the following models. Model 1 presents the unadjusted results controlling only for age and energy intake. Model 2 was adjusted for variables in model 1 plus smoking status, alcohol consumption, physical activity, hormone therapy use, osteoporosis, LM and height. Model 3 was adjusted for variables in model 2 but LM was replaced by FM. Longitudinal analyses were adjusted for vitamin D and calcium supplementation (study group) to control for plausible vitamin

D effect on physical performance; as well as PF and MS baseline measures to account for differential subsequent changes in physical performance depending on the initial physical performance measures. Comparing model 2 and 3 provided opportunity to evaluate if LM and FM differently associate with PFs and MS as suggested by previous studies (4,44,45).

All statistical analysis were executed using SPSS software version 21 for Windows (IBM Corp., Armonk, NY). Result was considered significant if a P value was < 0·05.

Results

The participants were 65·3- 71·6 years old (mean (±SD) age was 68 ± 1·9), and mean energy intake was 6560 ± 1556 kJ/d (Table 1). Total protein intake was 68·2 g/d which constituted to 17% of total energy intake and corresponded to 0·96 g/ kg BW. The minimum protein intake reported was 0.24 g/kg BW and the maximum 2.25 g/kg BW. Also, 30% of women had protein intake ≤ 0·8 g/ kg BW, 48 % were in the moderate range of 0·8-1·19 g/ kg BW, while 22% consumed protein ≥ 1·2 g/ kg BW. Higher protein intake was significantly associated with higher energy intake and lower carbohydrate intake as % of energy, but higher carbohydrate intake as g/d.

In total, 8 % of women had osteoporosis, 42% had hypertension, 3 % had diabetes, 6% had rheumatoid arthritis, 3 % had depression, 12% had hip arthrosis, 28 % had knee arthrosis and 21·8 % reported fall accident in past 12 months. However, no significant associations between reported diseases and protein intake g/kg BW were observed. Mean duration of hormone therapy was 11 years and time passed after menopause was 18 years. Women with higher protein intake reported more frequent use of hormone therapy, weighed less and had lower BMI as compared to moderate and lower intake. Among body composition measurements FM, LM and LM index were significantly lower in higher protein intake. Women in higher protein intake had significantly higher RSMI than the lower protein intake group.

In the Table 2 differences of baseline characteristics between non-sarcopenic and sarcopenic participants are presented. Sarcopenic group (n = 127) had significantly lower mean weight (-13·2%), BMI (-12·7%), FM (-16·0%) and LM (-12·0%) as compared to non-sarcopenic group (n =369). Average protein intake was similar in sarcopenic and non-sarcopenic group, 17·6 ± 2·9 % and 17·9 ± 3·1 % of energy, respectively.

Significant differences in physical performance measures between women with higher protein intake and those with lower protein intake at the baseline and over 3-year follow-up were detected (Table 3). At the baseline after adjustment for selected factors previously described as associated with physical performance (model 2) those with higher protein intake as compared to those with moderate and lower intake had greater GS/BM (*P* = 0·001), knee extension/BM (*P* = 0·003), longer one leg stance performance (*P*= 0·047), better chair rise performance (*P* = 0·043), faster WS 10m pace (*P* = 0·005), squat completion (*P* = 0·019) and squat to the ground completion (*P* = 0·001), and higher SPPB score (*P* = 0·004). Overall results were no longer significant after controlling for FM (model 3). Results for the prospective analysis showed that those with higher protein intake had less decline in GS/BM (*P* = 0·027), one leg stance performance duration (*P* = 0·024) and had increased tandem walk speed (*P* = 0·024), which were no longer significant after controlling for FM.

In linear regression analyses with physical performance measures and SPPB as the dependent measures, results from models including energy-adjusted fat intake (g/d), or energy-adjusted carbohydrate intake (g/d) as determinant instead of protein showed no significant contribution for fat (g/d) and carbohydrate (g/d) (data not sown).

