Interaction of recommended levels of physical activity and protein intake is associated with greater physical function and lower fat mass in older women: Kuopio Osteoporosis Risk Factor- (OSTPRE) and Fracture-Prevention Study

Samu Sjöblom1*, Joonas Sirola1,2, Toni Rikkonen1, Arja T. Erkkilä3, Heikki Kröger1,2, Sarang L. Qazi1 and Masoud Isanejad1,4*

1Kuopio Musculoskeletal Research Unit, University of Eastern Finland, Kuopio, Finland
2Department of Orthopedics and Traumatology, Kuopio University Hospital, Kuopio, Finland
3Institute of Public Health and Clinical Nutrition, University of Eastern Finland, Kuopio, Finland
4Department of Musculoskeletal Biology, Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool, UK

(Submitted 20 March 2019 – Final revision received 8 December 2019 – Accepted 20 December 2019 – First published online 8 January 2020)

Abstract

The aim of the study was to investigate whether the interaction of physical activity (PA) and protein intake is associated with physical function (PF). The women from the Osteoporosis Risk Factor and Fracture Prevention Study (n 610) completed a questionnaire on lifestyle factors and PA and underwent PF and body composition measurements at baseline (BL) and over 3 years of follow-up (3y-FU). PA was categorised according to WHO cut-off PA = 0, 0 < PA < 2·5 and PA ≥ 2·5 h/week. Protein intake was calculated from the 3-d food record at baseline and categorised according to the Nordic Nutrition Recommendations <1·1 and ≥1·1 g/kg body weight (BW). The results showed in univariate ANOVA at the baseline and at the 3-year follow-up, women with high PA ≥ 2·5 h/week and protein intake ≥ 1·1 g/kg BW had higher grip strength adjusted for BMI, higher mean number of chair rises, faster mean walking speed, higher modified mean short physical performance battery score and lower mean fat mass compared with other interaction groups. High PA and protein intake were associated with lower BMI despite significantly higher energy intake. In conclusion, higher PA and protein intake interaction was associated with greater PF and lower fat mass, but the association with relative skeletal muscle index and muscle mass was inconclusive. The present study gives noteworthy information for preventing sarcopenia.

Key words: Nutrition: Protein intake: Physical activity: Skeletal muscle: Sarcopenia

Ageing is accompanied with loss of muscle mass and muscle strength, which results diminished physical function (PF) and debilitating consequences such as fall and mortality in older people(1). The exact mechanism of PF decline in older people is yet to be investigated. In this context, identifying the most effective intervention strategy to preserve PF in older people has been of high public health interest(2,3).

Among the most important health behaviours are those that involve physical activity (PA) and diet to preserve muscle mass and PF in older life(4–6). Physical inactivity is linked to the loss of PF and muscle mass in older people(7). Physical inactivity is linked to the loss of PF and muscle mass in older people(7). Studies have shown that alterations in PF and muscle fibres in older adults remain responsive to functional demands such as physical exercise(8). Therefore, it has been suggested that regular PA can partially prevent progression of the loss of PF and muscle mass related to inactivity(9,10). The influence of PA on muscle has been described in relation to several of the factors acting on muscle in age-related imbalance processes, and exercise may up-regulate the metabolism of muscle synthesis(10).

In addition, a growing body of evidence indicates that increased dietary protein intake in the older population may prevent the loss of PF and muscle mass(11–13). Furthermore, increased dietary protein intake can enhance the effects of PA on functional ability(14,15). In a study among adults, functional tasks that benefitted most from a higher-protein diet (≥1·2 v. <0·8 g/kg per d) were doing heavy work at home (e.g. shoveling, or washing windows, walls or floors), walking half a mile, going up and down stairs, stooping/kneeling/crouching and lifting heavy items(16). The exact mechanism is unclear, but it

Abbreviations: BW, body weight; FM, fat mass; NNR, Nordic Nutrition Recommendation; OSTPRE-FPS, Osteoporosis Risk Factor and Fracture Prevention Study; PA, physical activity; PF, physical function; RCT, randomised controlled trial; RSMI, relative skeletal muscle index; SPPB, short physical performance battery; UNIANOVA, univariate ANOVA.

* Corresponding authors: Masoud Isanejad, email masoud.isanejad@liverpool.ac.uk; Samu Sjöblom, email samus@uef.fi
has been suggested that exercise-induced improvement of protein synthesis may be due to nutrient-stimulated vasodilation and nutrient delivery to muscle\(^\text{(17)}\). Although the interaction effect of PA and protein intake may prevent the decline of muscle mass and PF in older people, observational studies are scarce\(^\text{(18)}\).

Most previous studies have evaluated the association of PA and protein intake using population cut-offs (\(\text{ntiles}\)) or as a continuous variable. Although this approach is warranted, formulating PA and protein intake recommendations for older people, to preserve PF and muscle mass based on the results, is somewhat challenging. The WHO has recommended that adults aged 65 years and older ‘should do at least 150 min of moderate-intensity aerobic PA throughout the week to improve cardiorespiratory and muscular fitness, bone and functional health, reduce the risk of non-communicable disease, depression and cognitive decline’\(^\text{(19)}\). Furthermore, the Nordic Nutrition Recommendations (NNR 2012) outline a protein intake in the range of 1.1–1.3 g/kg BW (minimum 1.1 g/kg BW) to preserve PF in older adults\(^\text{(19)}\). However, data from observational studies using such recommendations for PA and protein intake in older adults are scarce.

In the present study, we investigated the association of PA independently and in an interaction with protein intake in relation to PF and muscle mass in older postmenopausal women. This approach provided an opportunity to study PA according to the WHO cut-off and protein intake according to the NNR 2012 recommendations.

### Materials and methods

#### Study population

Data for the present study were collected from the Osteoporosis Risk Factor and Prevention – Fracture Prevention Study (OSTPRE-FPS), which began in 2003 in Kuopio, Finland\(^\text{(20)}\). OSTPRE-FPS was a randomised population-based double-blinded controlled trial with a 3-year follow-up involving 3432 women (aged 66–71 years). The primary aim of the study was to determine whether vitamin D and Ca supplementation would be effective in preventing falls and fractures in postmenopausal women.

The power analysis was performed based on the incidence of fractures\(^\text{(20)}\). A subsample of 750 women was randomly selected at the baseline (the supplementation and control groups both had 375 subjects) and underwent body composition, clinical, physical and laboratory tests. There was no \textit{a priori} power analysis to calculate the size of the subsample of 750 women randomly selected from the 3432 women at the baseline.

The baseline measurements took place between February 2003 and May 2004 and the follow-up measurements between January 2006 and May 2007 (follow-up time 2.8 (sd 0.4) years)\(^\text{(21)}\). Between the randomisation and the actual start of the intervention, 237 subjects withdrew and ten women stopped participating in the study for various reasons during the 3-year follow-up period. At the end of the trial (n 593), 306 and 287 subjects in the intervention and control groups of the subsample, respectively, completed the follow-up. The present study was a \textit{post hoc} analysis of the subjects from the OSTPRE-FPS study. The final analytical data set for the present study comprised 608 women who had baseline and follow-up data for the self-administered questionnaire regarding PA. However, the 3-d dietary food record was only available at the baseline for 554 women (data for fifty-four food records were missing due to not returning the food record or returning an incomplete one, which resulted in missing values).

All clinical measurements were performed in the Kuopio Musculoskeletal Research Unit of the Clinical Research Centre 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.

#### Questionnaires

The OSTPRE-FPS baseline questionnaire in 2003–2004 contained questions on income per month (euros), age at menopause (years), chronic diseases and years of hormone therapy. In addition, questions about current smoking and tobacco use (no/yes), previous falls, prescribed medications and use of self-reported vitamin D and Ca supplements were asked.

