

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

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1 **Abstract**

2 Dietary protein intake might be beneficial to physical function (PF) in elderly. We examined the
3 cross-sectional and prospective associations of protein intake g/kg body weight (BW), fat mass
4 (FM) and lean mass (LM) with PF in 554 women aged 65.3-71.6 year belonging to OSTPRE-FPS
5 study. Participants filled a questionnaire on lifestyle factors and 3-day food record in 2002. Body
6 composition was measured by dual-energy X-ray absorptiometry and PF measures were performed
7 at baseline and at 3 year follow-up. Sarcopenia was defined using European Working Group on
8 Sarcopenia in Older People criteria. At the baseline women with higher protein intake (≥ 1.2 g/ kg
9 BW) had better performance in hand grip strength/body mass (GS/BM) ($P=0.001$), knee
10 extension/BM ($P=0.003$), one leg stance ($P=0.047$), chair rise ($P=0.043$), squat ($P=0.019$), squat to
11 the ground ($P=0.001$), faster walking speed 10 m ($P=0.005$) and higher short physical performance
12 battery score ($P=0.004$), than those with moderate and lower intakes (0.81-1.19 and ≤ 0.8 g/ kg
13 BW, respectively). In follow-up results, higher protein intake was associated with less decline in
14 GS/BM, one leg stance and tandem walk 6 m over 3years. Overall, results were no longer
15 significant after controlling for FM. Associations were detected between protein intake and PF in
16 non-sarcopenic women, but not in sarcopenic women except for change of GS ($P=0.037$). Further,
17 FM but not LM was negatively associated with PF measures ($P<0.050$). This study suggests that
18 higher protein intake and lower FM might be positively associated with PF in elderly women.

19 **Introduction**

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

29 Indeed, new evidence shows that adequate dietary protein is beneficial to support good health,
30 promote recovery from illness, and maintain LM in older adults ⁽⁷⁻¹¹⁾. It also has positive association
31 with MS and PF ⁽¹²⁻¹⁵⁾. However, the adequacy of current recommended dietary allowance (RDA)
32 ⁽¹⁶⁾ for protein 0.8 g/ kg per body weight (BW) has been questioned recently regarding that it might
33 not be enough to maintain the LM and prevent functional decline among elderly ^(5,17,18). To this end,
34 recent reviews and consensus statements have suggested that a protein intake between 1.0 and 1.5
35 g/kg/day may confer health benefits beyond those afforded by simply meeting the minimum ^(5,19). It
36 might be inappropriate also to generalize the protein intake requirements based on healthy young
37 men to older adults ⁽¹⁸⁾. PROT-AGE Study Group recommendations for dietary protein intake in
38 healthy older adults is an average in the range of 1.0 to 1.2 g/kg BW ⁽¹¹⁾. Further, Nordic nutrition
39 recommendation 2012 (NNR) for elderly also suggested protein intake in the range of 1.1-1.3 g/kg
40 BW (1.2 g/kg BW for planning purposes on population level) ⁽²⁰⁻²²⁾.

41 Ageing is accompanied with changes in body composition with a gradual increase in the proportion
42 of fat mass (FM) and decline in LM ⁽²³⁾. LM is the main reservoir of protein in human body and it
43 has a significant role in movement and posture, regulation of metabolism, and storage of energy and
44 nitrogen ⁽²⁴⁾. Previous studies supported the correlation between decreased LM and impaired
45 physical performance ⁽²⁵⁾. In a study by Pedrero et al. ⁽²⁶⁾ elderly men and women with sarcopenic
46 obesity showed lower physical fitness levels compared to non-sarcopenic subjects ⁽²⁷⁾. Notably,
47 older individuals have an attenuated muscle protein synthetic response after the ingestion of dietary
48 protein and amino acids. This resistance to the usually anabolic effect of protein on myofibrillar
49 protein synthesis (MPS) may partially contribute to the age-related decline in LM ⁽²⁸⁾. Because of
50 metabolic changes associated with ageing, elderly persons may produce less LM than younger

51 people from the same amount of ingested protein ⁽²⁹⁾. It is recommended, therefore, that in cases of
52 acute illness or psychological stress or sarcopenia higher protein intake is required ⁽³⁰⁾.