Further, we examined the association of protein intake with physical performance measures according to sarcopenia status (Table 4). Results of model 2 showed that among non-sarcopenic women protein intake was positively associated with GS/BM (β = 0·35 and *P*= 0·001), knee extension/BM (β = 0·25 and *P* =0·008), one leg stance performance (β= 0·26 and *P*= 0·001), chair rises (β = 0·15 and *P*= 0·039), WS 10 m (β = 0·30 and *P*< 0·001), ability to squat (β = 0·18 and *P*= 0·003), squat to the ground (β = 0·29 and *P*= 0·001) and also with SPPB score (β = 0·32 and *P*< 0·001) at the baseline. However, significant associations were lost after controlling for FM. Results of the prospective analysis indicated that higher protein intake in non-sarcopenic women was in positive relationship with changes of one leg stance performance (β = 0·14 and *P*= 0·037) and standing with eyes closed (β = 0·23 and *P*= 0·001). No significant associations between protein intake and physical performance measures were observed among sarcopenic women, except for GS/BM change (β = 0·23 and *P*= 0·037) and a non-significant relation with chair rise change (β = 0·27 and *P*= 0·064), which were lost after controlling for selected confounders and FM.

The associations between total body FM and LM with physical performance measures and changes in them are shown in Table 5. After adjustment for LM and factors previously described as associated with physical performance, FM was negatively correlated with GS/BM, GS, knee extension/BM (only at the baseline), one leg stance, chair rises, WS 10m, squat, squat to the ground and SPPB score at the baseline and over 3-year follow-up (β ≥ -0·07 and *P* ≤ 0·050). FM was also negatively associated with change of standing with closed eyes 10 seconds (β = -0·22 and *P* < 0·001). Further, LM was positively associated with GS, knee extension and one leg stance performance at the baseline as well as with GS changes over 3- year follow-up ($\beta \geq 0{\cdot}06$ and $P \leq 0{\cdot}025$). Results remained significant after controlling for FM.

Discussion

This study examined cross-sectional and prospective associations of protein intake (g/kg BW) and body composition (FM and LM) with different PF and MS measures in 554 elderly women belonging to the OSTPRE-FPS study. Associations of protein intake with PF and MS were also evaluated according to sarcopenia status. However, the significant associations were lost in the final models due to high collinearity of FM with physical performance. Our findings supported the hypothesis that higher protein intake than the current RDA (0·8 g/ kg BW), might be associated with better PF and MS among elderly women. Further, present study showed that the total body FM was negatively associated with physical performance tests, while total body LM was positively associated with GS, knee extension and one leg stance.

In recent years, there has been increased support for the contention that the current daily allowance (0·8 g/kg BW) for protein is insufficient to promote optimal health and preserve physical performance in the elderly (5,12,13,18,45-47). Consistently, in our cross-sectional findings, those women with higher protein intake performed better in many of the physical performance measures as compared to those who had moderate and lower protein intakes. The higher protein intake category had greater GS/BM, knee extension/BM, longer one leg stance, better chair rises performance, faster WS 10 m, better squat and squat to the ground ability, and higher SPPB score. The prospective results showed also that women in higher protein intake group had less decline in GS/BM and one leg stance performance, and had the highest increased chair rises performance over 3-year follow-up. No significant differences were observed between protein intake categories and WS 10 m and tandem walk speed 6 m prospectively. Thus, it might be that higher protein intake (g/kg BW) can be more related to preserving MS rather than mobility, which may partially explain the protein-frailty association. However, these associations were no longer significant after adjustment for FM.

Findings of study by Gregorio et al.(13) among 387 healthy women aged 60 to 90 years, showed that those in the lower protein intake < 0·8 g/ kg BW category performed less well in the single leg stance test than those in the higher protein intake ≥ 0·8 g/ kg BW category. They also walked eight feet at a slower pace and their SPPB score was lower than in women in the higher protein category. Further, Lemieux et al.(45) indicated that among 72 postmenopausal women, higher protein intake ≥ 1·2 g/kg BW was positively correlated to GS and knee extension. Women's Health Initiative clinical and observational study (12), was conducted in 134961 participants, aged 50 to 79 years for average 7 years of follow-up. Results showed that mean GS at baseline was slightly higher among women with higher calibrated daily protein intake (using urinary nitrogen protocol to estimate protein consumption over 24-h period), and these women experienced smaller decline in GS over time than those with low calibrated protein intake. Additionally, women in the highest quintile of calibrated protein intake completed on average 0·5 more chair rise at baseline than women in the lowest quintile. In contrast, there was no significant association between calibrated protein intake and the timed 6-meter walk in either cross-sectional or prospective analyses. Furthermore, the same results were shown when protein intake was expressed as g/kg BW.