#### Body composition measurements

Total body dual-energy X-ray absorptiometry was used by specially trained nurses to measure muscle mass and fat mass (FM) in 2003–2007. The dual-energy X-ray absorptiometry measurements were carried out using the same Lunar Prodigy, adhering to the imaging and analysis protocols provided by the manufacturer (Lunar Co.)\(^\text{(22)}\). The height and weight of participants were measured in light indoor clothing without shoes at the baseline and at the 3-year follow-up, and BMI was calculated as kg/m\(^2\). Appendicular lean mass is the sum of lean mass in arms and legs, whereas the relative skeletal muscle index (RSMI) was calculated as appendicular lean mass divided by height squared (kg/m\(^2\)).

#### Physical function measurements

The detailed explanation of PF measurements in these data has been published previously\(^\text{(22,24)}\). PF measures were assessed by trained nurses at the baseline and at the 3-year follow-up session, including hand grip strength (kg), number of chair rises in 30 s, ability to squat, knee extension (Newtons but converted into kg), walking speed 10 m (m/s), tandem walk for 6 m (m/s), standing with closed eyes for 10 s and one leg stance performance for 30 s. The follow-up variable of knee extension was excluded from the analysis because of an unexpected increase in measured extension force and/or possible data entry errors, which could not be traced due to the long period between this secondary analysis and the time data recorded in 2001–2003. This issue was explained in these data\(^\text{(24)}\). Grip strength (kg) was measured using the dominant hand while sitting on a bench, with the forearm flexed from the elbow at a 90-degree angle, near the torso. A total of three attempts were recorded, with approximately 30 s of resting time between the tests. Close attention was paid to making all three attempts in a similar, fixed posture (JAMAR® hand-grip dynamometer; Sammons Preston), and then mean grip...
Examples that have been stated by the WHO and NNR include climbing stairs, walking 4.8 km/h, snow clearing, lawn mowing, dancing, gardening, hiking and swimming.

**Dietary and protein intake**

Dietary intake was assessed using a 3-d food record at the baseline. A questionnaire and instructions were sent to participants beforehand, and they were returned at the baseline visit. Participants were instructed to record their diet and everything they ate and drank, and to evaluate the amount of food using household measures for 3 consecutive days, with 2 d during the week and 1 d during the weekend (Saturday or Sunday)\(^8\). In the case of uncertainties in the food record, a nutritionist called the participant for more information. Nutrient intakes were calculated using Nutrica dietary analysis software (version 2.5, Finnish Social Insurance Institute), based on the national database of the Finnish Social Insurance Institution. Assessment of underreporting has previously been described, and no participants were excluded due to low energy intake\(^9\). Total protein intake was calculated first as g/d and was then further expressed as g/kg BW. In these data, we previously introduced the cut-offs for protein intake according to NNR 2012\(^19\). In the present study, accordingly, women were categorised by protein intake (g/kg BW) according to NNR 2012 (<1.1 \(\text{v} < 1.1 \frac{\text{g}}{\text{kg BW}}\)).

**Confounders**

Data regarding lifestyle were self-reported at the baseline. The variables of interest included income per month (euros), marital status (married, divorced, widowed and not married), smoking status (never, past and current), medical history (diseases and surgeries), medications (including hormone therapy) and time since menopause\(^23\). Number of chronic diseases, including hypertension, hyperlipidaemia, CHD, diabetes, arthritis, osteoporosis, depression, chronic kidney disease and cancer were reported. Self-ambulatory status was defined as normal if women were (a) fully capable of moving, (b) capable of moving but unable to run or (c) capable of walking 1 km at the most. The status was defined as restricted when women were (a) capable of walking 100 m at the most, (b) moving only indoors or (c) incapable of moving.

**Statistical analysis**

All statistical analyses were executed using SPSS software version 25 for Windows (IBM Corp.). All tests were two-sided, and a \(P\) value of <0.05 was considered significant. To account for the possible intervention effect, we conducted preliminary analyses and there was neither a significant effect on the outcome of interests nor an interaction with PA and protein intake. Thus, data were pooled for the total population (intervention and control group) in year 3 of the follow-up. However, follow-up analyses were further adjusted for the randomised controlled trials (RCT) study group (Ca and vitamin D intervention). Pearson’s correlation coefficient \(r\) was calculated for the relationships between PA, protein intake, the interaction between these variables and PF measures. The PF measures \(r^2\) accounted for by the three independent variables (i.e. PA, protein intake and...
PA interaction with protein intake) were calculated using multiple regression analysis (presented in online Supplementary Table S1). This computation provided an estimate of the respective contribution of the three independent variables to the PF measures.

Baseline characteristics were tested using ANOVA for continuous variables and \( \chi^2 \) test for categorical variables, according to PA groups (0, 0 < PA < 2.5, and PA ≥ 2.5 h/week) stratified for protein intake cut-off (<1.1 and ≥1.1 g/kg BW). For the main analysis, PA was introduced to two main models, first as a categorical variable (PF and muscle mass) based on the values of multivariate regression, which is an extension of simple linear regression. This model could predict the value of the outcome variable (PF and muscle mass) based on the values of multiple independent variables, including independent variables (PA alone and in interaction with protein intake) and covariates\(^{(27)}\). The outcome variables were PF, total body lean mass, RSMI and total body FM at baseline, at 3 years, and for their absolute changes over 3 years of follow-up.

**Interaction analysis of physical activity and protein intake**

An interaction term was introduced by multiplying the continuous variables of PA h/week and protein intake g/kg BW, and this was used as an independent variable in multiple regression analyses, with multiple PF measures and muscle mass as dependent variables. The interaction was statistically significant \((P < 0.040)\). For interaction analysis, we merged the two groups of PA = 0 and 0 < PA < 2.5 h/week into one, as PA < 2.5, to provide a balanced number of subjects in each group, because the initial descriptive analysis showed only four women belonging to the PA = 0 and protein ≥1.1 g/kg BW group. The final four interaction groups were: (a) PA < 2.5 and protein intake <1.1 g/kg BW, (b) PA < 2.5 and protein intake ≥1.1 g/kg BW, (c) PA ≥ 2.5 h/week and protein intake <1.1 g/kg BW and (d) PA ≥ 2.5 h/week and protein intake ≥1.1 g/kg BW. Interaction between continuous PA at 1-h intervals and protein intake was significant in relation to PF, and PF measures \((P > 0.75, P < 0.040)\), except for the ability to squat to the ground and tandem walk speed.

UNIANOVA was used to calculate mean values and standard deviations (PA and protein intake interaction as a categorical variable), and multiple regression analysis was used to calculate the standard \( \beta \)-coefficient and SE (PA and protein intake interaction as a continuous variable) in the models, where PF measures, total body LM, total body FM and RSMI were set as dependent variables.

The final analytical sample for interaction analysis was performed for 554 women because there were fifty-four (total number = 608) women without a dietary food record and they could not be included in the interaction analysis. However, our prior analysis showed no significant differences in the baseline characteristics variables (presented in Table 1) between