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

59 **Subjects and methods**

60 *Study design and participants*

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

77 *Body composition measurements*

78 Height and weight of participants were measured in light indoor clothing without shoes, body mass
79 index (BMI) was calculated by weight (kg) divided in height squared meter. FM and LM were
80 measured by dual-energy X-ray absorptiometry (DXA) by specially trained nurses. The DXA
81 measurements carried out using the same Lunar Prodigy adhering to the imaging and analysis
82 protocols provided by the manufacturer (Lunar Co., Madison, WI, USA) ⁽³²⁾. DXA is currently a

83 common tool suitable for estimation of body composition in terms of evaluating the ratio between
84 fat, muscle, and bone in different parts of the body⁽³³⁾. DXA also has been showed to be superior to
85 bioimpedance for estimation of the body composition⁽³⁴⁾.

86 *Physical performance measurements*

87 Physical performance measures were assessed by trained nurses at the baseline and at year 3,
88 consisting of three main domains: (1) MS: hand grip strength (GS) (kPa), number of chair rises in
89 30 seconds, ability to squat and ability to squat to the ground and knee extension (kPa). (2) Mobility
90 test: walking speed (WS) 10 m (m/s) and tandem walk for 6 m (m/s); and (3) Balance ability:
91 standing with closed eyes for 10 seconds and one leg stance performance for 30 seconds. GS was
92 measured in a controlled sitting position with a pneumatic hand-held dynamometer (Martin
93 Vigorimeter, Germany) by calculating the mean of three successive measurements from the
94 dominant hand. To standardize, GS and knee extension were further expressed as a ratio to body
95 mass (BM) (FM+LM) which have been suggested to be better predictor of GS and knee extension
96 alone^(35,36). The chair rise test was conducted if participant was able to stand at least once without
97 using arms from a straight-backed, non-padded, armless chair. Any measurement errors were
98 excluded from the statistical analysis⁽³⁷⁾. The follow-up variable of knee extension was excluded
99 from analysis due to unexpected increase in measured extension force and/or possible data entry
100 errors. Further, based on EWGSOP definition short physical performance battery (SPPB) score was
101 calculated using three individual measures of physical performance including WS 10 m (m/s), chair
102 rises in 30 seconds and one leg stance performance categorized in quartiles⁽³⁸⁾. Each quartile was
103 scored on scale of 1-4 points with the total score ranging to 12; higher scores of SPPB indicates
104 better performance. Further, absolute changes in PF and MS measures were calculated by
105 subtracting the baseline measures from those measured at year 3. The magnitude of meaningful
106 changes in physical performance measures as well as SPPB have been evaluated previously, and
107 these measures are consistently used as preferred indicators of physical performance in older adults
108^(2,38,39).

109 *Diagnosis of sarcopenia*

110 Relative skeletal muscle index (RSMI) was calculated as the sum of the nonfat, nonbone skeletal
111 muscle in arms and legs divided by the square of height (m²). Women were subdivided into
112 quartiles according to their RSMI values: (1) 5.3–6.3 kg/m², (2) 6.3–6.7 kg/m², (3) 6.7–7.2 kg/m²
113 and (4) 7.2–9.3 kg/m². Baumgartner et al.⁽²³⁾ reported that the sarcopenia cutoff point was 5.45
114 kg/m², which was calculated as two standard deviations below the mean in young reference
115 population. However, in our study there were only six women whose RSMI was less than 5.45

116 kg/m². Accordingly, we decided to use the lowest quartile below 6.3 kg/m² as cutoff in the present
117 study⁽³⁷⁾. The study population was divided into quartiles also for their GS: (1) < 22.3 kPa, (2)
118 22.3–25.7 kPa, (3) 25.7–28.7 kPa and (4) 28.7–40 kPa. Physical performance test was assessed by
119 measuring WS by a 10-meter-WS test in a controlled situation and the WS was divided into
120 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
121 were not able to walk were allocated into the group of the lowest quartile. A woman was classified
122 as sarcopenic if she belonged to the lowest quartile of RSMI and the lowest quartile of either GS or
123 WS or both. A non-sarcopenic woman did not belong to the lowest quartile of any measurement
124 (RSMI, GS or WS), whereas pre-sarcopenic women were in the lowest quartile of RSMI but not in
125 the lowest quartile of any other outcome measure. Non-classified women belonged to the lowest
126 quartile of either GS or WS or both, but not to that of RSMI.