A new finding was that among non-sarcopenic women at the baseline, protein intake (g/kg BW) was in positive relationship with GS/BM, knee extension/BM, one leg stance ability, chair rises performance, WS 10m, ability to squat and squat to the ground and SPPB. Protein intake in these women was also associated with preserving physical performance over 3 years follow-up, including one leg stance and standing with eyes closed 10s. No such an association was observed in sarcopenic women except a positive relationship between protein intake and GS change. Thus consistent to our hypothesis the positive association of protein intake (g/kg BW) with PF was more pronounced in non-sarcopenic than in sarcopenic women. It has been suggested that older individuals suffering from illness, physiological stress or sarcopenia are required to consume higher protein intake (1·2-1·5 g/ kg BW) as compared to healthy older people (1-1·2 g/ kg BW) (30). However, we could not explore this due to the threshold of protein intake in this data between sarcopenic and non-sarcopenic women.

A preponderance of evidence now suggests that aging might result in the stimulation of MPS becoming resistance to the anabolic effect of hyperaminoacidemia, particularly at lower protein intakes (24,30,48-50). It was shown in study by Moore et al.(28) that the relative quantity of ingested protein required to maximize MPS is greater in older as compared with younger men (18) . However, it is unestablished whether elderly individuals with greater LM have higher capacity of MPS as compared to those with lower LM. Besides, previous research indicates that protein from different sources (animal and plant protein) may have different effects on physical performance (51,52). However, this study did not find any significant association between animal and plant protein intake with PF and MS measures.

Declines in LM might predict a reduction in muscle force and performance (1,48). It has also been shown that FM is associated with functional decline and muscle weakness in elderly individuals (35,44,53). In this study, total body FM was in strong negative correlation with all PF and MS measures at the baseline and changes in them at 3 years except for knee extension, tandem walk and standing with eyes closed at the baseline; while LM was positively correlated with GS and change in it, knee extension and one leg stance. Therefore, these findings accompanied with the loss of significant associations between protein intake and physical performance measures after controlling for FM but not LM, suggest that FM and LM may have opposite association with PF and MS in elderly women. There are different pathways through which fatness might be related to LM and muscle strength (54). However, more studies are needed to disentangle the relationship between FM and physical performance.

It is well known that adequate energy intake is required to optimally utilize dietary protein to maintain physical performance rather than as energy source (13). It was to our surprise that those with higher energy and protein intake had a lower weight. The actual cause is uncertain but this might be due to higher physical activity level in higher protein category, and also possible underreporting of total energy and fat intake in those with higher BMI (55). Worthy of note is that LM index (LM/ height (m^2)) and RSMI are both used as indicators of muscle mass in the diagnosis of sarcopenia (2). However, in this study protein intake showed the same association with LM index and RSMI, thus we used RSMI as clinical indicator of sarcopenia as adapted by EWGSOP (2).

A limitation of this study was that the study population consists of only elderly women and therefore caution should be taken when generalizing the findings to elderly men. The 3-day food records method has been described as a suitable instrument for assessing energy and protein intake in elderly people (56,57). The latter study has also validated protein intake against urinary nitrogen studies in both community dwelling and institutionalized elderly people (57). However, a single 3-day dietary record at the baseline might not be an appropriate method to capture long term effect of protein intake. Albeit we covered a wide selection for several known confounders that might influence physical performance, other factors such as health status, habitual physical activity level and/or dietary habits in participants in different protein intake categories might have affected the observed results. Lastly, based on the observational nature of our study we cannot establish a causal association.

An additional analyses in the present data showed no significant effect of vitamin D (800 IU) and calcium supplementation (1000 mg) on MS and PF and longitudinal analysis were controlled for study group receiving those. The availability of multiple standardized physical performance measures at baseline as well as over a 3-year period added significant strength to our study. Dynamometric measures of GS as a physical marker of lower limb strength and knee extension for a variety of functional tasks, such as walking, chair rising and stair climbing, particularly are predominate for the quantification of physical performance in older adults (36,58). The introduced protein intake categories in present study took into account the newer intake recommendations for elderly, which have not been used in the previous studies.

Conclusion

It is appropriate to focus on the relationship between protein intake, and MS and PF in the elderly because this group is most vulnerable to nutritional deficiencies. This cohort study suggests that higher protein intake and lower FM might be positively associated with MS and PF in elderly women. However, further research is required to establish causal association.

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Conflict of interests

The authors have no relevant interests to declare.

