

**Interaction of recommended levels of physical activity and protein intake is associated with greater physical function, and lower fat mass in older women: Kuopio OSTPRE- Fracture Prevention Study**

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**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 (OSTPRE-FPS, n=610) completed a questionnaire on lifestyle factors and PA, and underwent PF and body composition measurements at baseline (BL) and over three years of follow-up (3y-FU). PA was categorized according to World Health Organization cut-off PA=0,  $0 < PA < 2.5$  and  $PA \geq 2.5$  h/week). Protein intake was calculated from 3-day food record at baseline and categorized according to the Nordic Nutrition Recommendations  $< 1.1$  and  $\geq 1.1$  g/kg body weight. The results showed in UNIANOVA analysis at the baseline and at the three-year follow-up, women with high  $PA \geq 2.5$  h/week and protein intake  $\geq 1.1$  g/kg BW had higher grip strength adjusted for BMI, higher mean number of chair rises, faster mean walking speed, higher modified mean SPPB score, and lower mean FM (**Table 4, and Figure 1**) compared to 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 physical function, and lower FM but the association with RSMI and muscle mass was inconclusive. This study gives noteworthy information for preventing sarcopenia.

**Keywords:** NUTRITION; PROTEIN INTAKE; PHYSICAL ACTIVITY; SKELETAL MUSCLE; SARCOPENIA

## Introduction

Aging 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 <sup>(1)</sup>.

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 <sup>(2,3)</sup>.

Among the most important health behaviours are those that involve physical activity (PA) and diet to preserve muscle mass and PF in older life <sup>(4-6)</sup>. Physical inactivity is linked to the loss of PF and muscle mass in older people <sup>(7)</sup>. Studies have shown that alterations in PF and muscle fibres in older adults remain responsive to functional demands such as physical exercise <sup>(8)</sup>. Therefore, it has been suggested that regular PA can partially prevent progression of the loss of PF and muscle mass related to inactivity <sup>(9,10)</sup>. 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 upregulate the metabolism of muscle synthesis <sup>(8)</sup>.

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 <sup>(11-13)</sup>. Furthermore, increased dietary protein intake can enhance the effects of PA on functional ability <sup>(14,15)</sup>. In a study among adults, functional tasks that benefitted most from a higher-protein diet ( $\geq 1.2$  vs.  $< 0.8$  g/kg/day) were doing heavy work at home (e.g. shovelling, or washing windows, walls, or floors), walking half a mile, going up and down stairs, stooping/kneeling/crouching, and lifting heavy items<sup>(16)</sup>. The exact mechanism is unclear, but it has been suggested that exercise-induced improvement of protein synthesis may be due to nutrient-stimulated vasodilation and nutrient delivery to muscle <sup>(17)</sup>. 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 <sup>(5)</sup>.

Most previous studies have evaluated the association of PA and protein intake using population cut-offs (n-tiles) 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 minutes of moderate-intensity aerobic physical activity throughout the week to improve cardiorespiratory and muscular fitness, bone and functional health, reduce the risk of non-communicable disease, depression and cognitive decline”<sup>(18)</sup>. Furthermore, the Nordic Nutrition Recommendations (NNR 2012) outline a protein intake in the range of 1.1–1.3 g/kg body weight (minimum 1.1 g/kg BW) to preserve PF in older adults<sup>(19)</sup>. However, data from observational studies using such recommendations for PA and protein intake in older adults are scarce.

In this study, we investigated the association of PA independently and in a 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.

## **Material and methods**

### *Study population*

Data for the present study was collected from the Osteoporosis Risk Factor and Prevention - Fracture Prevention Study (OSTPRE-FPS), which began in 2003 in Kuopio, Finland<sup>(20)</sup>. OSTPRE-FPS was a randomized population-based double-blinded controlled trial with a three-year follow-up involving 3,432 women (aged 66 to 71 years). The primary aim of the study was to determine whether vitamin D and calcium supplementation would be effective in preventing falls and fractures in postmenopausal women.

The power analysis was performed based on the incidence of fractures<sup>(20)</sup>. 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 a priori power analysis to calculate the size of the subsample of 750 women randomly selected from the 3,432 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 (mean (SD) follow-up time 2·8 (0·4) years).<sup>(21)</sup> Between the randomization and the actual start of the intervention, 237 subjects withdrew, and ten women stopped participating in the study for various reasons during the three-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. This study was a post-hoc analysis of the subjects from the OSTPRE-FPS study. The final analytical data set for this study comprised 608 women who had baseline and follow-up data for the self-administered questionnaire regarding PA. However, the 3-day dietary food record was only available at the baseline for 554 women (data for 54 food records was 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 calcium supplements were asked.

### *Body composition measurements*

Total body dual-energy X-ray absorptiometry (DXA) was used by specially trained nurses to measure muscle mass and FM in 2003-2007. The DXA measurements were carried out using the same Lunar Prodigy, adhering to the imaging and analysis protocols provided by the manufacturer (Lunar Co., Madison, WI, USA) <sup>(22)</sup>. The height and weight of participants were measured in light indoor clothing without shoes at the baseline and at the three-year follow-up, and BMI was calculated as  $\text{kg/m}^2$ . 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 ( $\text{kg/m}^2$ ).

#### *Physical functions measurements*

The detailed explanation of PF measurements in this data has been published previously.<sup>(23,24)</sup> PF measures were assessed by trained nurses at the baseline and at the three-year follow-up session, including hand grip strength (kg), number of chair rises in 30 seconds, ability to squat, knee extension (Newtons but converted into kg), walking speed 10 m (m/s), tandem walk for 6m (m/s), standing with closed eyes for 10 seconds, and one leg stance performance for 30 seconds. 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 this data <sup>(24)</sup>. 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 (JAMARTM handgrip dynamometer; Sammons Preston, Bolingbrook, IL), and then mean grip strength was calculated. The interaction correlation coefficient for grip strength measurements was 0.93. To standardize the measurement, grip strength was further expressed as a ratio to the body mass index. The chair rise test was conducted if the participant could rise at least once without using their arms

from a straight-backed, non-padded, armless chair. The maximum number of chair rises was recorded by trained nurses. The women were asked to walk the 10 m distance first at normal pace and then the 6 m distance in a tandem position. The time was recorded, and the walking speed was calculated as m/s. The women who were not able to walk were given a value of zero (14 women at the baseline and 25 women in the three-year measurement).

