The Journal of Nutrition, Health and Aging 28 (2024) 100002

Contents lists available at ScienceDirect

The Journal of Nutrition, Health and Aging

journal homepage: www.elsevier.com/locate/jnha

Original Article

Animal and vegetable protein intake and malnutrition in older adults: a multicohort study

Adrián Carballo-Casla [a,b,c,*], Mercedes Sotos-Prieto [a,b,d,e], Esther García-Esquinas [b,f], Ellen A Struijk [a,b], Francisco Félix Caballero [a,b], Amaia Calderón-Larrañaga [c], Esther Lopez-Garcia [a,b,d], Fernando Rodríguez-Artalejo [a,b,d], Rosario Ortolá [a,b,*]

[a] *Department of Preventive Medicine and Public Health, Universidad Autónoma de Madrid/Idipaz, Calle del Arzobispo Morcillo 4, 28029 Madrid, Spain*
[b] *Center for Networked Biomedical Research in Epidemiology and Public Health (CIBERESP), Avenida de Monforte de Lemos 3-5, 28029 Madrid, Spain*
[c] *Aging Research Center, Department of Neurobiology, Care Sciences and Society, Karolinska Institutet & Stockholm University, Tomtebodavägen 18 A SE-171 77 Stockholm, Sweden*
[d] *IMDEA Food Institute. CEI UAM + CSIC, Carretera de Canto Blanco 8, 28049 Madrid, Spain*
[e] *Department of Environmental Health, Harvard T.H. Chan School of Public Health, 665 Huntington Avenue, Boston, MA 02115, USA*
[f] *National Center of Epidemiology, Instituto de Salud Carlos III, Avenida de Monforte de Lemos 5, Hall 12, 28029 Madrid, Spain*

ARTICLE INFO

Keywords:
Recommended dietary allowance
Protein supplement
Biological value
Plant protein
Vegetarian
Legume
Poultry
Inflammation
Environmental impact
One health

ABSTRACT

Objectives: Malnutrition is a global concern in older adults, as it negatively affects morbidity and mortality. While higher animal protein intake may help prevent and treat malnutrition, it might also increase the risk of chronic diseases and death. Conversely, vegetable protein intake might have a lower anabolic effect and not be as effective to improve nutritional status. We studied whether animal and vegetable protein intake are associated with changes in nutritional status in older adults.
Design: We used pooled data from two Spanish cohorts: the Seniors-ENRICA 1 and Seniors-ENRICA 2.
Settings and participants: 2,965 community-dwelling adults aged 62–92 years.
Measurements: Protein intake was estimated at baseline via an electronic, validated diet history. Nutritional status was assessed at baseline and after 2.6 years with the GLIM (Global Leadership Initiative on Malnutrition) phenotypic criteria: weight loss, low body mass index, and reduced muscle mass. The odds of improvements in nutritional status were assessed with logistic regression models, extensively adjusted for potential confounders.
Results: Higher animal and vegetable protein intake were associated with improvements in nutritional status [odds ratios (95% confidence intervals) per 0.25 g/kg/day were 1.15 (1.00, 1.32) and 1.77 (1.35, 2.32), respectively]. Cereal protein intake drove most of the latter association [2.07 (1.44, 2.98)]. Replacing 0.25 g/kg/day of total animal protein, meat, or fish protein (but not dairy or egg protein) with vegetable protein was associated with improvements in nutritional status [1.54 (1.13, 2.09), 1.70 (1.20, 2.41), and 1.77 (1.18, 2.64), respectively].
Conclusions: Higher animal and, especially, vegetable protein intake were associated with improvements in nutritional status in older adults. Replacing total animal protein, meat, or fish protein with vegetable protein may help improve malnutrition.