Author Contribution

H. Kröger and M. Tuppurainen designed the original OSTPRE-FPS study. M. Isanejad, A. Erkkilä, J. Sirola and J. Mursu. planned the present study together and collaborated on drafting the manuscript. M. Isanejad carried out the statistical analysis, and summarized the results in tables. H. Kröger, M. Tuppurainen and T. Rikkonen critically revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

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Table 1. Baseline characteristics of the participants in different protein intake categories (g/kg BW).

	≤ 0·8 g/kg body weight (n=171)		0·81-1·19 g/kg body weight (n=269)		≥ 1·2 g/kg body weight (n=112)		*P**
Demographic	Mean	SD	Mean	SD	Mean	SD	
Age (year)	68·0 [a]	1·9	67·8 [b]	1·9	67·7	1·8	0·003
Weight (kg)	79·1 [a]	12·2	71·5 [b]	10·8	66·0	10·6	0.001
Height (cm)	158·6	5·3	158·8	5·2	158·4	5·3	0·202
Body mass index (kg/m^2)	29·9 [a]	4·4	27·1 [b]	3·9	25·3	3·4	0.001
Osteoporosis (%)	10·5		9·4		6·1		0·088
Diabetes (%)	2·9		2·5		3·6		0·560
Depression (%)	5·3		1·9		2·9		0·211
Rheumatoid arthritis (%)	8·2		4·4		4·7		0·217
Fall in last 12 months (%)	22·8		21·6		21·4		0·942
Hormone therapy use (%)	46·9 [a]		44·4 [b]		61·9		0·009
Physical activity†	100·2	112·6	106·4	72·5	111·4	140·3	0·536
Body composition							
Fat mass (kg)	34·2 [a]	8·4	28·1 [b]	8·0	24·4	7·3	0·001
Lean mass (kg)	41·3 [a]	4·5	40·1 [b]	4·4	39·1	4·0	0·035
Lean mass index (kg/m^2)	16·4 [a]	1·7	15·9 [b]	1·4	15·6	1·2	0·037
Relative skeletal muscle index (kg/m^2)	6·5 [a]	0·7	6·7 [b]	0·6	6·6	0·5	0·036
Fat mass to lean mass ratio	0·82	0·17	0·70	0·18	0·62	0·17	0·164
Dietary factors							
Energy intake (kJ/d)	5388 [a]	1251	6699 [b]	1125	8008	933	0·001
Crude protein (g/d)	51·4 [a]	10·3	65·0 [b]	10·2	83·4	14·1	0·001
Protein (% of energy)	16·4	3·1	17·4	2·5	18·6	3·1	0·001
Carbohydrate (g/d)	165·7	45·5	187·6	37·0	219·1	46·3	0·001
Carbohydrate (% of energy)	50·6 [a]	5·9	48·8	5·5	48·0	5·7	0·036
Fat (g/d)	43·6	14·5	53·9	15·1	63·1	18·2	0·203
Fat (% of energy)	30·8	5·4	31·3	5·6	31·1	5·7	0·112

*ANCOVA and chi-square tests were used to evaluate the differences between participants' characteristics and dietary intake with protein intake categories as expressed per body weight according to different recommendations.

†Includes walking, gardening, cycling, cross-country skiing, and other more strenuous activity, times/month × strenuousness.

[a] Means that lowest category was significantly different than middle and highest categories after Tukey's post hoc test.

[b] Means that middle category was significantly different than highest category after Tukey's post hoc test

Table 2. Baseline characteristics of the participants according to sarcopenia status.

	Non-sarcopenic (n= 369)		Sarcopenia (n= 127)		*P**
Demographic	Mean	SD	Mean	SD	
Age (year)	67·7	1·8	67·9	1·9	0·007
Weight (kg)	74·7	12·1	64·8	8·8	0·001
Height (cm)	158·6	5·2	158·7	5·5	0·117
Body mass index (kg/m^2)	28·3	4·1	24·7	3·1	0·001
Osteoporosis (%)	7·2		10·3		0·143
Diabetes (%)	4·2		0·7		0·021
Depression (%)	2·6		5·7		0·190
Rheumatoid arthritis (%)	5·6		5·7		0·997
Fall in last 12 months (%)	20·7		23·8		0·560
Hormone therapy use (%)	49·0		53·9		0·581
Physical activity†	108·5	112·3	104·6	85·3	0·472
Body composition					
Fat mass (kg)	30·0	8·8	25·2	7·1	0·001
Lean mass (kg)	41·4	4·1	36·4	2·5	0·001
Lean mass index (kg/m^2)	16·4	1·3	14·4	0·7	0·001
Relative skeletal muscle index (kg/m^2)	7·0	0·5	5·9	0·2	0·001
Fat mass to lean mass ratio	0·72	0·19	0·69	0·18	0·004
Dietary factors					
Energy intake (kJ/d)	6539	1518	6614	1564	0·001
Protein (g/kg body weight)	0·94	0·28	1·04	0·30	0·021
Protein (% of energy)	17·9	3·1	17·6	2·9	0·020
Carbohydrate (g/d)	192·3	47·8	197·2	48·7	0·002
Carbohydrate (% of energy)	48·8	5·7	49·5	6·1	0·006
Fat (g/d)	53·6	17·6	55·0	19·6	0·001
Fat (% of energy)	30·8	5·5	31·8	5·6	0·002