| Table 1. Baseline characteristics and dietary factors of the participants according to physical activity (PA) (h/week) groups* (Mean values and standard deviations; numbers and percentages) |
|---------------------------------------------------------------|
| **Inactive: PA = 0 h/week** | **Insufficient: 0 < PA < 2.5 h/week** | **Sufficient: PA ≥ 2.5 h/week** |
| (n = 77) | (n = 166) | (n = 365) |
| PA (h/week) | 0 | 0 | 1.2 | 0.69 | 6.1 | 4.7 | 0.0001 |
| PA range (h/week) | 0–0 | 0 | 1.1–1.3 | 0.69 | 6.1 | 4.7 | 0.0001 |
| Age (years) | 67.7 | 1.9 | 68.0 | 1.8 | 67.7 | 1.8 | 0.415 |
| BMI (kg/m^2) | 29.0 | 4.7 | 27.9 | 4.5 | 26.8 | 3.8 | 0.0001 |
| Time since menopause (years) | 19.4 | 7.4 | 18.6 | 5.2 | 18.4 | 5.4 | 0.364 |
| Income (euros/month) | 787 | 269 | 845 | 308 | 880 | 294 | 0.173 |
| Number of chronic diseases | 1.4 | 3.0 | 1.5 | 1.2 | 1.4 | 1.1 | 0.074 |
| Mobility status | | | | | | | 0.211 |
| Normal | | | | | | | | |
| n | 40 | 136 | 324 | | | | |
| % | 80.0 | 88.9 | 96.1 | | | | |
| Restricted | | | | | | | | |
| n | 10 | 17 | 13 | | | | |
| % | 20.0 | 11.1 | 3.9 | | | | |
| Current smoker | | | | | | | 0.596 |
| n | 2 | 10 | 14 | | | | |
| % | 3.8 | 6.5 | 4.2 | | | | |
| Current hormone therapy | | | | | | | 0.411 |
| n | 8 | 35 | 80 | | | | |
| % | 15.1 | 22.6 | 23.3 | | | | |
| Dietary factors and food groups | | | | | | | | |
| Energy intake (kJ) | 5816 | 1494 | 6552 | 1498 | 6665 | 1556 | 0.001 |
| Alcohol (g/d) | 6.0 | 4.2 | 9.0 | 4.6 | 10.0 | 3.7 | 0.111 |
| Protein (g/d) | 59.7 | 17.9 | 68.1 | 16.8 | 69.2 | 18.3 | 0.001 |
| Protein (g/kg body weight) | 0.78 | 0.25 | 0.95 | 0.25 | 0.99 | 0.27 | <0.0001 |
| Carbohydrate (g/d) | 174.6 | 47.6 | 192.7 | 45.9 | 196.1 | 49.0 | 0.007 |
| Fat (g/d) | 47.2 | 16.4 | 56.4 | 18.7 | 17.9 | 0.96 | 0.019 |

*ANOVA was used to calculate means and standard deviations, and \( \chi^2 \) tests for categorical variables to calculate n and percentages according to PA groups. \( P \)-values are significant two-tailed.
those that were and were not included in the interaction analyses (data not shown).

**Covariates and adjustment for analytical models**

We initially assessed known covariates of sarcopenia based on the literature. Furthermore, covariates were selected based on their multicollinearity and the predictive values alone, which led to the selection of the following models. For both multiple regression analysis and UNIANOVA, the first model was adjusted for age and energy intake (kJ). The second model was adjusted for the variables in model 1 plus smoking (yes, no), hormone therapy (yes, no), rheumatoid arthritis, baseline height (m), income per month (euros) and intensity of PA. Follow-up analyses were adjusted for changes in PF variables and RCT study group (intervention with Ca and vitamin D supplementation).

**Results**

Table 1 shows the baseline characteristics according to PA groups. PA was reported to be equal or higher than 2.5 h/week in 365 women. Those with PA ≥ 2.5 h/week had significantly lower BMI and FM. Energy and protein intake were both higher in women with higher PA. The baseline characteristics according to the PA and protein intake interaction groups are reported in Table 2. High PA and protein intake were associated with lower BMI and higher energy intake. Half of the women received Ca (1000 mg) and vitamin D (17.5 μg) during the 3-year follow-up. Results showed no significant effect of vitamin D and Ca supplementation on PF or muscle mass between the intervention and control group.

Univariate analysis indicated that, for PF and muscle mass measures, the most significant correlation was with PA and the protein intake interaction value (online Supplementary Table S1). PA was not associated with lean mass and RSMI, whereas protein intake was inversely associated with lean mass and RSMI.

**Physical activity and physical function**

In cross-sectional analysis, continuous PA at 1-h intervals was positively associated with higher number of chair rises (standardised β 0.248, \( P = 0.040 \)), walking speed (standardised β 0.107, \( P = 0.005 \)), modified SPPB (standardised β 0.118, \( P = 0.022 \)), ability to squat (standardised β 0.185, \( P = 0.001 \)) and lower FM (standardised β −0.196, \( P < 0.001 \)). Similarly, the cross-sectional analysis results of UNIANOVA showed that the means for walking speed, standing on one leg and modified SPPB were significantly higher in the high PA group as a categorical variable compared with the low PA group after adjusting for confounders. At the 3-year follow-up, higher continuous PA (1-h intervals and PA ≥ 2.5 h/week) was significantly associated with walking speed, ability to squat and lower FM (\( P < 0.048 \)) (Table 3).

**Physical activity and protein intake interaction**

At the baseline, after adjusting for the confounders in regression analysis, continuous PA at 1-h intervals and protein intake interaction were associated with higher grip strength adjusted for BMI (β 0.140 and \( P = 0.001 \)), faster walking speed over 10 m (β 0.205 and \( P < 0.001 \)), longer standing on one leg for a maximum of 30 s (β 0.106 and \( P = 0.014 \)), more frequent ability to squat (β 0.118 and \( P = 0.050 \)), higher modified SPPB score (β 0.177, \( se = 0.003 \) and \( P < 0.001 \)) and lower FM (β −0.206, and \( P < 0.001 \)) (Table 4). Follow-up analysis further showed that continuous PA at 1-h intervals and protein intake interaction were associated with higher grip strength adjusted for BMI (β 0.087, \( se = 0.003 \) and \( P = 0.046 \)), increased repetitions of higher number of chair rises (β 0.190, \( se = 0.004 \) and \( P = 0.001 \)), faster walking speed over 10 m (β 0.143 and \( P = 0.002 \)), more frequent ability to squat (β 0.105 and \( P = 0.022 \)), higher modified SPPB score (β 0.089 and \( P = 0.043 \)) and lower FM (β −0.198 and \( P < 0.001 \)). The only significant association of PA and protein intake interaction as a continuous variable was with FM change (β −0.095 and \( P = 0.040 \)).

Further, in UNIANOVA at the baseline and at the 3-year follow-up, women with high PA ≥ 2.5 h/week and protein intake ≥ 1.1 g/kg BW had higher grip strength adjusted for BMI mean (1-1 (SD 0-2)), higher mean number of chair rises, faster mean walking speed, higher modified mean SPPB score and lower mean FM (Table 4, and Fig. 1) compared with the other interaction groups.

Women with highest PA and protein intake had significantly greater RSMI at the baseline (\( P < 0.001 \)). To the contrary, at 3 years of follow-up, highest value for RSMI and LM was detected for women with lowest PA and protein intake (\( P < 0.001 \)). When using continuous variables for PA and protein intake, the association with LM and RSMI was not significant for baseline or at 3 years of follow-up.

**Discussion**

The present study provides an opportunity to assess the interaction of PA according to the WHO recommendation and protein intake according to NNR with muscle mass and PF. The foregoing analysis indicates that the interaction of PA 2.5 h/week with protein intake ≥ 1.1 g/kg BW was positively associated with PF at most measured sites and lower FM in older women. The results remained significant after adjusting for a number of relevant confounders. Significant associations in the prospective analyses remained at 3-years follow-up with walking speed, squat ability and lower FM, possibly due to small changes in the outcome measures over a relatively short follow-up (3 years).

The interaction of PA and protein intake was not clearly associated with RSMI and lean mass. Previous epidemiological studies have shown that quadriceps, grip strength, ability to squat and walking speed have been determined as significant predictors of mortality 28–30, and ability to squat and walking speed were associated with higher risk of fracture 31.