1. Introduction

1.1. Background & rationale

Malnutrition (i.e., undernutrition) is a global concern that negatively affects a wide array of outcomes: from activities of daily living, physical function, and rehabilitation effectiveness, to morbidity and mortality [1,2]. Malnutrition may be caused by a combination of reduced nutrient intake or assimilation and varying degrees of inflammation, leading to altered body composition and diminished biological function [1]. This condition is potentially reversible, and intervention trials targeting protein and/or energy intake have shown benefits on weight, muscle mass and strength, physical function, quality of life, hospitalization, and mortality [2,3]. In high-income countries, much of the malnutrition burden is carried by the older adult population, where prevalence rates could be as high as 28%, 18%, and 9% in the hospital, residential care, and

---

* Corresponding authors.
E-mail addresses: adrian.carballo@uam.es, adrian.carballo.casla@ki.se (A. Carballo-Casla), rosario.ortola@uam.es (R. Ortolá).

http://doi.org/10.1016/j.jnha.2023.100002
Received 16 October 2023; Received in revised form 17 November 2023; Accepted 19 November 2023

community settings, respectively [4]. This is likely because both reduced food consumption (e.g., due to sensory declines, changes in hormones and neurotransmitters, declines in social health, or psychological problems) and chronic organ diseases that lead to inflammation are most common in this population subgroup [1,5,6].

Aging is also characterized by multiple physiological changes, which alter protein utilization and requirements [5]. One of these is anabolic resistance, meaning that protein synthesis may not be sufficient with the dietary allowances recommended for younger adults, potentially leading to decreased muscle mass and strength during old age [7]. This is supported by the fact that protein intake above these levels has been shown to increase muscle protein synthesis and lean mass in older adults [8]. In line with said findings, most guidelines set protein requirements of healthy individuals over 65 years at 1.0–1.2 grams/kilogram of body weight/day (g/kg/day), and advocate for additional increases for those with acute or chronic diseases, injuries, or malnutrition [5]. Still, many older adults fall short of these requirements. In a pooled dataset from North American and European countries, the prevalence of protein intake below recommendations was 47% and 71% when using 1.0 and 1.2 g/kg/day cut-offs, respectively [9].

While higher protein intake may help prevent and treat malnutrition, it might as well increase the risk of cardiovascular disease, cancer, and all-cause mortality [10,11]. However, the main driver of these associations seems to be animal protein, while plant protein intake has been linked with lower risk of death [10,12]. On the other hand, it is generally acknowledged that animal-based proteins have a higher anabolic effect than vegetable proteins due to their better digestibility and essential amino acid content [8], so their intake could translate into a lower risk of malnutrition than that of vegetable protein.

1.2. Objectives

To shed light on the role of protein sources on malnutrition, we studied whether vegetable and animal protein intake were associated with changes in nutritional status in a pooled sample of community-dwelling older adults. We delved deeper into these associations by examining the role of protein intake from the main vegetable and animal sources.

2. Methods

2.1. Study design and participants

We used data from the Seniors-ENRICA 1 and Seniors-ENRICA 2 studies (ClinicalTrials.gov Identifiers: NCT01133093 and NCT03541135), two Spanish cohorts of community-dwelling individuals aged ≥60 years and ≥65 years, respectively. The Seniors-ENRICA 1 cohort was set up in 2012 and participants were followed-up in 2014–2015 [13,14]. Those in the Seniors-ENRICA 2 cohort were recruited in 2015–2017 and followed-up in 2018–2019 [14,15].

Data collection was very similar in both cohorts and waves. Data on socio-demographic, lifestyle, and morbidity variables were collected via computer-assisted telephone interviews, while trained personnel conducted home-based, electronic, validated diet histories (to assess food consumption) and a set of physical examinations (including grip strength, bioelectrical impedance, weight and height measurements, and blood draws) [13,16]. The Clinical Research Ethics Committee of the “La Paz” University Hospital in Madrid approved the research protocol of each cohort, and all participants gave written informed consent.

2.2. Variables

2.2.1. Protein intake

Food consumption was assessed with a validated, face-to-face, electronic diet history, where subjects could report up to 860 foods and recipes habitually consumed during the previous year [13,17]. Portion sizes were estimated with the help of 127 photographs and household measures. To convert food consumption into protein intake, the diet history used data from six food composition tables from Spain and five tables from other countries [17]. According to the food source, proteins were deemed of vegetable (cereals, legumes, nuts, and other vegetable foods) or animal origin (dairy, meat, eggs, fish, and other animal foods). The diet history was validated against seven 24-h recalls over one year, and the mean correlation coefficients for vegetable and animal protein were both 0.62 [17].