The short physical performance battery (SPPB) may indicate an individual's physical ability <sup>(25)</sup>. The modified SPPB score was calculated based on the European Working Group on Sarcopenia definition. A modified SPPB was calculated using three individual measures of PF assessment, including: 1) walking speed over 10 m (m/s), 2) chair rises in 30 seconds, and 3) standing on one leg. These three PF variables were further categorized into quartiles, and each quartile was scored on a scale of 1–4 points. Individuals unable to complete a task received a score of 0. The total SPPB score ranged from 0 to 12, with higher scores referring to better physical performance.

### *Physical activity*

Within the baseline questionnaires, PA data was collected on the amounts of different types of household, leisure activities, and exercise engaged in, including winter and summer seasons at the baseline and over three years. Household and leisure activities that were asked included wood work, hunting, fishing, major work at home such as renovation and house chores. The exercise that women reported included walking, cycling, skiing, swimming, aerobic exercise, ball sports (e.g. football, volleyball, indoor hockey etc.), skating, bowling, floorball, gymnastics, and rowing. However, among the household, leisure and exercise variables the most common activities reported were skiing, walking, cycling, swimming, and aerobic exercise, explaining over 90% of the weekly PA in this data (data not shown), which was used to form the PA variable. These types of activities are normally referred to as aerobic exercise. However, because the intention of our questionnaire at the time of data collection was to estimate on overall PA level, we refer to these activities as PA

rather than aerobic exercise. The reported amount of weekly PA at those time points was used to form a long-term PA variable by summing up the average weekly PA at the baseline.

Our secondary analysis showed that there was a significant deviation in reported intensity within and between study subjects. Therefore, intensity of exercise was introduced to models as a covariate rather than forming the final PA variable. The women were then categorized into the following groups: inactive (0 h/week), insufficient ( $0 < PA < 2.5$  h/week), and sufficient ( $PA \geq 2.5$  h/week); the cut-offs were adapted according to WHO recommendations for amount of PA per week in older adults<sup>(18)</sup>. In addition, the continuous PA variable was introduced in one-unit increments to account for normality. In NNR 2012, the recommendation for PA is identical to the WHO recommendation, which is 150 minutes of moderate-intensity PA per week. 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 three-day 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 three consecutive days, with two days during the week and one day during the weekend (Saturday or Sunday)<sup>(26)</sup>. 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, Turku, Finland), 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<sup>(24)</sup>. Total protein intake was calculated first as g/d and was then further expressed as g/kg BW. In this data, we previously introduced the cut-offs for protein intake



according to NNR 2012.<sup>(19)</sup> In this study, accordingly, women were categorized by protein intake (g/kg BW) according to NNR 2012 (<1·1 vs. ≥ 1·1 g/kg BW).

### *Confounders*

Data regarding lifestyle was 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<sup>(21)</sup>. Number of chronic diseases, including hypertension, hyperlipidemia, coronary heart disease, 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., Armonk, NY). 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 was pooled for the total population (intervention and control group) in year three of the follow-up. However, follow-up analyses were further adjusted for the RCT study group (calcium 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) was calculated using multiple regression analysis (presented in Supplementary Table 1). 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-square test for categorical variables, according to PA groups (0,  $0 < PA < 2.5$ , and  $PA \geq 2.5$  h/week) stratified for protein intake cut-off ( $< 1.1$  and  $\geq 1.1$  g/kg BW). For the main analysis, PA was introduced to two main models, first as a categorical variable to UNIANOVA for calculated means and standard deviations (SD), and second as a continuous variable in multiple regression analysis for calculated standard coefficient  $\beta$  and standard error (SE). SPSS software provided the multiple 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 <sup>(27)</sup>. 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 PA 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  $\geq 1.1$  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  $\geq 1.1$  g/kg BW, (c)  $PA \geq 2.5$  h/week and protein intake  $< 1.1$  g/kg BW, and (d)  $PA \geq 2.5$  h/week and protein intake  $\geq 1.1$  g/kg BW. Interaction between continuous PA at one-hour intervals and protein intake was significant in relation to FM, and PF measures ( $\beta > 0.75$ ,  $P < 0.040$ ), except for the ability to squat to the ground and tandem walk speed. UNIANOVA was used to calculate means and SD (PA and protein intake interaction as a categorical variable), and multiple regression analysis was used to calculate the standard coefficient

$\beta$  and SE (PA and protein intake interaction as a continuous variable) in the models where PF measures, total body LM, total body fat mass, and RSMI were set as dependent variables.

The final analytical sample for interaction analysis was performed for 554 women, because there were 54 (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 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 their 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 (Kcal). 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 calcium 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  $\geq 2.5$  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 women received calcium (1000 mg) and vitamin D (700 IU) during the 3-year follow-up. Results showed no significant effect of vitamin D and calcium 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 (Supplementary Table 1). PA was not associated with lean mass and RSMI whereas protein intake was inversely associated with lean mass and RSMI.

### *Physical activity and PF*

In cross-sectional analysis, continuous PA at one-hour intervals was positively associated with higher number of chair rises (standardized  $\beta=0.248$ ,  $P=0.040$ ), walking speed (standardized  $\beta=0.107$ ,  $P=0.005$ ), modified SPPB (standardized  $\beta=0.118$ ,  $P=0.022$ ), ability to squat (standardized  $\beta=0.185$ ,  $P=0.001$ ), and lower FM (standardized  $\beta=-0.196$ ,  $P<0.001$ ). 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 to the low PA group after adjusting for confounders. At the three-year follow-up, higher continuous PA (one-hour intervals and  $PA \geq 2.5$  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 one-hour intervals and protein intake interaction were associated with higher grip strength adjusted for BMI ( $\beta=0.140$ , and  $P=0.001$ ), faster walking speed over 10 m ( $\beta=0.205$ , and  $P<0.001$ ), longer standing on one leg for a maximum of 30 s ( $\beta=0.106$ , and  $P=0.014$ ), more frequent ability to squat ( $\beta=0.118$ , and  $P=0.050$ ), higher modified SPPB score ( $\beta=0.177$ ,  $SE=0.003$  and  $P<0.001$ ), and lower FM ( $\beta=-0.206$ , and  $P<0.001$ ) **Table 4**. Follow-up analysis further showed that continuous PA at one-hour intervals and protein intake interaction were associated with higher grip strength adjusted for BMI ( $\beta=0.087$ ,  $SE=0.003$  and  $P=0.046$ ), increased repetitions of higher number of chair rises ( $\beta=0.190$ ,  $SE=0.004$  and  $P=0.001$ ), faster walking speed over 10 m ( $\beta=0.143$ , and  $P=0.002$ ), more frequent ability to squat ( $\beta=0.105$ , and  $P=0.022$ ), higher modified SPPB score ( $\beta=0.089$  and  $P=0.043$ ), and lower FM ( $\beta=-0.198$ , and  $P<0.001$ ). The only significant association of PA and protein intake interaction as a continuous variable was with FM change ( $\beta=-0.095$ , and  $P=0.040$ ).