There might be several mechanisms underlying the decline in PF in older people. Reduced strength with age may be a result of a combination of loss of muscle mass and neural control 32. Ageing muscles are susceptible to reduced number of motor neurons which are mainly responsible of generating muscle strength 33. In addition, muscle strength and PF in older people
Table 2. Baseline characteristics and dietary factors of the participants according to physical activity (PA) (h/week) groups stratified for protein intake cut-off* (Mean values and standard deviations; numbers and percentages)

| Protein intake < 1·1 g/kg body weight | Protein intake ≥ 1·1 g/kg body weight |
|--------------------------------------|--------------------------------------|
|                                      | PA = 0 h/week (n 45) | 0 < PA < 2·5 h/week (n 111) | PA ≥ 2·5 h/week (n 231) | PA = 0 h/week (n 4) | 0 < PA < 2·5 h/week (n 50) | PA ≥ 2·5 h/week (n 113) | P |
| Age (years)                          | Mean 67·9             | Mean 67·9                     | Mean 67·8                   | Mean 67·9             | Mean 67·9                     | Mean 67·8                   | 0·553 |
|                                      | SD 2·2                | SD 1·8                        | SD 1·8                      | SD 2·0                | SD 1·8                        | SD 1·8                      |       |
| BMI (kg/m²)                          | Mean 30·5             | Mean 30·4                     | Mean 29·1                   | Mean 30·4             | Mean 29·1                     | Mean 27·6                   | 0·001 |
|                                      | SD 4·5                | SD 4·5                        | SD 4·6                      | SD 4·5                | SD 4·6                        | SD 4·6                      |       |
| Time since menopause (years)         | Mean 19·4             | Mean 19·2                     | Mean 19·1                   | Mean 19·2             | Mean 19·1                     | Mean 17·5                   | 0·282 |
|                                      | SD 7·6                | SD 7·1                        | SD 5·2                      | SD 7·1                | SD 6·2                        | SD 17·5                     |       |
| Income (euros/month)                 | Mean 747              | Mean 987                      | Mean 879                    | Mean 987              | Mean 880                      | Mean 893                    | 0·734 |
|                                      | SD 286                | SD 255                        | SD 297                      | SD 286                | SD 339                        | SD 292                      |       |
| Number of chronic diseases           | Mean 1·5              | Mean 1·7                      | Mean 1·5                    | Mean 1·7              | Mean 1·4                      | Mean 1·2                    | 0·409 |
|                                      | SD 1·0                | SD 1·2                        | SD 1·0                      | SD 1·2                | SD 1·2                        | SD 1·3                      |       |
| Mobility status                      | Normal                | 32                              | 93                          | 212                    | 8                              | 43                          | 112   |
| n%                                   | 76·2                  | 84·5                           | 94·6                        | 100                   | 100                            | 99·1                        |       |
| Restricted                            | 10                   | 17                             | 12                          | 0                     | 0                             | 1                          |       |
| n%                                   | 23·8                  | 15·5                           | 5·4                         | 0                     | 0                             | 0·9                        |       |
| Current smoker                       | 2                    | 4·7                            | 9                            | 8·2                   | 5·8                           | 0                          | 2·3   |
| n%                                   | 72                   | 6                              | 55                          | 12·5                  | 20·5                          | 22·1                       | 0·644 |
| Dietary hormone therapy              | Current smoker        | 0·472                          | 0·472                       | 0·472                 | 0·472                         | 0·472                       |       |
|                                      | n%                   | 15·6                           | 23·4                        | 23·8                  | 12·5                          | 20·5                       | 22·1  |
| Dietary factors and food groups      | Energy intake (kJ)    | 5581                           | 1473                        | 6029                  | 1230                          | 6113                        | 1343  | 0·025 |
| Alcohol (g/d)                        | 4·8                  | 1·2                            | 7·7                         | 1·1                   | 10·7                          | 1·8                         | 12·3  | 17·0  |
| Protein (g/d)                        | 55·7                 | 15·6                           | 61·8                        | 11·9                  | 60·5                          | 13·4                        | 81·8  | 14·5  |
| Protein (g/kg body weight)           | 0·69                 | 0·2                            | 0·81                        | 0·14                  | 0·83                          | 0·16                        | 1·2   | 0·2   |
| Carbohydrate (g/d)                   | 168                  | 47                             | 179                         | 40                    | 182                           | 42                          | 214   | 42    |
| Fat intake (g/d)                     | 46·2                 | 15·5                           | 49·6                        | 15·8                  | 50·3                          | 16·9                        | 55·6  | 17·2  |

* ANOVA was used to calculate means and standard deviations, and χ² tests for categorical variables to calculate n and percentages according to physical activity groups stratified for protein intake.
Table 3. Physical function and body composition in physical activity (PA) (h/week) groups* (Mean values and standard deviations; β values with their standard errors)

| PA = 0 h/week (n 77) | 0 < PA < 2.5 h/week (n 166) | PA ≥ 2.5 h/week (n 365) | PA at 1-h intervals (n 608) |
|----------------------|--------------------------------|-------------------------|----------------------------|
| Mean (SD)            | Mean (SD)                      | Mean (SD)               | P†                         |
| Hand grip strength (kg) |                                |                         |                            |
| Baseline             | 26.0 (5.1)                     | 25.6 (4.7)              | −0.006                     |
| At 3 years           | 25.2 (6.3)                     | 24.8 (6.1)              | 0.060                      |
| Hand grip strength adjusted for BMI (kg/m²) |                                |                         | 0.391                      |
| Baseline             | 0.90 (0.24)                    | 0.93 (0.22)             | −0.031                     |
| At 3 years           | 0.90 (0.24)                    | 0.93 (0.22)             | 0.501                      |
| Knee extension (Newtons) |                                |                         | 0.886                      |
| Baseline             | 275 (89)                       | 288 (82)                | 0.079                      |
| Chair rises in 20 s  |                                |                         | 0.986                      |
| Baseline             | 6.2 (3.0)                      | 7.5 (2.6)               | 0.248                      |
| At 3 years           | 7.2 (4.2)                      | 8.1 (3.1)               | 0.047                      |
| Tandem walk over 6 m (s) |                                |                         | 0.046                      |
| Baseline             | 17.0 (10.0)                    | 19.4 (8.1)              | 0.057                      |
| At 3 years           | 19.8 (4.4)                     | 20.4 (7.0)              | 0.002                      |
| Walking speed over 10 m (m/s) |                                |                         | 0.031                      |
| Baseline             | 1.4 (0.2)                      | 1.5 (0.3)               | 0.017                      |
| At 3 years           | 1.4 (0.2)                      | 1.6 (0.3)               | 0.009                      |
| Standing on one leg for maximum 30 s (s) |                                |                         | 0.050                      |
| Baseline             | 13.2 (11.1)                    | 17.6 (10.2)             | 0.113                      |
| At 3 years           | 15.2 (12.0)                    | 17.0 (11.5)             | 0.011                      |
| Modified short physical performance battery (calculated score) |                                |                         | 0.124                      |
| Able to perform squat (% of women) |                                |                         | 0.095                      |
| Baseline             | 5.2 (1.5)                      | 5.8 (1.7)               | 0.118                      |
| At 3 years           | 7.8 (2.4)                      | 7.6 (1.6)               | 0.009                      |
| Lean mass (kg)       |                                |                         | 0.021                      |
| Baseline             | 41.2 (4.0)                     | 40.4 (4.3)              | 0.039                      |
| At 3 years           | 41.3 (4.9)                     | 40.7 (4.7)              | 0.004                      |
| Relative skeletal muscle index (kg/m²) |                                |                         | 0.057                      |
| Baseline             | 6.8 (0.7)                      | 6.8 (0.6)               | 0.046                      |
| At 3 years           | 6.6 (0.9)                      | 6.7 (0.6)               | 0.007                      |
| Fat mass (kg)        |                                |                         | 0.012                      |
| Baseline             | 33.1 (10.0)                    | 30.0 (7.7)              | −0.196                     |
| At 3 years           | 31.8 (12.1)                    | 30.1 (8.4)              | 0.022                      |

UNIANOVA, univariate ANOVA.
1 UNIANOVA was used to calculate mean values and adjusted standard deviations. Multiple regression analysis was used to calculate β-coefficient and standard error. Model 1 was adjusted for age and energy intake. Model 2 was adjusted for age, energy intake (kL), smoking (yes, no), hormone therapy (yes, no), rheumatoid arthritis, baseline height (m), income per month (euros) and intensity of PA.