2.2.2. Malnutrition

Malnutrition was assessed according to the Global Leadership Initiative on Malnutrition (GLIM), which was launched in 2016 to build a global consensus on diagnostic criteria for malnutrition in adults in clinical settings, and proposed three phenotypic criteria (involuntary weight loss, low body mass index, and reduced muscle mass) and two etiologic criteria (reduced food consumption or nutrient assimilation, and inflammation) [1].

Metrics for grading malnutrition as moderate or severe also followed the GLIM initiative [1]. First, it was considered that any weight loss of 5–10 % within the previous 3 months or 10–20 % at or beyond 12 months was moderate, and that of >10% within the previous 3 months or >20% at or beyond 12 months was severe [1]. Second, body mass index (measured with electronic scales and stadiometers) $<20\,kg/m^2$ if <70 years or $<22\,kg/m^2$ if ≥70 years was considered moderately low, and $<18.5\,kg/m^2$ if <70 years or $<20\,kg/m^2$ if ≥70 years was severely low [1]. Third, muscle mass deficit was deemed moderate if grip strength (assessed with dynamometers) was <27 kg in men and <16 kg in women [1,18], and severe if, in addition to low strength, muscle mass was reduced (assessed with bioelectrical impedance and anthropometry). We used several metrics to this end: appendicular skeletal muscle mass <20 kg in men or <15 kg in women, appendicular skeletal muscle mass index $<7\,kg/m^2$ in men or $<5.5\,kg/m^2$ in women, fat-free mass index $<17\,kg/m^2$ in men or $<15\,kg/m^2$ in women, mid-arm circumference ≤21 cm, or calf circumference <31 cm [1,18]. Of note is that grading malnutrition as moderate or severe required at least one phenotypic criterion that met that grade [1].

As for the etiologic criteria, reduced food consumption or nutrient assimilation meant that energy intake was ≤50% of requirements, there had been any reduction in food consumption in the previous 3 months, or there was any chronic gastrointestinal condition that adversely impacted nutrient assimilation or absorption. On the other hand, inflammation was deemed to impact nutritional status if a selection of chronic diseases was present or supportive laboratory measures were altered.

Further information on the operationalization of the malnutrition criteria in the Seniors-ENRICA 1 and Seniors-ENRICA 2 cohorts is available in Supplemental Appendix 1. The data sources used for every criterion for the diagnosis of malnutrition at every time point and cohort can be found in Supplemental Table 1.

Since only the malnutrition phenotypic criteria are used for severity grading in the GLIM initiative, while the etiological criteria were arguably conceived for clinical settings, we restricted the main analyses to the phenotypic criteria for the diagnosis of malnutrition [1].

According to the changes in nutritional status from baseline to follow-up, study participants were classified into two groups: (1) those with improvements in nutritional status, defined as the transition from moderate malnutrition to no malnutrition, or from severe malnutrition to moderate malnutrition or no malnutrition; and (2) those with no change or worsening nutritional status, defined as the transition from no malnutrition to moderate or severe malnutrition, or from moderate malnutrition to severe malnutrition.

2.2.3. Potential confounders

We considered six sets of possible confounders of the study associations. Regarding sociodemographic characteristics, we gathered data on sex, age, living conditions (reported difficulty to make ends meet, from very difficult to very easy), self-reported living arrangements (alone, with a spouse/partner, or other), and educational level (primary or less, secondary, or university) [13]. Since protein intake may be correlated with energy intake even after accounting for body weight, energy intake (kcal/day) was taken into account [17]. We also considered lifestyle variables, specifically tobacco smoking (never, former, or current), alcohol consumption (never, former, or current), recreational physical activity (Metabolic Equivalents of task-hours/week), estimated with the validated EPIC-cohort questionnaire [15], and sedentary behavior, defined as time spent watching television (hours/week) and assessed with the Nurses' Health Study questionnaire [15]. We gathered data on several morbid conditions: diabetes (either treatment with antidiabetic drugs, blood glucose levels $\geq$126 mg/dL, or physician-diagnosed diabetes), cardiovascular disease (coronary heart disease, stroke, or heart failure), chronic obstructive pulmonary disease, musculoskeletal disease (osteoarthritis, arthritis, or hip fracture), cancer, and depression (requiring medical treatment). Diagnoses could be self-reported or taken from electronic health records (if available) [13]. Limitations in instrumental activities of daily living were assessed with the Lawton and Brody scale [19]. The cutoff for dependence was set at $\leq$7 instrumental activities of daily living for women and $\leq$4 for men [19]. Finally, we accounted for diet quality using data on fruit, vegetable, and sugar-sweetened beverage consumption, which were taken from the diet history [17].