127 *Dietary intakes*

128 Dietary intake was collected by using 3-day food record at the baseline. A questionnaire and
129 instructions were sent to the participants beforehand, and they were returned on the visiting day.
130 Participants were advised to fill the questionnaire for 3 consecutive days, including 2 days during
131 the week and one day in the weekend (Saturday or Sunday). Participants were instructed to write
132 down everything they ate and drank and to evaluate the amount of food consumed using household
133 measures. In case of uncertainties in the food record, a nutritionist called the participant for
134 additional information⁽⁴⁰⁾. To assess the underreporting the ratio of energy intake to estimated basal
135 metabolic rate was calculated based on BW according to equations given by Department of Health
136 in the UK⁽⁴¹⁾. The ratio of energy intake to basal metabolic rate cutoff value for under-reporting was
137 chosen to be 1.49, as derived from Goldberg et al.⁽⁴²⁾ and Black⁽⁴³⁾ and none of the participants was
138 excluded from the analyses. Nutritional intake from food was calculated using Nutrica program
139 (version 2.5, Finnish social insurance institute, Turku, Finland). Collected data provided
140 calculations of animal and plant sources of protein in addition to total protein intake.

141 *Potential confounders*

142 All lifestyle related information was gathered by the self-administered questionnaire. The
143 questionnaire included questions on age, hormone therapy use (never used or used), time since
144 menopause (year), smoking status (never, former and current), self-reported calcium and vitamin D
145 supplementation and alcohol consumption (portions/week). Total physical activity was based on
146 self-reported amounts of sports, recreation and miscellaneous activities, including walking, jogging,
147 skiing, cycling, swimming, aerobic exercise, ball sports and other more strenuous activities. Women
148 were asked how many days they performed each activity per month. The sum of each activity days

149 during all twelve months were summed and divided by 12 in order to obtain the mean activity level
150 per month. Furthermore, the mean activity level was multiplied by self-reported of strenuousness of
151 the exercise (the scale was 1 (low) to 4 (strenuous))⁽³⁷⁾.

152 *Statistical analysis*

153 Protein intake was reported as crude protein intake per BW (g/kg BW). Protein intake was
154 categorized based on three different nutrition recommendations, RDA⁽¹⁶⁾ (≤ 0.8 g/ kg BW), PROT-
155 AGE Study Group recommendation⁽¹¹⁾ (0.81 - 1.19 g/ kg BW), and NNR recommendation (≥ 1.2
156 g/ kg BW)⁽²⁰⁾. For the purpose of this study, these three categories were referred to as lower,
157 moderate and higher intake, respectively. Continuous variables were compared across the protein
158 intake categories using ANOVA and ANCOVA and categorical variables using chi-square tests.

159 Mean and SD of PF and MS measures at the baseline and absolute changes in them were tested in
160 the ANCOVA across the categories of protein intake. Multiple linear regression or logistic
161 regression models were used to calculate (β) and 95% CI of PF and MS measures at the baseline
162 and changes in them across categories of protein intake. Tests for a linear trend across categories of
163 protein intake were conducted by using the median value in each category as a continuous variable
164 in the linear and logistic regression models. Pair wise comparisons of the group means were
165 performed with Tukey's post hoc test. Linear and logistic regression analyses evaluated the
166 association of FM and LM with PF and MS measures at the baseline and over 3-year follow-up. We
167 examined further the association of protein intake g/kg BW with PF measures at baseline and over
168 3-year follow-up according to sarcopenia status. To achieve balanced numbers of participants in the
169 stratified analysis and to evaluate our secondary hypothesis, women were classified as sarcopenic if
170 they belonged to pre-sarcopenia, sarcopenia and severe sarcopenia (lowest quartile of RSMI) and
171 non-sarcopenic group was compiled from normal and non-classified groups (normal RSMI).

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

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

186 All statistical analysis were executed using SPSS software version 21 for Windows (IBM Corp.,
 187 Armonk, NY). Result was considered significant if a P value was < 0.05.