† Mean values and standard deviations calculated from UNIANOVA adjusted for variables in model 1.
‡ Mean values and standard deviations calculated from UNIANOVA adjusted for variables in model 2.
§ P calculated from multiple regression analysis, regression coefficient adjusted for variables in model 2.

Adequate PA has been considered as an effective way to prevent PF decline in older people, but previous epidemiological studies defined PA different to our study as well as using different cutoffs for PA[25–26,29], which should be noted. For instance, in a cohort study by Hillson et al., PA was self-reported, by frequency of participation in ‘mildly energetic’ (e.g. walking, weeding, general housework); ‘moderately energetic’ (e.g. dancing, cycling, leisurely swimming); and vigorous PA (e.g. running, hard swimming, squash), where active men and women had on average higher PF at follow-up, compared with their sedentary counterparts[20]. The results from the InCHIANTI study (men and women aged 65 years or older, n 1149) showed that physical inactivity was associated with decline in modified SPPB score and disability when compared with physically active participants[20]. In their study, participants were allocated to different groups for PA level.

The response categories were (i) minimal, (ii) light: performed 2–4 h/week not accompanied by sweating, (iii) moderate: performed 1–2 h/week accompanied by sweating or light PA not accompanied by sweating for more than 4 h/week, (iv) moderate: PA: performed ≥3 h/week accompanied by sweating and (v) physical exercise: performed regularly that required maximal strength and endurance several times per week.

There is some evidence to suggest PA in interaction with higher protein intake[39] which may be an effective strategy to prevent PF decline in older people, especially because inadequate protein intake is common in older adults, even among the physically active[40]. The Framingham Offspring observational study...
Table 4. Association of physical activity (PA) and protein intake interaction as categorical and continuous variables with physical function assessment at baseline and 3-year follow-up*  
(Mean values and standard deviations; \( \beta \) values with their standard errors)

|                     | PA < 2.5 h/week; protein < 1.1 g/kg g/kg BW (n = 147) | PA < 2.5 h/week; protein ≥ 1.1 g/kg g/kg BW (n = 46) | PA ≥ 2.5 h/week; protein < 1.1 g/kg g/kg BW (n = 240) | PA ≥ 2.5 h/week; protein ≥ 1.1 g/kg g/kg BW (n = 119) | PA and protein intake interaction continuous variable (n = 552) |
|---------------------|----------------------------------------------------------|-------------------------------------------------------|--------------------------------------------------------|--------------------------------------------------------|--------------------------------------------------|
|                     | Mean | SD  | Mean | SD  | Mean | SD  | Mean | SD  | Mean | SD  | \( \beta \) | SE   | \( P^\dagger \) | \( P^\ddagger \) | \( P^\S \) |
| Hand grip strength (kg) Baseline | 26.1 | 0.5 | 24.2 | 0.9 | 25.8 | 0.4 | 24.1 | 0.6 | 0.020 | 0.125 | 0.282 | 0.205 | 0.638 |
| At 3 years | 24.9 | 6.1 | 25.2 | 5.6 | 25.2 | 4.8 | 24.1 | 5.0 | 0.008 | 0.05 | 0.718 | 0.896 | 0.852 |
| Change | -1.0 | 0.4 | -0.78 | -1.15 | -1.32 | -0.97 | -0.29 | -0.47 | -0.030 | 0.053 | 0.274 | 0.250 | 0.511 |
| Hand grip strength adjusted for BMI (kg/m²) Baseline | 0.89 | 0.02 | 1.0 | 0.4 | 0.95 | 0.1 | 1.1 | 0.2 | 0.140 | 0.003 | 0.019 | 0.047 | 0.001 |
| At 3 years | 0.83 | 0.25 | 0.88 | 0.24 | 1.00 | 0.25 | 0.92 | 0.20 | 0.087 | 0.003 | 0.001 | 0.001 | 0.046 |
| Change | -0.04 | 0.01 | 0.02 | 0.03 | -0.05 | 0.01 | 0.04 | 0.01 | -0.069 | 0.002 | 0.083 | 0.046 | 0.139 |
| Knee extension (Newtons) Baseline | 291 | 7.8 | 277 | 13.2 | 304 | 5.9 | 289 | 8.5 | 0.090 | 0.929 | 0.211 | 0.115 | 0.054 |
| Chair rises in 20 s Baseline | 6.9 | 0.3 | 8.2 | 0.5 | 7.8 | 0.2 | 8.6 | 0.3 | 0.050 | 0.048 | 0.035 | 0.048 | 0.273 |
| At 3 years | 7.7 | 3.1 | 8.6 | 3.1 | 8.4 | 3.3 | 9.8 | 2.6 | 0.190 | 0.030 | 0.008 | 0.002 | 0.001 |
| Change | 0.81 | 0.04 | 0.00 | 0.07 | 0.02 | 0.03 | -0.09 | 0.03 | 0.088 | 0.045 | 0.913 | 0.897 | 0.057 |
| Tandem walk speed over 6 m (m/s) Baseline | 0.29 | 0.04 | 0.34 | 0.07 | 0.35 | 0.03 | 0.32 | 0.04 | 0.049 | 0.003 | 0.603 | 0.779 | 0.304 |
| At 3 years | 0.31 | 0.12 | 0.33 | 0.11 | 0.33 | 0.10 | 0.33 | 0.10 | 0.048 | 0.142 | 0.441 | 0.489 | 0.302 |
| Change | -0.07 | 0.04 | -0.04 | 0.07 | -0.07 | 0.03 | 0.03 | 0.04 | 0.042 | 0.004 | 0.786 | 0.788 | 0.396 |
| Walking speed over 10 m (m/s) Baseline | 1.50 | 0.03 | 1.73 | 0.06 | 1.69 | 0.02 | 1.79 | 0.04 | 0.205 | 0.004 | <0.001 | 0.010 | <0.001 |
| At 3 years | 1.47 | 0.03 | 1.54 | 0.02 | 1.55 | 0.05 | 1.62 | 0.03 | 0.143 | 0.004 | 0.027 | 0.039 | 0.002 |
| Change | -0.03 | 0.03 | -0.17 | 0.05 | -0.11 | 0.02 | -0.13 | 0.03 | -0.586 | 0.004 | 0.570 | 0.486 | 0.558 |
| Standing on one leg for maximum 30 s Baseline | 15.6 | 1.0 | 19.2 | 2.0 | 19.0 | 0.8 | 22.0 | 1.2 | 0.106 | 0.110 | 0.002 | 0.012 | 0.014 |
| At 3 years | 16.1 | 0.9 | 16.8 | 0.7 | 18.0 | 1.5 | 20.1 | 1.1 | 0.098 | 0.017 | 0.071 | 0.078 | 0.289 |
| Change | 0.5 | 0.3 | 0.02 | 0.22 | -2.3 | 0.9 | -3.0 | 1.4 | 0.012 | 0.118 | 0.418 | 0.361 | 0.801 |
| Able to perform squat (% of women) Baseline | 55 | 77 | 67 | 93 | 0.118 | 0.002 | 0.003 | 0.048 | 0.050 |
| At 3 years | 83 | 88 | 91 | 94 | 0.105 | 0.003 | 0.241 | 0.113 | 0.022 |
| Change | 20 | -15 | 29 | -10 | 0.057 | 0.004 | 0.343 | 0.330 | 0.218 |
| Modified SPPB (calculated score) Baseline | 5.4 | 1.7 | 6.2 | 1.9 | 6.2 | 1.5 | 6.5 | 1.7 | 0.177 | 0.040 | <0.001 | <0.001 | <0.001 |
| At 3 years | 7.3 | 1.8 | 7.4 | 1.8 | 7.6 | 1.6 | 8.2 | 1.6 | 0.089 | 0.020 | 0.018 | 0.010 | 0.043 |
| Change | 1.8 | 2.2 | 2.2 | 1.4 | 1.8 | 1.5 | 3.1 | 2.1 | -0.125 | 0.027 | 0.112 | 0.074 | 0.098 |
| Lean mass (kg) Baseline | 41.0 | 4.5 | 38.8 | 3.5 | 40.4 | 4.3 | 39.0 | 3.8 | 0.096 | 0.022 | 0.686 | 0.710 | 0.121 |
| At 3 years | 41.2 | 5.0 | 40.3 | 3.4 | 40.2 | 3.0 | 39.1 | 3.2 | 0.178 | 0.012 | <0.001 | <0.001 | 0.311 |
| Change | 0.2 | 0.1 | -0.1 | 0.2 | 0.07 | 0.11 | 0.07 | 0.17 | -0.025 | 1.82 | 0.432 | 0.421 | 0.592 |
| RSMI (kg/m²) Baseline | 6.5 | 0.4 | 6.7 | 0.6 | 6.8 | 0.5 | 7.0 | 0.6 | 0.069 | 0.007 | <0.001 | <0.001 | 0.127 |
| At 3 years | 6.8 | 0.8 | 6.6 | 0.6 | 6.4 | 0.5 | 6.6 | 0.6 | 0.057 | 0.008 | <0.001 | <0.001 | 0.277 |
| Change | 0.0 | 0.4 | 0.1 | 0.2 | -0.1 | 0.3 | 0.0 | 0.3 | 0.023 | 0.005 | 0.434 | 0.537 | 0.626 |
Table 4. (Continued)