*Independent sample t-test and chi-square tests were used to evaluate the differences between participant's characteristics according to sarcopenia status.

†Includes walking, gardening, cycling, cross-country skiing, and other more strenuous activity, times/month × strenuousness.

Table 3. Physical performance measures in protein intake categories at the baseline and over 3-year follow-up.

Physical performance measures	≤ 0·8 g/kg BW (n=171)		0·81-1·19 g/kg BW (n=269)		≥ 1·2 g/kg BW (n=112)		P_{trend} - value		
	Mean	SD	Mean	SD	Mean	SD	Model 1*	Model 2[‡]	Model 3[†]
Hand grip strength/body mass (kPa/kg)									
Baseline	0·32 [a]	0·08	0·37 [b]	0·06	0·40	0·01	< 0·001	0·001	0·342
Change[§]	-1·51 [a]	6·70	-0·79 [b]	3·68	-0·68	3·42	0·020	0·027	0·779
Hand grip strength (kPa)									
Baseline	25·96 [a]	7·04	26·23 [b]	4·88	24·53	4·56	0·029	0·657	0·135
Change	-1·51	6·70	-0·79	3·68	-0·68	3·43	0·538	0·358	0·967
Knee extension/body mass (kPa/kg)									
Baseline	3·71 [a]	1·13	4·34 [b]	1·25	4·47	1·32	0·080	0·003	0·799
Knee extension (kPa)									
Baseline	282·07	81·73	307·01	85·70	285·99	77·19	0·104	0·822	0·240
One leg stance 30 s									
Baseline	15·79 [a]	10·90	19·31 [b]	10·28	21·54	9·42	< 0·001	0·047	0·804
Change	-1·64 [a]	10·02	-1·50 [b]	10·89	-0·96	10·48	0·007	0·024	0·993
Chair rises									
Baseline	7·87 [a]	6·97	7·84 [b]	2·86	8·41	2·20	0·042	0·043	0·720
Change	0·12 [a]	6·07	0·83 [b]	2·82	1·15	2·68	0·001	0·725	0·111
Tandem walk speed 6 m (m/s)									
Baseline	0·30	0·09	0·34	0·37	0·33	0·12	0·675	0·959	0·254
Change	0·02	0·11	-0·15	0·42	0·03	0·11	0·992	0·024	0·483
Walking speed 10 m (m/s)									
Baseline	1·53 [a]	0·31	1·67 [b]	0·32	1·72	0·28	< 0·001	0·005	0·668
Change	-0·11	0·24	-0·10	0·33	-0·11	0·29	0·505	0·486	0·712
Standing with eyes closed 10 s (%)									
Baseline	94·1 [a]		95·6 [b]		97·0		0·050	0·381	0·412
Change	-5·54		-5·19		-4·94		0·646	0·873	0·100
Ability to squat (%)									
Baseline	91·1 [a]		94·3 [b]		97·0		0·027	0·019	0·191

Change	-0·08 [a]		0·32 [b]		0·21		0·012	0·100	0·503
Ability to squat to the ground (%)									
Baseline	58·0 [a]		69·8 [b]		78·7		< 0·001	0·001	0·080
Change	-0·02		-0·01		-0·06		0·202	0·309	0·690
Short physical performance battery score									
Baseline	5·52 [a]	1·82	6·28 [b]	1·87	6·51	1·77	< 0·001	0·004	0·586
Change	1·35	0·21	1·55	0·14	1·57	0·24	0·968	0·908	0·845

BW, Body weight.