188 **Results**

189 The participants were 65.3- 71.6 years old (mean (\pm SD) age was 68 ± 1.9), and mean energy intake
 190 was 6560 ± 1556 kJ/d (Table 1). Total protein intake was 68.2 g/d which constituted to 17% of total
 191 energy intake and corresponded to 0.96 g/ kg BW. The minimum protein intake reported was 0.24
 192 g/kg BW and the maximum 2.25 g/kg BW. Also, 30% of women had protein intake ≤ 0.8 g/ kg
 193 BW, 48 % were in the moderate range of 0.8-1.19 g/ kg BW, while 22% consumed protein ≥ 1.2 g/
 194 kg BW. Higher protein intake was significantly associated with higher energy **intake and lower**
 195 **carbohydrate intake as % of energy, but higher carbohydrate intake as g/d.**

196 In total, 8 % of women had osteoporosis, 42% had hypertension, 3 % had diabetes, 6% had
 197 rheumatoid arthritis, 3 % had depression, 12% had hip arthrosis, 28 % had knee arthrosis and 21.8
 198 % reported fall accident in past 12 months. However, no significant associations between reported
 199 diseases and protein intake g/kg BW were observed. Mean duration of hormone therapy was 11
 200 years and time passed after menopause was 18 years. Women with higher protein intake reported
 201 more **frequent** use of hormone therapy, weighed less and had lower BMI as compared to moderate
 202 and lower intake. Among body composition measurements FM, LM and LM index were
 203 significantly lower in higher protein intake. **Women in higher protein intake had significantly higher**
 204 **RSMI than the lower protein intake group.**

205 In the Table 2 differences of baseline characteristics between non-sarcopenic and sarcopenic
 206 participants are presented. Sarcopenic group (n = 127) had significantly lower mean weight (-
 207 13.2%), BMI (-12.7%), FM (-16.0%) and LM (-12.0%) as compared to non-sarcopenic group (n
 208 =369). Average protein intake was similar in sarcopenic and non-sarcopenic group, 17.6 ± 2.9 %
 209 and 17.9 ± 3.1 % of energy, respectively.

210 Significant differences in physical performance measures between women with higher protein
 211 intake and those with lower protein intake at the baseline and over 3-year follow-up were detected
 212 (Table 3). At the baseline after adjustment for selected factors previously described as associated
 213 with physical performance (model 2) those with higher protein intake as compared to those with

214 moderate and lower intake had greater GS/BM ($P = 0.001$), knee extension/BM ($P = 0.003$), longer
 215 one leg stance performance ($P = 0.047$), better chair rise performance ($P = 0.043$), faster WS 10m
 216 pace ($P = 0.005$), squat completion ($P = 0.019$) and squat to the ground completion ($P = 0.001$),
 217 and higher SPPB score ($P = 0.004$). Overall results were no longer significant after controlling for
 218 FM (model 3). Results for the prospective analysis showed that those with higher protein intake had
 219 less decline in GS/BM ($P = 0.027$), one leg stance performance duration ($P = 0.024$) and had
 220 increased tandem walk speed ($P = 0.024$), which were no longer significant after controlling for
 221 FM.

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

226 Further, we examined the association of protein intake with physical performance measures
 227 according to sarcopenia status (Table 4). Results of model 2 showed that among non-sarcopenic
 228 women protein intake was positively associated with GS/BM ($\beta = 0.35$ and $P = 0.001$), knee
 229 extension/BM ($\beta = 0.25$ and $P = 0.008$), one leg stance performance ($\beta = 0.26$ and $P = 0.001$), chair
 230 rises ($\beta = 0.15$ and $P = 0.039$), WS 10 m ($\beta = 0.30$ and $P < 0.001$), ability to squat ($\beta = 0.18$ and $P =$
 231 0.003), squat to the ground ($\beta = 0.29$ and $P = 0.001$) and also with SPPB score ($\beta = 0.32$ and $P <$
 232 0.001) at the baseline. However, significant associations were lost after controlling for FM. Results
 233 of the prospective analysis indicated that higher protein intake in non-sarcopenic women was in
 234 positive relationship with changes of one leg stance performance ($\beta = 0.14$ and $P = 0.037$) and
 235 standing with eyes closed ($\beta = 0.23$ and $P = 0.001$). No significant associations between protein
 236 intake and physical performance measures were observed among sarcopenic women, except for
 237 GS/BM change ($\beta = 0.23$ and $P = 0.037$) and a non-significant relation with chair rise change ($\beta =$
 238 0.27 and $P = 0.064$), which were lost after controlling for selected confounders and FM.