| PA intake interaction | Mean | SD | Mean | SD | Mean | SD | Mean | SD | β | SE | P† | P‡ | P§ |
|-----------------------|------|----|------|----|------|----|------|----|----|----|----|----|----|
| PA < 2.5 h/week;      |      |    |      |    |      |    |      |    |    |    |    |    |    |
| protein < 1.1 g/kg g/kg BW (n 147) | 33.6 | 8.2 | 23.8 | 6.6 | 29.4 | 8.4 | 24.2 | 6.2 | -0.206 | 0.098 | >0.001 | >0.001 | >0.001 |
| At 3 years            |      |    |      |    |      |    |      |    | 0.198 | 0.097 | >0.001 | >0.001 | >0.001 |
| Change                |      |    |      |    |      |    |      |    | 0.095 | 3.431 | 0.487 | 0.605 | 0.040 |

PA and protein intake interaction, continuous variable (n 552)

Fat mass (kg)

Baseline 33.6 ± 8.2
At 3 years 33.0 ± 9.4
Change 0.05 ± 3.12

More studies are needed to figure out the effectiveness of different PA and protein intake on physical performance. Previous studies have shown the benefit of PA and exercise to increase muscle mass in older adults (43-46). While PA was not associated with muscle mass, our results remained inconclusive regarding association of PA and protein intake with PF measures in older adults regardless of preserving or increasing muscle mass or body fat changes in older women (45). Furthermore, our PA included household duties and other light PA which may not increase RSMI but could maintain or improve lower muscle mass or body fat changes in older women (47). In addition, less PA results in a loss of significance in the association of PA with PF measures. However, we did not find an association between PA and protein intake interaction and a change in PF measures. In older people, the decline in muscle strength is related to the loss of muscle mass, but not the decline in muscle mass per se (48). This may be due to the fact that the decline in muscle mass is not due to the loss of muscle mass per se, but rather the loss of muscle mass due to the loss of muscle fibers and muscle mass (49). In addition, the loss of muscle mass is not due to the loss of muscle mass per se, but rather the loss of muscle mass due to the loss of muscle fibers and muscle mass (49).

BW, body weight; SPPB, short physical performance battery; RSMI, relative skeletal muscle index; UNIANOVA, univariate ANOVA.

1 UNIANOVA was used to calculate mean values and standard deviations adjusted. Multiple regression analysis was used to calculate β coefficient and standard error. Model 1 was adjusted for age and energy intake. Model 2 was adjusted for age, energy intake (kJ), smoking (yes, no), hormone therapy (yes, no), rheumatoid arthritis, baseline height (m), income per month (euros) and intensity of PA. At 3 years of follow-up, analyses were adjusted for absolute changes in physical function, muscle mass and study group (Ca and vitamin D intervention).

† Mean values and standard deviations calculated from UNIANOVA adjusted for variables in model 1.

‡ Mean values and standard deviations calculated from UNIANOVA adjusted for variables in model 2.

§ Calculated from multiple regression analysis, regression coefficient adjusted for variables in model 2.

β- coefficient and standard error. Model 1 was adjusted for age and energy intake. Model 2 was adjusted for age, energy intake (kJ), smoking (yes, no), hormone therapy (yes, no), rheumatoid arthritis, baseline height (m), income per month (euros) and intensity of PA. At 3 years of follow-up, analyses were adjusted for absolute changes in physical function, muscle mass and study group (Ca and vitamin D intervention).
Fig. 1. Univariate ANOVA (UNIANOVA) was used to calculate mean values and adjusted standard deviations. Multiple regression analysis was used to calculate β-coefficients and standard errors for physical function measures, lean mass, relative skeletal muscle index (RSMI) and fat mass according to physical activity (PA) and protein intake interaction groups: activity and protein intake interaction groups as follows: (i) PA < 2.5 h/week and protein intake < 1 g/kg body weight (BW) (n 147); (ii) PA < 2.5 h/week and protein intake ≥ 1 g/kg BW (n 52); (iii) PA ≥ 2.5 h/week and protein intake ≥ 1 g/kg BW (n 119). Model 1 was adjusted for age and energy intake. Model 2 was adjusted for age, energy intake (kJ), smoking (yes, no), hormone therapy (yes, no), rheumatoid arthritis, baseline height (m), income per month (euros) and intensity of PA. At 3 years of follow-up, analyses were adjusted for absolute changes in physical function, muscle mass and study group (calcium and vitamin D intervention). P calculated from UNIANOVA adjusted for variables in model 1. * P calculated from UNIANOVA adjusted for variables in model 2. † P calculated from multiple regression analysis adjusted for variables in model 2. The follow-up variable of knee extension was excluded from the analysis because of an unexpected increase in measured extension force and/or possible data entry errors, which could not be traced due to the long period between this secondary analysis and the time data recorded in 2001–2003. This issue was explained in these data (24).
Standing on one leg for max 30 s

(g) Baseline
(g2) At 3 years

(h) Baseline
(h2) At 3 years

Modified SPPB (calculated score)

(i) Baseline
(i2) At 3 years

(j) Baseline
(j2) At 3 years

Lean mass (kg)

(k) Baseline
(k2) At 3 years

(l) Baseline
(l2) At 3 years

Fat mass (kg)

Fig. 1. (Continued).
The effect of protein supplementation and exercise intervention has also been evaluated in clinical intervention studies. A recent meta-analysis of RCT regarding the effects of protein supplementation on the body composition and PF of older people undergoing resistance exercise training concluded that compared with resistance exercise training alone, protein supplementation combined with resistance exercise training may have a stronger effect in preventing ageing-related muscle mass attenuation and leg strength loss in older people\(^{(39)}\). However, the Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases working group noted in 2018 that protein supplementation intake has the potential to slow muscle mass loss, but evidence of the functional benefits of supplementation is mixed\(^{(39)}\). It appears that the interaction with protein supplementation intake and physical exercise requires further investigation. It is noteworthy that randomised control trials using protein supplementation and exercise intervention are mostly limited to a short follow-up period and may not be able to capture the effect of habitual PA and protein intake on PF and muscle mass in older adults. In addition, while multiple studies have been based on protein intake supplementation, a limited number of studies have used the habitual dietary intake of protein.