Further, in UNIANOVA analysis at the baseline and at the three-year follow-up, women with high  $PA \geq 2.5$  h/week and protein intake  $\geq 1.1$  g/kg BW had higher grip strength adjusted for BMI mean ( $1.1 \pm 0.2$ ), higher mean number of chair rises, faster mean walking speed, higher modified mean SPPB score, and lower mean FM (**Table 4, and Figure 1**) compared to 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

This 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 interaction of physical activity 2·5 h/week with protein intake  $\geq 1\cdot1$  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 (three years). The interaction of PA and protein intake was not clearly associated with RSMI and lean mass. Previous epidemiologic studies have showed that quadriceps, grip strength, ability to squat and walking speed have been determined as significant predictors of mortality<sup>(28-30)</sup>, and ability to squat and walking speed was associated with higher risk of fracture<sup>(31)</sup>.

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<sup>(32)</sup>. Ageing muscles are susceptible to reduced number of motor neurons which are mainly responsible of generating muscle strength<sup>(33)</sup>. In addition, muscle strength and PF in older people can be influenced by the hormonal changes such as insulin-like growth factor-I, which declines with age<sup>(34,35)</sup>.

### *PA and interaction of PA and protein intake with PF*

Adequate PA has been considered as an effective way to prevent PF decline in older people, but previous epidemiologic studies defined PA different to our study as well as using different cut-offs for PA<sup>(36-38)</sup>, 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 physical activity (e.g., running, hard swimming, squash), were active men and women had on

average higher PF at follow-up, compared to their sedentary counterparts<sup>(38)</sup>. 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 to physically active participants<sup>(36)</sup>. In their study participants were allocated to different groups for PA level

The response categories were (i) minimal (ii), light: performed 2–4 hours per week not accompanied by sweating (iii), moderate: performed 1 to 2 hours per week accompanied by sweating or light physical activity not accompanied by sweating for more than 4 hours per week (iv), moderate physical activity: performed  $\geq 3$  hours per 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<sup>(39)</sup> 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<sup>(40)</sup>. The Framingham Offspring observational study among middle-aged adults showed that active subjects with higher intakes of animal or plant protein source foods (red meat, poultry, fish, dairy, soy, nuts, seeds, and legumes) had higher skeletal muscle mass and a 35% lower risk of functional decline<sup>(41)</sup>. However, dietary protein intake was not reported in the study. Results of our study detected several positive associations in the interaction of PA and protein intake with PF measures. However, we did not find an association between PA and protein intake interaction with changes in PF variables. This might be mostly due to the rather short follow-up period, which may have limited the ability of this study to capture changes in PF. In addition, our study population age had a mean of 65 years, and PF decline might not have been happening at a rapid pace at this age<sup>(42)</sup>. Furthermore, the full adjustment resulted in a loss of significance in the association of PA with PF measures, but not in the cumulative association of PA and protein intake with PF measures, suggesting a strong

association between these two factors. There are no studies in literature why ordinary physical activity (including different household duties, gardening, climbing chairs etc.) increase the demand for protein intake. More studies are needed to figure out the effectiveness of different physical activities to demand for protein and physical performance.

*PA and interaction of PA and protein intake with muscle mass*

Previous studies have shown the benefit of PA and exercise to increase muscle mass in older adults<sup>(7,43,44)</sup>. While PA was not associated with muscle mass, our results remained inconclusive regarding association of interaction of PA and protein intake, where highest value for LM and RSMI was detected among women with lowest PA and protein intake at the three-year follow-up, to the contrary at baseline highest value for RSMI was belonged to women with highest PA and protein intake. This may be explained by the argument, that the ageing muscles loss of motor neurones may results in an increase in size of remaining motor units along with higher type 1 fibres preservation which means a possible preservation of muscle mass with relatively fewer type 2 fibres, thus lower strength. Our finding may suggest that higher PA and protein intake can be beneficial to PF measures in older adults regardless of preserving or increasing muscle mass<sup>(33)</sup>. However, this aspect is difficult to study without muscle biopsy and further studies are warranted. Furthermore our PA included household duties and other lighter PA which may not increase RSMI but could maintain or increase PF.

Subsequently, this finding may suggest that, higher PA may improve PF and muscle strength when compared to muscle mass and it may show its benefit by reducing FM in older women. In a resistance training program<sup>(45)</sup> 10 meter walking speed improvement after an 8-week was associated with increased lower limb muscular strength and muscle quality, but not with muscle mass or body fat changes in older women<sup>(45)</sup>. Furthermore, studies in older people indicated that the decline in muscle strength exceeds the decline in mass,<sup>(46-48)</sup> and higher FM can predict lower muscle quality<sup>(47)</sup>. In this data was also previously presented that a greater FM was adversely associated with



multiple PF<sup>(49)</sup>. However, further studies are warranted to reveal the association between PA and protein intake with muscle quality rather than sheer body composition.

The effect of protein supplementation and exercise intervention have been also evaluated in clinical intervention studies. A recent meta-analysis of randomized controlled trials regarding the effects of protein supplementation on the body composition and PF of older people undergoing resistance exercise training concluded that, compared to 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.<sup>(50)</sup>

However, the Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases working group (ESCEO) 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<sup>(51)</sup>. It appears that the interaction with protein supplementation intake and physical exercise requires further investigation. It is noteworthy that randomized 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 this 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 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 DXA 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.

DXA has been shown to provide accurate estimations of body composition <sup>(52)</sup>. The percentage of frail people in this data was not considerably high. As indicated by Isanejad et al. (2019), among the subjects of this study, according to the frailty phenotype definition by Fried et al. (2001), 8.1% (n=36) of the women were classified as frail. <sup>(53)</sup>.