239 The associations between total body FM and LM with physical performance measures and changes
 240 in them are shown in Table 5. After adjustment for LM and factors previously described as
 241 associated with physical performance, FM was negatively correlated with GS/BM, GS, knee
 242 extension/BM (only at the baseline), one leg stance, chair rises, WS 10m, squat, squat to the ground
 243 and SPPB score at the baseline and over 3-year follow-up ($\beta \geq -0.07$ and $P \leq 0.050$). FM was also
 244 negatively associated with change of standing with closed eyes 10 seconds ($\beta = -0.22$ and $P <$
 245 0.001). Further, LM was positively associated with GS, knee extension and one leg stance

246 performance at the baseline as well as with GS changes over 3- year follow-up ($\beta \geq 0.06$ and $P \leq$
247 0.025). Results remained significant after controlling for FM.

248 **Discussion**

249 This study examined cross-sectional and prospective associations of protein intake (g/kg BW) and
250 body composition (FM and LM) with different PF and MS measures in 554 elderly women
251 belonging to the OSTPRE-FPS study. Associations of protein intake with PF and MS were also
252 evaluated according to sarcopenia status. However, the significant associations were lost in the final
253 models due to high collinearity of FM with physical performance. Our findings supported the
254 hypothesis that higher protein intake than the current RDA (0.8 g/ kg BW), might be associated
255 with better PF and MS among elderly women. Further, present study showed that the total body FM
256 was negatively associated with physical performance tests, while total body LM was positively
257 associated with GS, knee extension and one leg stance.

258 In recent years, there has been increased support for the contention that the current daily allowance
259 (0.8 g/kg BW) for protein is insufficient to promote optimal health and preserve physical
260 performance in the elderly^(5,12,13,18,45-47). Consistently, in our cross-sectional findings, those women
261 with higher protein intake performed better in many of the physical performance measures as
262 compared to those who had moderate and lower protein intakes. The higher protein intake category
263 had greater GS/BM, knee extension/BM, longer one leg stance, better chair rises performance,
264 faster WS 10 m, better squat and squat to the ground ability, and higher SPPB score. The
265 prospective results showed also that women in higher protein intake group had less decline in
266 GS/BM and one leg stance performance, and had the highest increased chair rises performance over
267 3-year follow-up. No significant differences were observed between protein intake categories and
268 WS 10 m and tandem walk speed 6 m prospectively. Thus, it might be that higher protein intake
269 (g/kg BW) can be more related to preserving MS rather than mobility, which may partially explain
270 the protein-frailty association. However, these associations were no longer significant after
271 adjustment for FM.

272 Findings of study by Gregorio et al.⁽¹³⁾ among 387 healthy women aged 60 to 90 years, showed that
273 those in the lower protein intake < 0.8 g/ kg BW category performed less well in the single leg
274 stance test than those in the higher protein intake ≥ 0.8 g/ kg BW category. They also walked eight
275 feet at a slower pace and their SPPB score was lower than in women in the higher protein category.
276 Further, Lemieux et al.⁽⁴⁵⁾ indicated that among 72 postmenopausal women, higher protein intake \geq
277 1.2 g/kg BW was positively correlated to GS and knee extension. Women's Health Initiative

278 clinical and observational study⁽¹²⁾, was conducted in 134961 participants, aged 50 to 79 years for
279 average 7 years of follow-up. Results showed that mean GS at baseline was slightly higher among
280 women with higher calibrated daily protein intake (using urinary nitrogen protocol to estimate
281 protein consumption over 24-h period), and these women experienced smaller decline in GS over
282 time than those with low calibrated protein intake. Additionally, women in the highest quintile of
283 calibrated protein intake completed on average 0.5 more chair rise at baseline than women in the
284 lowest quintile. In contrast, there was no significant association between calibrated protein intake
285 and the timed 6-meter walk in either cross-sectional or prospective analyses. Furthermore, the same
286 results were shown when protein intake was expressed as g/kg BW.