* Model 1 was adjusted for age and total energy intake.

[†] Model 2 was adjusted for variables in model 1 plus smoking status, alcohol consumption (portions/week), physical activity level, hormone therapy use, osteoporosis, baseline height and lean mass.

[‡] Model 3 was adjusted for variables in model 2 but lean mass was replaced by fat mass.

[§] Longitudinal analyses were adjusted also for physical performance baseline variables and calcium and vitamin D intervention.

Tests for a linear trend across categories of protein intake were conducted by using the median value in each category as a continuous variable in the linear and logistic regression models. Median total protein intake for each category was 0·66, 0·9·8 and 1·34 g/ kg BW, respectively.

[a] Means that lowest category was significantly different than middle and highest categories after Tukey’s post hoc test.

[b] Means that middle category was significantly different than highest category after Tukey’s post hoc test.

Table 4· Effect of protein intake (g/kg body weight) and physical performance measures according to sarcopenia status.

	Non-sarcopenic (n=369)				Sarcopenic (n=127)			
Physical performance **measures**	regression coefficient (95% CI)	*P* Model 1*	*P* Model $2^{\ddagger}$	*P* Model $3^{\dagger}$	regression coefficient (95% CI)	*P* Model 1*	*P* Model $2^{\ddagger}$	*P* Model $3^{\dagger}$
Hand grip strength/body mass (kPa/kg)								
Baseline	0·35 (0·07, 0·15)	<0·001	<0·001	0·284	0·22 (0·04, 0·21)	0·041	0·320	0·806
Change§	0·09 (-0·48, 3·4)	0·138	0·237	0·666	0·20 (0·93, 11·06)	0·021	0·037	0·872
Hand grip strength (kPa)								
Baseline	-0·13 (-4·22, 0·17)	0·069	0·520	0·113	-0·23 (-4·22, 0·17)	0·114	0·850	0·334
Change	0·18 (-1·49, 1·94)	0·018	0·430	0·406	0·06 (-3·14, 5·07)	0·043	0·257	0·690
Knee extension/body mass (kPa/kg)								
Baseline	0·25 (0·72, 2·12)	<0·001	0·008	0·726	0·28 (0·28, 2·50)	0·014	0·053	0·533
Knee extension (kPa)								
Baseline	-0·04 (-6·17, 17·03)	0·613	0·683	0·562	-0·07 (-4·81, 70·81)	0·642	0·552	0·562
One leg stance 30 s								
Baseline	0·26 (5·62, 15·17)	<0·001	0·001	0·974	0·45 (-2·40, 14·20)	0·762	0·545	0·948
Change	0·14 (0·44, 9·60)	0·032	0·037	0·658	-0·48 (-10·0, 5·64)	0·718	0·489	0·055
Chair rises								
Baseline	0·15 (0·65, 4·13)	0·038	0·039	0·658	0·01 (-8·15, 1·82)	0·987	0·235	0·486
Change	0·20 (1·02, 3·59)	<0·001	0·182	0·653	0·27 (0·02, 5·22)	0·064	0·126	0·228
Tandem walk speed 6 m (m/s)								
Baseline	0·31 (-0·05, 0·09)	0·687	0·560	0·989	-0·23 (-0·62, 0·20)	0·133	0·667	0·972
Change	0·02 (-0·05, 0·06)	0·682	0·692	0·793	-0·13 (-0·04, 0·11)	0·616	0·844	0·728
Walking speed 10 m (m/s)								
Baseline	0·30 (0·17, 0·48)	<0·001	<0·001	0·161	0·11 (-0·11, 0·36)	0·769	0·267	0·429
Change	0·23 (-0·02, 0·24)	0·119	0·854	0·324	-0·01 (-0·28, 0·24)	0·784	0·608	0·978
Standing with eyes closed 10 s (%)								
Baseline	0·04 (-0·06, 0,13)	0·514	0·305	0·850	-0·11 (-0·14, 0·05)	0·383	0·564	0·650
Change	0·23 (0·62, 2·37)	0·001	0·001	0·096	0·13 (-0·47, 1·54)	0·297	0·246	0·557
Ability to squat (%)								