2.3. Statistical methods

2.3.1. Study size

From the 5,793 participants at baseline, 141 (2.4%) died and 1,933 (33.4%) were lost to follow-up. From the remaining 3,718 participants, we further excluded 753 (13.0%) with inadequate data (210 had no information on diet, 714 on malnutrition, and 215 on potential confounders; note that some participants lacked data in more than one variable). Hence, the analytical sample comprised 2,965 individuals. Baseline characteristics of the participants, by inclusion status in the analytical sample, are shown in Supplemental Table 2.

2.3.2. Main analyses

The associations of vegetable and animal protein intake with improvements in nutritional status were summarized with odds ratios (OR) and their 95% confidence interval (CI), obtained from logistic regression models. We used two *a priori* incrementally adjusted models to control for potential confounding: the first, adjusted for sociodemographic variables and energy intake, and the second, additionally adjusted for lifestyle, morbidity, dependence in instrumental activities of daily living, and diet quality. The pooled analyses were also adjusted for cohort.

To account for the potential effect of body size on nutrient intake, protein was expressed as g/kg/day, in line with most guidelines on

Table 1
Baseline characteristics of the participants in the pooled sample, by quartiles of animal and vegetable protein intake.

	Animal protein intake				Vegetable protein intake			
	Quartile 1[a]	Quartile 2[a]	Quartile 3[a]	Quartile 4[a]	Quartile 1[b]	Quartile 2[b]	Quartile 3[b]	Quartile 4[b]
n	741	740	741	740	741	740	741	740
Sex-Men, n (%)	374 (50.5)	389 (52.6)	375 (50.6)	319 (43.1)*	372 (50.2)	387 (52.3)	384 (51.8)	314 (42.4)*
Age (years)	71.8 (5.00)	71.7 (5.07)	71.1 (4.95)	71.2 (5.10)*	71.6 (5.24)	71.5 (4.94)	71.2 (4.79)	71.4 (5.16)
Living conditions (make ends meet) [c]	4.39 (1.07)	4.53 (1.00)	4.55 (0.94)	4.58 (0.97)*	4.48 (1.01)	4.47 (1.08)	4.50 (0.99)	4.59 (0.91)
Living arrangements, n (%)								
Alone	163 (22.0)	156 (21.1)	154 (20.8)	150 (20.3)	168 (22.7)	155 (20.9)	137 (18.5)	163 (22.0)*
With a spouse/partner	566 (76.4)	579 (78.2)	577 (77.9)	584 (78.9)	559 (75.4)	582 (78.6)	597 (80.6)	568 (76.8)
Other	12 (1.62)	5 (0.68)	10 (1.35)	6 (0.81)	14 (1.89)	3 (0.41)	7 (0.94)	9 (1.22)
Educational level, n (%)								
Primary or less	429 (57.9)	415 (56.1)	409 (55.2)	390 (52.7)	400 (54.0)	400 (54.1)	432 (58.3)	411 (55.5)
Secondary	149 (20.1)	166 (22.4)	175 (23.6)	160 (21.6)	159 (21.5)	174 (23.5)	148 (20.0)	169 (22.8)
University	163 (22.0)	159 (21.5)	157 (21.2)	190 (25.7)	182 (24.6)	166 (22.4)	161 (21.7)	160 (21.6)