PA data was 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 <sup>(39)</sup>. Another limitation of this method is over-reporting 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 this 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 this study that are important to take into consideration. For example, although the 3-day food record method has been described as a suitable instrument for assessing energy and protein intake in older people, <sup>(54)</sup> a repeated measure of 3-day 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 <sup>(55)</sup>. However, none of the participants met the threshold to be excluded from the analyses based on

the basal metabolic rate cut-offs indicated by the dietary reference values for food energy and nutrients for the United Kingdom <sup>(56)</sup>. Finally, we have controlled for several confounders, but the possibility of other confounding factors may exist.

It is worth noting that although RCTs are of high value, they are not able to capture lifestyle-related variables such as diet, protein intake, and physical activity. They are usually short by nature and are conducted in a controlled situation. Thus, observational studies such as this may provide information beyond RCTs to reflect how habitual diet and PA may interactively be associated interactively with PF. Furthermore, a novel approach in this 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**

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### **Disclosures**

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**Figure legends**

UNIANOVA was used to calculate means  $\pm$  SD adjusted. Multiple regression analysis was used to calculate coefficient  $\beta$  and standard error for physical function measures, lean mass, relative skeletal muscle index, and fat mass according to physical activity and protein intake interaction groups: activity and protein intake interaction groups as follows: (i) PA < 2.5 h/week and protein intake < 1.1 g/kg BW (n=147); (ii) PA < 2.5 h/week and protein intake  $\geq$  1.1 g/kg BW (n=46); (iii) PA  $\geq$  2.5 h/week and protein intake < 1.1 g/kg BW (n=240); (iv) PA  $\geq$  2.5 h/week and protein intake  $\geq$  1.1 g/kg BW (n=119).

Model 1 was adjusted for age and energy intake. Model 2 was adjusted for age, energy intake (Kcal), 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 this data.<sup>(49)</sup>