287 A new finding was that among non-sarcopenic women at the baseline, protein intake (g/kg BW)
288 was in positive relationship with GS/BM, knee extension/BM, one leg stance ability, chair rises
289 performance, WS 10m, ability to squat and squat to the ground and SPPB. Protein intake in these
290 women was also associated with preserving physical performance over 3 years follow-up, including
291 one leg stance and standing with eyes closed 10s. No such an association was observed in
292 sarcopenic women except a positive relationship between protein intake and GS change. Thus
293 consistent to our hypothesis the positive association of protein intake (g/kg BW) with PF was more
294 pronounced in non-sarcopenic than in sarcopenic women. It has been suggested that older
295 individuals suffering from illness, physiological stress or sarcopenia are required to consume higher
296 protein intake (1.2-1.5 g/ kg BW) as compared to healthy older people (1-1.2 g/ kg BW)⁽³⁰⁾.
297 However, we could not explore this due to the threshold of protein intake in this data between
298 sarcopenic and non-sarcopenic women.

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

308 Declines in LM might predict a reduction in muscle force and performance^(1,48). It has also been
309 shown that FM is associated with functional decline and muscle weakness in elderly individuals
310^(35,44,53). In this study, total body FM was in strong negative correlation with all PF and MS

311 measures at the baseline and changes in them at 3 years except for knee extension, tandem walk and
312 standing with eyes closed at the baseline; while LM was positively correlated with GS and change
313 in it, knee extension and one leg stance. Therefore, these findings accompanied with the loss of
314 significant associations between protein intake and physical performance measures after controlling
315 for FM but not LM, suggest that FM and LM may have opposite association with PF and MS in
316 elderly women. There are different pathways through which fatness might be related to LM and
317 muscle strength ⁽⁵⁴⁾. However, more studies are needed to disentangle the relationship between FM
318 and physical performance.

319 It is well known that adequate energy intake is required to optimally utilize dietary protein to
320 maintain physical performance rather than as energy source ⁽¹³⁾. It was to our surprise that those
321 with higher energy and protein intake had a lower weight. The actual cause is uncertain but this
322 might be due to higher physical activity level in higher protein category, and also possible
323 underreporting of total energy and fat intake in those with higher BMI ⁽⁵⁵⁾. Worthy of note is that
324 LM index (LM/ height (m²)) and RSMI are both used as indicators of muscle mass in the diagnosis
325 of sarcopenia ⁽²⁾. However, in this study protein intake showed the same association with LM index
326 and RSMI, thus we used RSMI as clinical indicator of sarcopenia as adapted by EWGSOP ⁽²⁾.

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

338 An additional analyses in the present data showed no significant effect of vitamin D (800 IU) and
339 calcium supplementation (1000 mg) on MS and PF and longitudinal analysis were controlled for
340 study group receiving those. The availability of multiple standardized physical performance
341 measures at baseline as well as over a 3-year period added significant strength to our study.
342 Dynamometric measures of GS as a physical marker of lower limb strength and knee extension for
343 a variety of functional tasks, such as walking, chair rising and stair climbing, particularly are

344 predominate for the quantification of physical performance in older adults ^(36,58). The introduced
345 protein intake categories in present study took into account the newer intake recommendations for
346 elderly, which have not been used in the previous studies.

347 **Conclusion**

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

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356 **Conflict of interests**

357 The authors have no relevant interests to declare.

358 **Author Contribution**

359 H. Kröger and M. Tuppurainen designed the original OSTPRE-FPS study. M. Isanejad, A. Erkkilä,
360 J. Sirola and J. Mursu. planned the present study together and collaborated on drafting the
361 manuscript. M. Isanejad carried out the statistical analysis, and summarized the results in tables. H.
362 Kröger, M. Tuppurainen and T. Rikkinen critically revised the manuscript for important intellectual
363 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)		<i>P</i> *
	Mean	SD	Mean	SD	Mean	SD	
Demographic							
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)		<i>P</i> *
	Mean	SD	Mean	SD	
Demographic					
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)		<i>P</i> _{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.

Physical performance measures	Non-sarcopenic (n=369)				Sarcopenic (n=127)			
	regression coefficient (95% CI)	<i>P</i> Model 1*	<i>P</i> Model 2‡	<i>P</i> Model 3†	regression coefficient (95% CI)	<i>P</i> Model 1*	<i>P</i> Model 2‡	<i>P</i> Model 3†
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	<i>P</i> Model 1*	<i>P</i> Model 2†	β	SE	<i>P</i> Model 1	<i>P</i> 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.