Tobacco smoking, n (%)								
Never	390 (52.6)	400 (54.1)	407 (54.9)	415 (56.1)	390 (52.6)	384 (51.9)	395 (53.3)	443 (59.9)
Former	274 (37.0)	271 (36.6)	268 (36.2)	261 (35.3)	280 (37.8)	281 (38.0)	273 (36.8)	240 (32.4)
Current	77 (10.4)	69 (9.32)	66 (8.91)	64 (8.65)	71 (9.58)	75 (10.1)	73 (9.85)	57 (7.70)
Alcohol consumption, n (%)								
Never	149 (20.1)	119 (16.1)	125 (16.9)	142 (19.2)	142 (19.2)	122 (16.5)	115 (15.5)	156 (21.1)
Former	74 (9.99)	79 (10.7)	79 (10.7)	86 (11.6)	76 (10.3)	78 (10.5)	83 (11.2)	81 (10.9)
Current	518 (69.9)	542 (73.2)	537 (72.5)	512 (69.2)	523 (70.6)	540 (73.0)	543 (73.3)	503 (68.0)
Physical activity (MET-hours/week)	24.4 (17.5)	25.6 (18.2)	26.2 (17.9)	27.2 (17.7)*	23.4 (18.5)	26.1 (17.5)	26.6 (17.5)	27.3 (17.6)*
Sedentary behavior (TV hours/week)	21.0 (11.6)	21.5 (10.5)	20.6 (10.5)	19.2 (9.84)*	21.2 (11.2)	20.4 (10.7)	20.9 (10.7)	19.7 (9.95)*
Energy intake (kcal/day)	1874 (360)	1953 (343)	2014 (382)	2129 (482)*	1840 (327)	1928 (347)	2031 (379)	2171 (478)*
Chronic diseases, n (%)								
Diabetes	165 (22.3)	161 (21.8)	144 (19.4)	125 (16.9)*	188 (25.4)	172 (23.2)	130 (17.5)	105 (14.2)*
Cardiovascular disease	103 (13.9)	84 (11.4)	84 (11.3)	85 (11.5)	85 (11.5)	93 (12.6)	99 (13.4)	79 (10.7)
Chronic lung disease	103 (13.9)	100 (13.5)	96 (13.0)	96 (13.0)	104 (14.0)	118 (15.9)	87 (11.7)	86 (11.6)*
Musculoskeletal disease	453 (61.1)	434 (58.6)	421 (56.8)	415 (56.1)	456 (61.5)	442 (59.7)	411 (55.5)	414 (55.9)*
Cancer	78 (10.5)	75 (10.1)	71 (9.58)	62 (8.38)	72 (9.72)	58 (7.84)	76 (10.3)	80 (10.8)
Depression	72 (9.72)	68 (9.19)	46 (6.21)	59 (7.97)	69 (9.31)	69 (9.32)	51 (6.88)	56 (7.57)
Dependence in IADL, n (%) [d]	48 (6.48)	46 (6.22)	44 (5.94)	34 (4.59)	66 (8.91)	32 (4.32)	43 (5.80)	31 (4.19)*
Food consumption (g/day)								
Fruits	350 (179)	341 (175)	338 (182)	349 (200)	316 (172)	334 (177)	349 (180)	380 (201)*
Vegetables	222 (127)	223 (126)	225 (119)	245 (137)*	200 (110)	220 (122)	238 (120)	257 (149)*
Sugar-sweetened beverages	61.6 (141)	49.3 (122)	41.1 (96.8)	37.3 (98.9)*	59.0 (122)	56.9 (139)	43.2 (102)	30.1 (94.2)*

Values are numbers (%) or means (standard deviations).

* P-value <0.05 for differences in means (ANOVA) or proportions (Pearson's chi-squared) across quartiles of animal or vegetable protein intake.

[a] Animal protein intake: Quartile 1, 0.27 to 0.68 g/kg/day; Quartile 2, 0.68 to 0.81 g/kg/day; Quartile 3, 0.81 to 0.96 g/kg/day; Quartile 4, 0.96–2.39 g/kg/day.

[b] Vegetable protein intake: Quartile 1, 0.05 to 0.35 g/kg/day; Quartile 2, 0.35 to 0.41 g/kg/day; Quartile 3, 0.41 to 0.49 g/kg/day; Quartile 4, 0.50–1.44 g/kg/day.