A novelty of the present study is that it provides the opportunity to investigate the association of PA independently and in combination with protein intake, according to WHO and NNR recommendations, respectively. We endeavoured to state previous studies which focused on the association of PA alone or by interaction with protein intake; however, it is important to note that PA is a term which has been used interchangeably with exercise or vice versa. We have mentioned the PA ascertainment method of each of these studies to provide easier comparison.

We used dual-energy X-ray absorptiometry measurement, which is a common tool suitable for the estimation of body composition in terms of evaluating the ratios of fat, muscle and bone in different parts of the body. Dual-energy X-ray absorptiometry has been shown to provide accurate estimations of body composition\(^{(39)}\). The percentage of frail people in these data was not considerably high. Among the subjects of the present study, according to the frailty phenotype definition by Fried et al., 8.1% (n 36) of the women was classified as frail\(^{(52)}\).

PA data were collected via questionnaires, which has some limitation. For practical reasons, PA questionnaires are currently the most commonly used assessment method in large population-based cohort studies to assess individual PA levels. However, the agreement between different PA questionnaires in correctly revealing individuals as physically active (e.g. meeting the adult PA recommendations of ≥ 2.5 h/week) is challenging\(^{(39)}\). Another limitation of this method is overreporting of PA, which may have led to an overestimation of subjects identifying themselves as sufficiently physical active when PA is assessed using self-reporting. In addition, capturing the intensity of PA by means of a questionnaire is susceptible to individuals’ perceptions, which can vary for a single individual or from person to person. Although all the questionnaires were checked by trained nurses, it may be that answers to the questionnaire regarding the intensity of PA refer more to health status and fitness level rather than strenuous PA. Therefore, the intensity of PA, as asked by the questionnaire in the present study, rather than exposure, was introduced as a covariate in the analysis models. It is recommended that further studies use PA validation methods to minimise such bias.

There are some other limitations to the present study that are important to take into consideration. For example, although the 3-d food record method has been described as a suitable instrument for assessing energy and protein intake in older people\(^{(39)}\), a repeated measure of 3-d dietary records at the follow-up could provide a more quantifiable measure to capture long-term protein intake. It is noteworthy that energy intake among our study population was relatively low, which may be due to underreporting (conscious or unconscious) or to actually reducing the typical level of food intake\(^{(53)}\). However, none of the participants met the threshold to be excluded from the analyses based on the BMR cut-offs indicated by the dietary reference values for food energy and nutrients for the UK\(^{(54)}\). Finally, we have controlled for several confounders, but the possibility of other confounding factors may exist.

It is worth noting that although RCT are of high value, they are not able to capture lifestyle-related variables such as diet, protein intake and PA. They are usually short by nature and are conducted in a controlled situation. Thus, observational studies such as this may provide information beyond RCT to reflect how habitual diet and PA may interactively be associated interactively with PF. Furthermore, a novel approach in the present study was to introduce the independent and interaction association of PA according to the WHO with protein intake according to NNR recommendations, which has previously not been studied.

**Conclusion**

In conclusion, our findings suggest that higher PA, as recommended by the WHO, in combination with adequate protein intake, as recommended by NNR 2012, may have a positive association with better PF in older adults. However, further studies with a longer follow-up period are warranted to confirm this finding.

**Acknowledgements**

The authors would like to express their appreciation to all the study nurses, secretaries and participants in the present study. The OSTPRE-FPS study was supported by the Finnish Cultural Foundation (2019) (the North Savo Regional Fund, the Hulda Tossavainen Foundation, Matti Kärkkäinen), the Sigrid Juselius Foundation (H. K. and T. R.), the Academy of Finland (M. I.) and a Kuopio University Hospital EVO grant. The present study was financially supported by grants from the Finnish Cultural Foundation (2019), Juho Vainio Foundation, the Otto. A Malm Foundation and the Yrjö Jahnsson Foundation to M. I.

M. I. conducted the statistical analyses and together with S. S. summarised them in the tables and figures. The first draft of the present study was written by S. S. and M. I. and J. S., T. R., A. T. E.
and H. K. revised this manuscript for scientific content. S. S. and M. I. take responsibility for the integrity of the data analysis. The authors of this study declare no conflicts of interest.

**Supplementary material**

For supplementary material referred to in this article, please visit https://doi.org/10.1017/S0007114520000045

**References**

1. Coats AJS, Forman DE, Haykowsky M, et al. (2017) Physical function and exercise training in older patients with heart failure. *Nat Rev Cardiol* 14, 550.

2. Fontana L, Kennedy BK, Longo VD, et al. (2014) Medical research: treat ageing. *Nature* 511, 405–407.

3. Dickinson JM & Volpi E (2013) Exercise and nutrition to target protein synthesis impairments in aging skeletal muscle. *Exerc Sport Sci Rev* 41, 216–223.

4. Galvani R, Michelli A, Landi F, et al. (2013) Current nutritional recommendations and novel dietary strategies to manage sarcopenia. *J Frailty Aging* 2, 38–53.

5. Beaudart C, Dawson A, Shaw SC, et al. (2013) Protein intake and physical activity in the prevention and treatment of sarcopenia: systematic review. *Osteoporosis Int* 28, 1817–1833.

6. Liu G, Chen Y, Yang Y, et al. (2017) Pilot study of the Mini Nutritional Assessment on predicting outcomes in older adults with type 2 diabetes. *Geriatr Gerontol Int* 17, 2485–2492.

7. Steffl M, Bohannon RW, Sontakova I, et al. (2017) Relationship between sarcopenia and physical activity in older people: a systematic review and meta-analysis. *Clin Interv Aging* 12, 835–845.

8. Pillard F, Laoudji-Chenivesse D, Carnac G, et al. (2011) Physical activity and sarcopenia. *Clin Geriatr Med* 27, 449–470.

9. Cesari M, Vellas B, Hsu FC, et al. (2011) A physical activity intervention to treat the frailty syndrome in older persons-results from the LIFE-P study. *J Gerontol A Biol Sci Med Sci* 70, 216–222.

10. Wu F, Wills K, Laslett LL, et al. (2017) Moderate-to-vigorous physical activity but not sedentary time is associated with musculoskeletal health outcomes in a cohort of Australian middle-aged women. *J Bone Miner Res* 32, 708–715.

11. Gregorio I, Brindisi J, Kleppinger A, et al. (2014) Adequate dietary protein is associated with better physical performance among post-menopausal women 60–90 years. *J Nutr Health Aging* 18, 155–160.

12. Paddon-Jones D, Campbell WW, Jacques PF, et al. (2015) Protein and healthy aging. *Am J Clin Nutr* 101, 1339S–1345S.

13. Nilsson A, Montiel Rojas D & Kadi F (2018) Impact of meeting different guidelines for protein intake on muscle mass and physical function in physically active older women. *Nutrients* 10, 1156.

14. Deutz NE, Bauer JM, Barazzoni R, et al. (2014) Protein intake and exercise for optimal muscle function with aging: recommendations from the ESPEN expert group. *Clin Nutr* 33, 929–936.

15. Houston DK, Tocco JA, Garcia K, et al. (2017) Protein intake and mobility limitation in community-dwelling older adults: the Health ABC Study. *J Am Geriatr Soc* 65, 1705–1711.

16. Mustafa J, Ellison RC, Singer MR, et al. (2018) Dietary protein and preservation of physiologicalfunctioning among middle-aged and older adults in the Framingham Offspring Study. *Am J Epidemiol* 187, 1411–1419.

17. Timmernner KL, Dhanani S, Glynn EL, et al. (2012) A moderate acute increase in physical activity enhances nutritive flow and the muscle protein anabolic response to mixed nutrient intake in older adults. *Am J Clin Nutr* 95, 1403–1412.