Figure 1

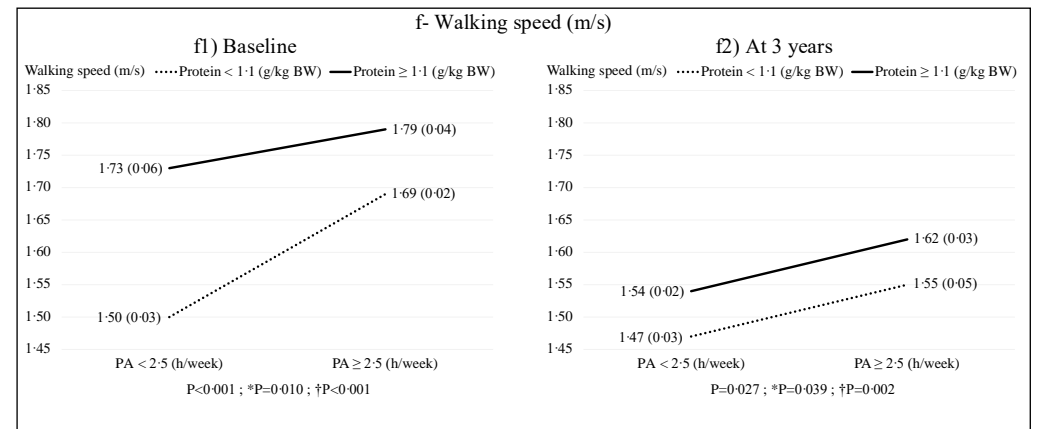
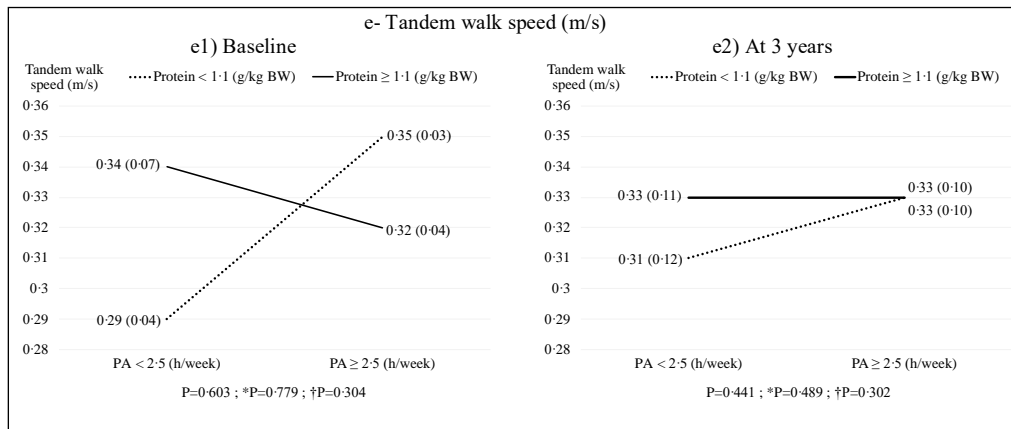
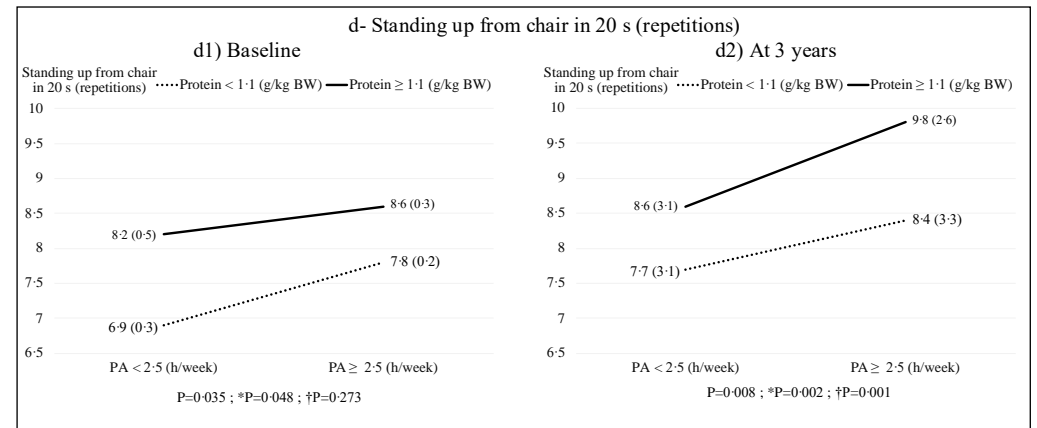
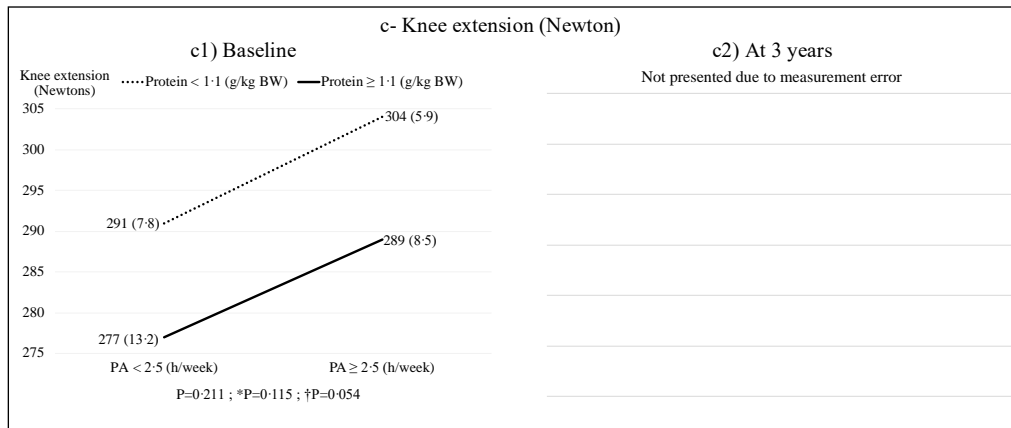
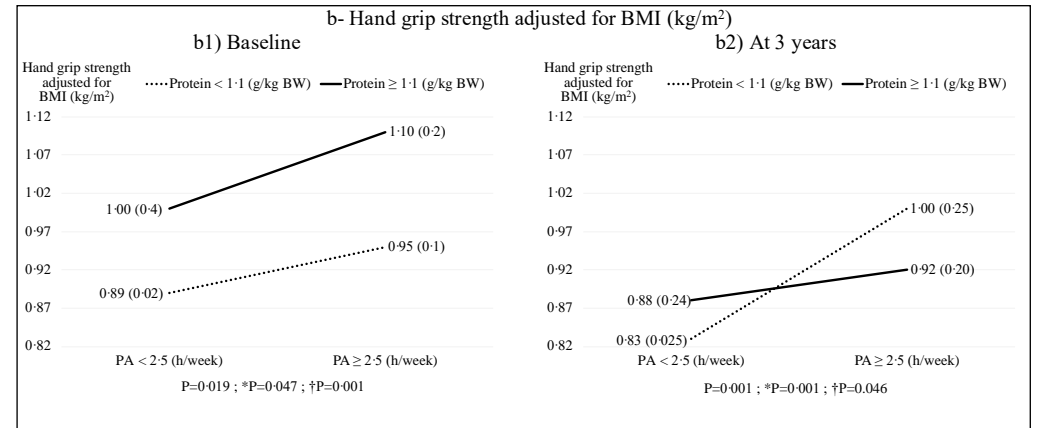
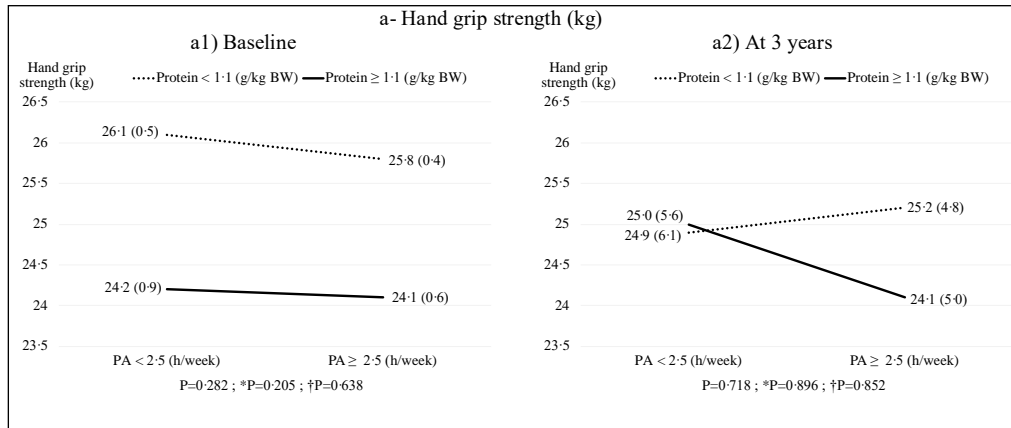