Baseline	0·18 (0·04, 0·25)	0·006	0·003	0·964	0·08 (-0·05, 0·09)	0·536	0·309	0·545
Change	0·09 (-0·03, 0·26)	0·134	0·190	0·528	0·15 (-0·10, 0·40)	0·256	0·123	0·578
Ability to squat to the ground (%)								
Baseline	0·29 (0·26, 0·68)	0·001	0·001	0·852	0·10 (-0·22, 0·52)	0·432	0·652	0·333
Change	0·59 (-0·11, 0·33)	0·340	0·389	0·224	0·04 (-0·30, 0·45)	0·682	0·381	0·677
Short physical performance battery score								
Baseline	0·32 (1·15, 2·86)	<0·001	<0·001	0·177	-0·05 (-1·89, 1·23)	0·722	0·214	0·132
Change	0·15 (0·09, 2·11)	0·032	0·301	0·919	-0·02 (-1·80, 1·59)	0·880	0·876	0·983

*Model 1 was adjusted for age and total energy intake.

† Model 2 was adjusted for variables in model 1 plus smoking status, alcohol consumption (portions/week), physical activity level, hormone therapy use, osteoporosis, study group and baseline height.

‡Model 3 was adjusted for variables in model 2 plus fat mass.

§ Longitudinal analyses were adjusted also for physical performance baseline variables and calcium and vitamin D intervention.

Table 5. Association of total body fat mass and lean mass with physical performance measures at the baseline and over 3-year follow-up.

Physical performance measures	Total body fat mass				Total body lean mass			
	β	SE	*P* Model 1[*]	*P* Model 2[†]	β	SE	*P* Model 1	*P* Model 2
Hand grip strength/body mass (kPa/kg)								
Baseline	-0·58	0·01	<0·001	<0·001	-0·01	0·01	<0·001	0·821
Change [§]	-0·33	0·01	<0·001	<0·001	0·04	0·01	0·079	0·429
Hand grip strength (kPa)								
Baseline	-0·10	0.03	0·754	0·029	0·21	0·01	<0·001	<0·001
Change	-0·09	0.02	0·622	0·050	0·11	0·01	0·014	0·002
Knee extension/body mass (kPa/kg)								
Baseline	-0·47	0·01	<0·001	<0·001	0·09	0·01	0·003	0·079
Knee extension (kPa)								
Baseline	0·02	0·46	0·570	0·094	0·26	0·01	0·002	<0·001
One leg stance 30 s								
Baseline	-0·28	0·05	<0·001	<0·001	0·06	0·01	<0·001	0·025
Change	-0.19	0·05	<0·001	<0·001	0·17	0·11	<0·001	0·119
Chair rises								
Baseline	-0·14	0·02	0·004	0·005	0·03	0·01	0·398	0·537
Change	-0·16	0·01	<0·001	<0·001	0·09	0·03	0·012	0·822
Tandem walk speed 6 m (m/s)								
Baseline	0·07	0·02	0·337	0·177	-0·03	0·04	0·580	0.266
Change	-0·01	0·01	0·666	0·865	-0·01	0·02	0·536	0·638
Walking speed 10 m (m/s)								
Baseline	-0·34	0·02	<0·001	<0·001	0·03	0·04	<0·001	0·502
Change	-0·13	0·02	0·003	0·017	-0·01	0·04	0·060	0·546
Standing with eyes closed 10 s (%)								
Baseline	-0·05	0·01	0·034	0·256	-0·09	0·03	0·017	0·118
Change	-0·22	0·02	<0·001	<0·001	-0·01	0·02	0·031	0·991
Ability to squat (%)								
Baseline	-0·23	0·01	<0·001	<0·001	0·02	0·03	0·005	0·721
Change	-0·16	0·02	<0·001	0·001	0·18	0·04	0·177	0·738
Ability to squat to the ground (%)								

Baseline	-0·33	0·03	<0·001	<0·001	0·01	0·06	<0·001	0·185
Change	-0·07	0·01	<0·001	<0·001	0.07	0·06	0·732	0·657
Short physical performance battery score								
Baseline	-0·32	0·01	<0·001	<0·001	0·01	0·02	<0·001	0·738
Change	-0·27	0·01	<0·001	<0·001	0·06	0·02	0·001	0·252

SPPB, short physical performance battery.

*Model 1 was adjusted for age, total energy intake, smoking status, alcohol consumption (portions/week), physical activity level, hormone therapy use, osteoporosis and height.

[†] Model 2 adjusted for variables in model 1, and lean mass and fat mass were adjusted for each other.

[§] Longitudinal analyses were adjusted also for physical performance baseline variables and calcium and vitamin D intervention.