18. World Health Organization (2018) *Physical Activity and Older Adults*. Geneva: WHO.

19. Nordic Council of Ministers (2014) *Nordic Nutrition Recommendations, 2012: Integrating Nutrition and Physical Activity*, 5th ed. Copenhagen: Nordic Council of Ministers.

20. Salovaara K, Tuppurainen M, Kärkkäinen M, et al. (2010) Effect of vitamin D3 and calcium on fracture risk in 65- to 71-year-old women: a population-based 3-year randomized, controlled trial – the OSTPRE-FPS. *J Bone Miner Res* 25, 1487–1495.

21. Kärkkäinen M, Tuppurainen M, Salovaara K, et al. (2010) Effect of calcium and vitamin D supplementation on bone mineral density in women aged 65–71 years: a 3-year randomized population-based trial (OSTPRE-FPS). *Osteoporos Int* 21, 2047–2055.

22. Lohman M, Tallroth K, Kettunen JA, et al. (2009) Reproducibility of dual-energy x-ray absorptiometry total and regional body composition measurements using different scanning positions and definitions of regions. *Metabolism* 58, 1663–1668.

23. Sjöblom S, Suuronen J, Rikkonen T, et al. (2013) Relationship between postmenopausal osteoporosis and the components of clinical sarcopenia. *Maturitas* 75, 175–180.

24. Isaneadj M, Mursu J, Sirola J, et al. (2016) Dietary protein intake is associated with better physical function and muscle strength among elderly women. *Br J Nutr* 115, 1281–1291.

25. Cruz-Jentoft AJ, Baeysens JP, Bauer JM, et al. (2010) Sarcopenia: European consensus on definition and diagnosis: report of the European Working Group on Sarcopenia in Older People. *Age Aging* 39, 412–423.

26. Erikkäi AT, Jarvinen R, Karvonen H, et al. (2012) Validation of a semi-quantitative FFQ using food records as a reference in older women in the Kuopio Fracture Prevention Study (OSTPRE-FPS). *Public Health Nutr* 15, 635–639.

27. Lecch NL, Barrett KC & Morgan GA (2005) *SPSS for Intermediate Statistics: Use and Interpretation*, 2nd ed. Mahwah, NJ: Lawrence Erlbaum Associates.

28. Hsu B, Meroni D, Blyth FM, et al. (2018) Total physical activity, exercise intensity, and walking speed as predictors of all-cause and cause-specific mortality over 7 years in older men: the Concord Health and Aging in Men Project. *J Am Med Dir Assoc* 19, 216–222.

29. Hardy SE, Perera S, Roumani YF, et al. (2007) Improvement in usual gait speed predicts better survival in older adults. *J Am Geriatr Soc* 55, 1727–1734.

30. Newman AB, Kupelian V, Visser M, et al. (2006) Strength, but not muscle mass, is associated with mortality in the health, aging and body composition study cohort. *J Gerontol A Biol Sci Med Sci* 61, 72–77.

31. Rikkonen T, Poole K, Sirola J, et al. (2018) Long-term effects of functional impairment on fracture risk and mortality in post-menopausal women. *Osteoporosis Int* 29, 2111–2120.

32. Lauritani F, Russo CR, Bandinelli S, et al. (2005) Age-associated changes in skeletal muscles and their effect on mobility: an operational diagnosis of sarcopenia. *J Appl Physiol (1985)* 98, 1851–1860.

33. Andersen JL (2003) Muscle fibre type adaptation in the elderly human muscle. *Scand J Med Sci Sports* 13, 40–47.

34. Tieland M, Trouwborst I & Clark BC (2018) Skeletal muscle performance and ageing. *J Cachexia Sarcopenia Muscle* 9, 3–19.

35. Gappola AR, Bandeen-Roche K, Wand GS, et al. (2001) Association of IGF-I levels with muscle strength and mobility in older women. *J Clin Endocrinol Metab* 86, 4139–4146.

36. Stenhofm S, Koster A, Valkenhurst H, et al. (2015) Association of physical activity history with physical function and mortality in old age. *J Gerontol A Biol Sci Med Sci* 71, 496–501.
37. Manini T & Pahor M (2008) Physical activity and maintaining physical function in older adults. *Br J Sports Med* **43**, 28–31.

38. Hillsdon MM, Brunner EJ, Guralnik JM, et al. (2005) Prospective study of physical activity and physical function in early old age. *Am J Prev Med* **28**, 245–250.

39. ten Haaf D, van Dongen E, Nuijten M, et al. (2018) Protein intake and distribution in relation to physical functioning and quality of life in community-dwelling elderly people: acknowledging the role of physical activity. *Nutrients* **10**, 506.

40. Ten Haaf D, De Regt MF, Visser M, et al. (2018) Insufficient protein intake is highly prevalent among physically active elderly. *J Nutr Health Aging* **22**, 1112–1114.

41. Bradlee ML, Mustafa J, Singer MR, et al. (2017) High-protein foods and physical activity protect against age-related muscle loss and functional decline. *J Gerontol A Biol Sci Med Sci* **73**, 88–94.

42. Skelton DA, GREIG CA, Davies JM, et al. (1994) Strength, power and related functional ability of healthy people aged 65–89 years. *Age Ageing* **23**, 371–377.

43. Zhao G, Li C, Ford ES, Fulton JE, et al. (2014) Leisure-time aerobic physical activity, muscle-strengthening activity and mortality risks among US adults: the NHANES linked mortality study. *Br J Sports Med* **48**, 244–249.

44. Montero-Fernandez N & Serra-Rexach JA (2013) Role of exercise on sarcopenia in the elderly. *Eur J Phys Rehabil Med* **49**, 131–143.

45. Santos L, Ribeiro AS, Schoenfeld BJ, et al. (2017) The improvement in walking speed induced by resistance training is associated with increased muscular strength but not skeletal muscle mass in older women. *Eur J Sport Sci* **17**, 488–494.

46. Visser M (2012) Epidemiology of muscle mass loss with age. In *Sarcopenia*, pp. 1–7 [AJ Cruz-Jentoft and JE Morley, editors]. Chichester: John Wiley & Sons Ltd.

47. Koster A, Ding J, Stenhollin S, et al. (2011) Does the amount of fat mass predict age-related loss of lean mass, muscle strength, and muscle quality in older adults? *J Gerontol A Biol Sci Med Sci* **66**, 888–895.

48. Delimónico MJ, Harris TB, Visser M, et al. (2009) Longitudinal study of muscle strength, quality, and adipose tissue infiltration. *Am J Clin Nutr* **90**, 1579–1585.

49. Liao C, Tsauo J, Wu Y, et al. (2017) Effects of protein supplementation combined with resistance exercise on body composition and physical function in older adults: a systematic review and meta-analysis. *Am J Clin Nutr* **106**, 1078–1091.

50. Robinson SM, Reginster J, Rizzoli R, et al. (2017) Does nutrition play a role in the prevention and management of sarcopenia? *Clin Nutr* **37**, 1121–1132.

51. Mijnarends DM, Meijers JM, Halfens RJ, et al. (2013) Validity and reliability of tools to measure muscle mass, strength, and physical performance in community-dwelling older people: a systematic review. *J Am Med Dir Assoc* **14**, 170–178.

52. Isanejad M, Sirola J, Rikkonen T, et al. (2019) Higher protein intake is associated with a lower likelihood of frailty among older women, Kuopio OSTPRE-Fracture Prevention Study. *Eur J Nutr* (epublication ahead of print version 7 May 2019).

53. Lührmann PM, Herbert BM, Gaster C, et al. (1999) Validation of a self-administered 3-day estimated dietary record for use in the elderly. *Eur J Nutr* **38**, 235–240.

54. Lupton JR, Brooks JA, Butte NF, et al. (2002) Protein and amino acids. In *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids*, pp. 589–768. Washington, DC: National Academies Press.