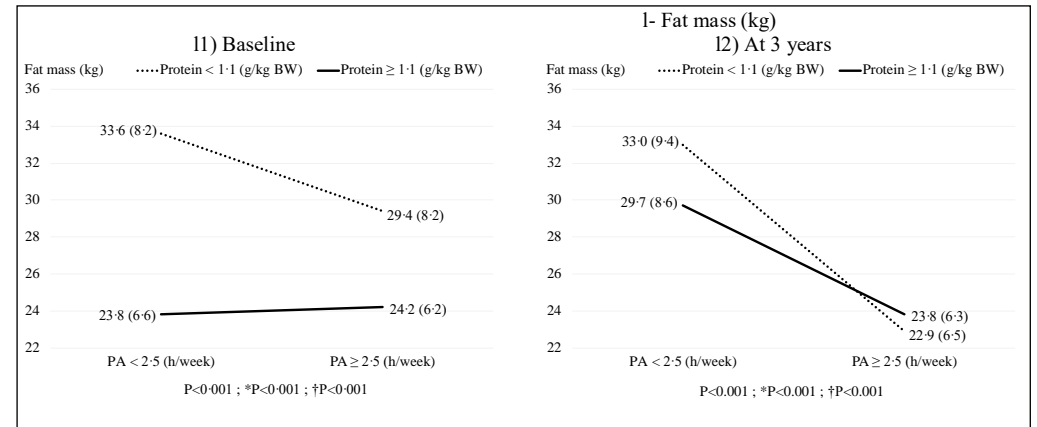
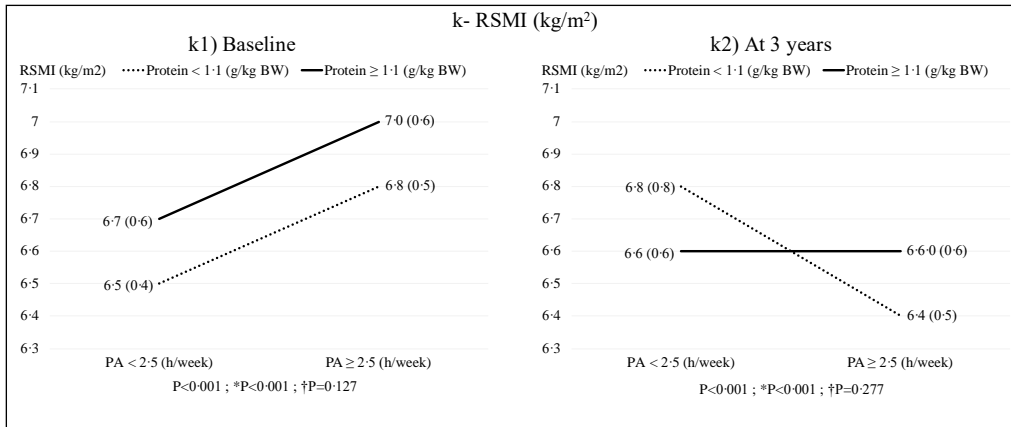
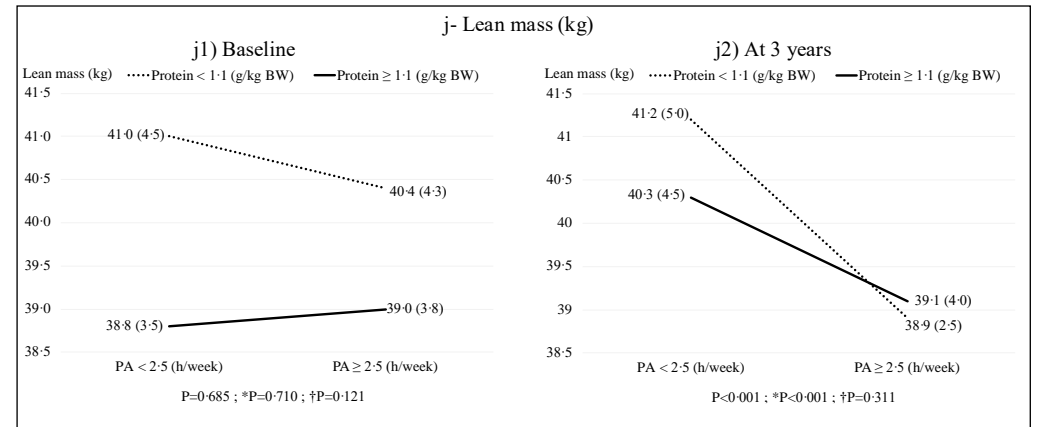
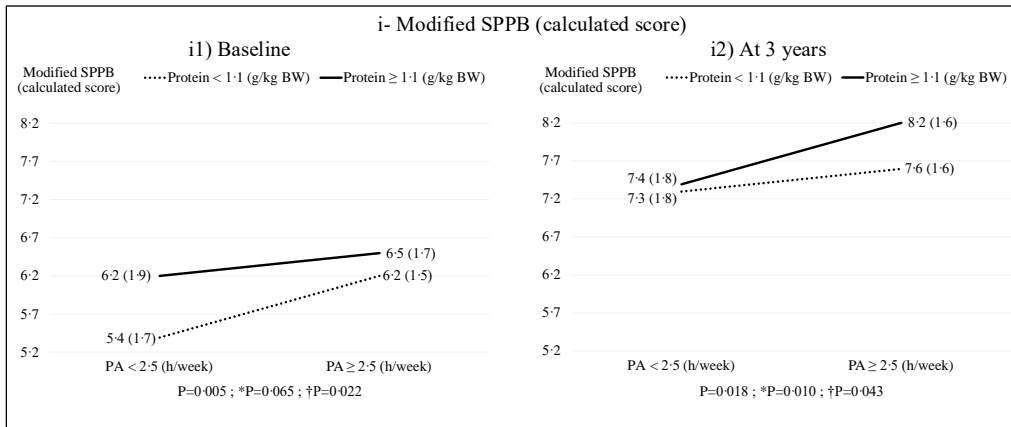
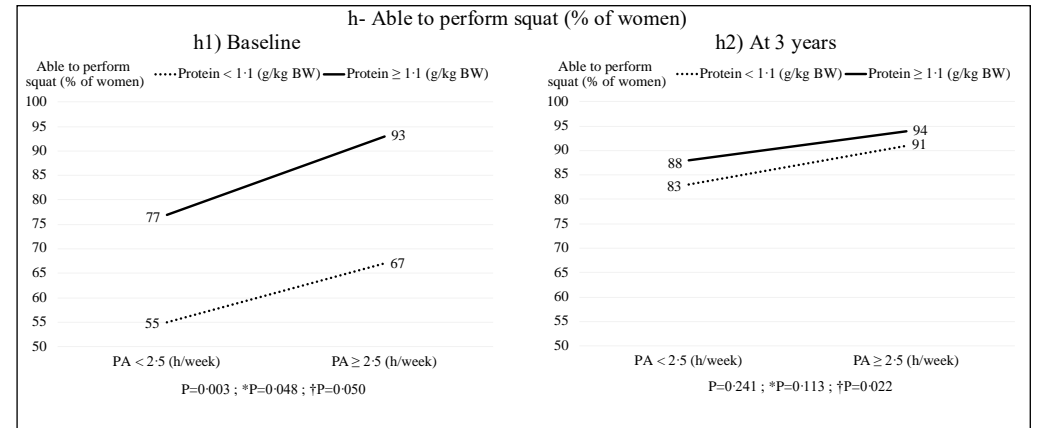
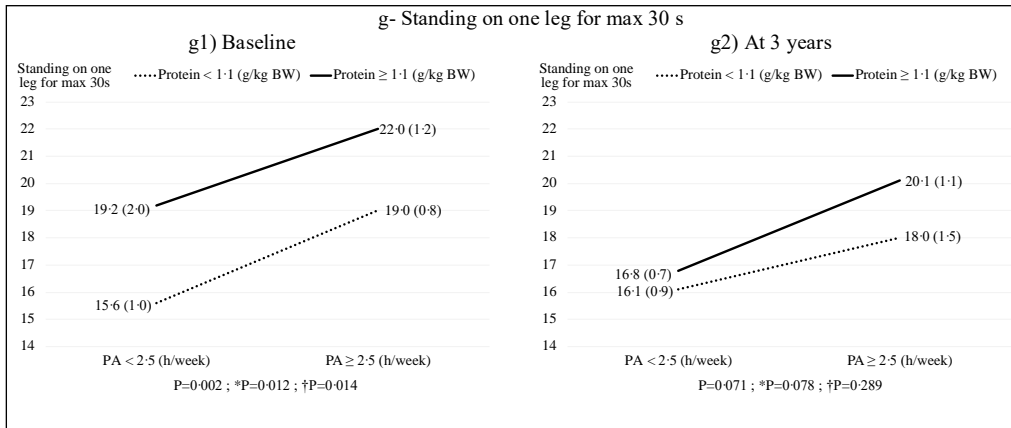


Figure 1



**Table 1.** Baseline characteristics and dietary factors of the participants according to physical activity (h/week) groups.

	Inactive PA=0 h/week, n=77	Insufficient 0<PA<2.5 h/week n=166	Sufficient PA≥2.5 h/week n=365	P
Physical activity (h/week)	0 (0)	1.2 (0.69)	6.1 (4.7)	0.0001
Physical activity range (h/week)	0-0	1.1-1.3	5-6	0.0001
Age (years)	67.7 (1.9)	68.0 (1.8)	67.7 (1.8)	0.415
Body mass index (kg/m <sup>2</sup> )	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 (1.0)	1.5 (1.2)	1.4 (1.1)	0.774
Mobility status <i>n</i> (%)				0.211
Normal	40 (80.0)	136 (88.9)	324 (96.1)	
Restricted	10 (20.0)	17 (11.1)	13 (3.9)	
Current smoker <i>n</i> (%)	2 (3.8)	10 (6.5)	14 (4.2)	0.596
Current hormone therapy <i>n</i> (%)	8 (15.1)	35 (22.6)	80 (23.3)	0.411
Dietary factors and food groups				
Energy intake (Kcal)	1390 (357)	1566 (358)	1593 (372)	0.001
Alcohol (g/d)	6.0 (4.2)	9.0 (4.6)	10.9 (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 square tests for categorical variables to calculate *n* and percentages according to physical activity groups. P values are significant 2-tailed.

**Table 2.** Baseline characteristics and dietary factors of the participants according to physical activity (h/week) groups stratified for protein intake cut-off.

	Protein intake <1.1 g/kg body weight			Protein intake ≥1.1 g/kg body weight			P
	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	
Age (years)	67.9±2.2	67.9±1.8	67.8±1.8	67.9±2.0	67.9±1.8	67.8±1.8	0.553
Body mass index (kg/m <sup>2</sup> )	30.5±4.5	29.1±4.6	28.6±4.0	30.4±4.5	29.1±4.6	27.6±4.0	0.001
Time since menopause (years)	19.4±7.6	18.4±4.9	18.4±5.2	19.2±7.1	19.1±6.2	17.5±4.7	0.282
Income (euros/month)	747±286	831±309	879±297	987±255	880±339	893±292	0.734
Number of chronic diseases	1.5±1.0	1.5±1.1	1.5±1.2	1.7±0.8	1.4±1.2	1.3±1.0	0.409
Mobility status n (%)							0.0001
Normal	32 (76.2)	93 (84.5)	212 (94.6)	8 (100)	43 (100)	112 (99.1)	
Restricted	10 (23.8)	17 (15.5)	12 (5.4)	0 (0)	0 (0)	1 (0.9)	
Current smoker n (%)	2 (4.7)	9 (8.2)	13 (5.8)	0 (0)	1 (2.3)	1 (0.9)	0.644
Current hormone therapy n (%)	7 (15.6)	26 (23.4)	55 (23.8)	1 (12.5)	9 (20.5)	25 (22.1)	0.472
Dietary factors and food groups							
Energy intake (Kcal)	1334±352	1441±294	1461±321	1709±177	1881±308	1865±319	0.025
Alcohol (g/d)	4.8±1.2	7.7±1.1	10.7±1.8	12.3±1.5	12.3±1.5	11.3±1.7	0.044
Protein (g/d)	55.7±15.6	61.8±11.9	60.5±13.4	81.8±13.7	86.2±14.5	86.9±13.6	0.033
Protein (g/kg body weight)	0.69±0.2	0.81±0.14	0.83±0.16	1.2±0.1	1.3±0.2	1.3±0.2	0.045
Carbohydrate (g/d)	168±47	179±40	182±42	214±33.3	224±42	226±48	0.137
Fat intake (g/d)	46.2±15.5	49.6±15.8	50.3±16.9	55.6±16.9	63.9±17.2	226±48	0.171

ANOVA was used to calculate means and standard deviations, and chi square 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 (h/week) groups.

	PA=0 h/week	0<PA<2.5 h/week	PA≥2.5 h/week	PA at one-hour intervals			
Number of participants	n=77	n=166	n=365	n=608	P	*P	†P
	Mean ± SD	Mean ± SD	Mean ± SD	β (SE)	Model 1	Model 2	Model 2
<b>Hand grip strength (kg)</b>							
Baseline	26.0±5.1	25.6±4.7	25.8±4.8	-0.006 (0.060)	0.391	0.501	0.886
At 3 years <sup>a</sup>	25.2±6.3	24.8±6.1	24.7±5.5	-0.031 (0.058)	0.698	0.478	0.443
<b>Hand grip strength adjusted for BMI (kg/m<sup>2</sup>)</b>							
Baseline	0.90±0.24	0.93±0.22	0.97±0.21	-0.007 (0.060)	0.387	0.486	0.867
At 3 years	0.90±0.24	0.93±0.22	0.97±0.21	-0.044 (0.002)	0.211	0.651	0.787
<b>Knee extension (Newton)</b>							
Baseline	275±89	288±82	302±82	0.079 (0.986)	0.118	0.121	0.075
<b>Chair rises in 20 s</b>							
Baseline	6.2±3.0	7.5±2.6	8.0±2.6	0.248 (0.047)	0.047	0.046	0.040
At 3 years	7.2±4.2	8.1±3.1	9.2±3.1	0.002 (0.031)	0.078	0.077	0.098
<b>Tandem walk over 6 m (s)</b>							
Baseline	17.0±10.0	19.4±8.1	19.6±8.5	0.027 (0.003)	0.605	0.349	0.549
At 3 years	19.8±4.4	20.4±7.0	20.1±10.4	0.038 (0.153)	0.973	0.588	0.406
<b>Walking speed over 10 m (m/s)</b>							
Baseline	1.4±0.2	1.5±0.2	1.7±0.2	0.107 (0.004)	0.015	0.012	0.005
At 3 years	1.4±0.2	1.5±0.3	1.6±0.3	0.017 (0.311)	0.099	0.050	0.048
<b>Standing on one leg for max 30 s (s)</b>							
Baseline	13.2±11.1	17.6±10.2	20.2±9.1	0.113 (0.105)	0.011	0.124	0.095
At 3 years	15.2±12.0	17.0±11.5	17.8±10.6	0.057 (0.097)	0.114	0.250	0.194
<b>Modified short physical performance battery (calculated score)</b>							
Baseline	5.2±1.5	5.8±1.7	6.4±1.9	0.118 (0.046)	0.005	0.065	0.022
At 3 years	7.8±2.4	7.6±1.6	7.6±1.7	0.009 (0.021)	0.387	0.433	0.840

**Able to perform squat (% of women)**

Baseline	43	71	72	0.185 (0.002)	0.027	0.039	0.001
At 3 years	76	89	93	0.039 (0.004)	0.001	0.005	0.007

**Lean mass (kg)**

Baseline	41.2±4.0	40.4±4.3	39.9±4.2	0.045 (0.037)	0.654	0.733	0.191
At 3 years	41.3±4.9	40.7±4.7	40.3±4.6	0.046 (0.039)	0.718	0.882	0.154

**Relative skeletal muscle index (kg/m<sup>2</sup>)**

Baseline	6.8±0.7	6.8±0.6	6.7±0.6	0.047 (0.017)	0.258	0.214	0.180
At 3 years	6.6±0.9	6.7±0.6	6.6±0.6	0.097 (0.008)	0.419	0.517	0.318

**Fat mass (kg)**

Baseline	33.1±10.0	30.0±7.7	27.4±8.4	-0.196 (0.222)	0.001	0.001	0.0001
At 3 years	31.8±12.1	30.1±8.4	27.6±8.9	-0.140 (0.237)	0.018	0.020	0.014

PA, physical activity; SD, standard deviation; SE, standard error; SPPB, short physical performance battery.

UNIANOVA was used to calculate means ± SD adjusted. Multiple regression analysis was used to calculate coefficient  $\beta$  and standard error.

Model 1 was adjusted for age and energy intake.

Model 2 was adjusted for age, energy intake (Kcal), 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 Means and SD calculated from UNIANOVA adjusted for variables in model 1.

\*P Means and SD calculated from UNIANOVA adjusted for variables in model 2.

†P calculated from multiple regression analysis, regression coefficient adjusted for variables in model 2.

Table 4. Association of PA and protein intake interaction as categorical and continuous variables with physical function assessment at baseline and 3-year follow-up.

Number of participants	PA<2.5 h/week, Protein <1.1 g/kg BW		PA<2.5 h/week, Protein ≥1.1 g/kg BW		PA≥2.5h/wee k, Protein <1.1 g/kg BW		PA≥2.5h/week Protein ≥1.1 g/kg BW		PA and protein intake interaction continuous variable	P	*P	†P
	n=147		n=46		n=240		n=119		n=552	Model 1	Model 2	Model 2
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	B (SE)			
<b>Hand grip strength (kg)</b>												
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	25.0	5.6	25.2	4.8	24.1	5.0	-0.008 (0.05)	0.718	0.896	0.852
Change	-1.0	0.42	0.34	0.78	-1.15	0.32	-0.29	0.47	-0.030 (0.053)	0.274	0.250	0.511
<b>Hand grip strength adjusted for BMI (kg/m<sup>2</sup>)</b>												
Baseline <sup>a</sup>	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
<b>Knee extension (Newton)</b>												
Baseline <sup>a</sup>	291	7.8	277	13.2	304	5.9	289	8.5	0.090 (0.929)	0.211	0.115	0.054
<b>Chair rises in 20 s</b>												
Baseline <sup>a</sup>	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	0.01	0.04	0.00	0.07	0.02	0.03	-0.09	0.03	0.088 (0.045)	0.913	0.897	0.057
<b>Tandem walk speed over 6 m (m/s)</b>												
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	-0.02	0.07	-0.03	0.03	0.03	0.04	-0.042 (0.004)	0.786	0.788	0.396
<b>Walking speed over 10 m (m/s)</b>												
Baseline <sup>a</sup>	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	-0.08	0.03	-0.17	0.05	-0.11	0.02	-0.13	0.03	-0.586 (0.004)	0.570	0.486	0.558
<b>Standing on one leg for max 30 s</b>												
Baseline <sup>a</sup>	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

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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	-0.3	1.2	-0.2	2.2	-2.3	0.9	-3.0	1.4	-0.012 (0.118)	0.418	0.361	0.801
<b>Able to perform squat (% of women)</b>												
Baseline <sup>a</sup>	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
<b>Modified SPPB (calculated score)</b>												
Baseline <sup>a</sup>	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	1.2	2.2	1.4	1.8	1.5	2.1	-0.125 (0.027)	0.112	0.074	0.098
<b>Lean mass (kg)</b>												
Baseline <sup>a</sup>	41.0	4.5	38.8	3.5	40.4	4.3	39.0	3.8	0.096 (0.022)	0.685	0.710	0.121
At 3 years	41.2	5.0	40.3	4.5	38.9	2.5	39.1	4.0	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
<b>RSMI (kg/m<sup>2</sup>)</b>												
Baseline <sup>a</sup>	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
<b>Fat mass (kg)</b>												
Baseline <sup>a</sup>	33.6	8.2	23.8	6.6	29.4	8.4	24.2	6.2	-0.206 (0.089)	<0.001	<0.001	<0.001
At 3 years	33.0	9.4	29.7	8.6	22.9	6.5	23.8	6.3	-0.198 (0.097)	<0.001	<0.001	<0.001
Change	0.05	3.12	0.08	3.21	-0.94	2.31	-0.19	2.18	-0.095 (3.431)	0.487	0.605	0.040

BW, body weight; PA, physical activity; SD, standard deviation; SE, standard error; SPPB, short physical performance battery.

UNIANOVA was used to calculate means  $\pm$  SD adjusted. Multiple regression analysis was used to calculate coefficient  $\beta$  and standard error.

Model 1 was adjusted for age and energy intake. Model 2 was adjusted for age, energy intake (Kcal), 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 Means and SD calculated from UNIANOVA adjusted for variables in model 1.

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†P calculated from multiple regression analysis, regression coefficient adjusted for variables in model 2.