[c] Living conditions: difficulty to make ends meet, from very difficult (1) to very easy (6).

[d] Instrumental activities of daily living.

protein requirements in older adults [5]. To assess dose-response relationships, protein intake was modeled in the analyses as: (1) a continuous variable (per 0.25 g/day/kg, roughly one standard deviation increment); (2) a categorical variable (quartiles, using the lowest as reference); and (3) a restricted cubic spline (knots located at the 10th, 50th, and 90th percentiles). Further details on variable categorization can be found in tables and figures.

Substitution models (i.e., logistic regression models including protein intake from all animal sources and vegetable protein intake) were used to examine the association between the theoretical replacement of total animal protein (and that from individual animal sources) with vegetable protein and improvements in nutritional status. Substitution coefficients were calculated by subtracting the coefficient for the corresponding animal source of protein from that for total vegetable protein intake.

2.3.3. Ancillary analyses

Additional analyses were conducted with similar models to those described in the previous section. First, the main analyses were stratified by etiologic criteria for the diagnosis of malnutrition. Since these criteria are partially based on the presence of certain chronic diseases, the analyses were not adjusted for morbidity. Second, we computed the associations of protein intake from animal subgroups with improvements in nutritional status. Third, we assessed the theoretical replacement of protein from animal subgroups with vegetable protein, as well as the replacement of total animal protein (and that from individual animal sources) with cereal protein (i.e., the main vegetable protein source).

We also conducted four sensitivity analyses. First, to better account for the overall health status of the participants, we adjusted the models for the frailty phenotype (i.e., unintentional weight loss, self-reported exhaustion, weakness, slow walking speed, and low physical activity) instead of limitations in instrumental activities of daily living [20]. Second, to minimize the potential for residual dietary confounding, we adjusted the analyses for the Mediterranean Diet Adherence Screener instead of fruits, vegetables, and sugar-sweetened beverages [21]. Fourth, since adjusting the models for total energy intake could potentially pose problems of estimation and interpretation, we also used the residual method, that is to say, we modeled the residuals from the regression of protein intake on energy intake instead of protein intake itself [22]. Fourth, since protein intake in the Seniors-ENRICA 1 cohort underwent substantial variation from 2008 to 2010 to 2012, and such changes have been linked with the accumulation of health deficits (including low grip strength, unintentional weight loss, and low body mass index) [23], we also studied whether changes in vegetable and animal protein intake were associated with improvements in nutritional status in this cohort (note that longitudinal dietary data were not available in the Seniors-ENRICA 2 cohort).

Finally, we examined if the cohorts, sex, age, living arrangements, physical activity, sedentary behavior, self-reported oral health, animal, vegetable, or total protein intake modified the main study associations by using Wald tests that compared models with and without interaction terms, defined as the product of these variables by vegetable or animal protein intake (expressed as g/kg/day).

Analyses were performed with the Stata 17 software (Stata Corp. 2021. Stata Statistical Software: Release 17. College Station, TX: Stata Corp LP).

Table 2

Odds Ratios (95% confidence interval) for the association of animal and vegetable protein intake with improvements in nutritional status over 2.6 years.

	Animal protein intake				
	Quartile 1[c]	Quartile 2[c]	Quartile 3[c]	Quartile 4[c]	Per 0.25 g/kg/day
Pooled sample					
Cases/n	75/741	70/740	66/741	81/740	292/2962
Model 1[a]	Ref.	1.02 (0.72,1.44)	0.99 (0.70,1.42)	1.27 (0.89,1.81)	1.14 (1.00,1.31)
Model 2[b]	Ref.	1.00 (0.70,1.43)	1.00 (0.69,1.43)	1.29 (0.90,1.85)	1.15 (1.00,1.32)*
Seniors-ENRICA-1					
Cases/n	42/350	42/342	40/338	53/365	177/1395
Model 1[a]	Ref.	1.12 (0.71,1.78)	1.15 (0.72,1.85)	1.55 (0.98,2.45)	1.22 (1.04,1.44)*
Model 2[b]	Ref.	1.10 (0.69,1.76)	1.17 (0.73,1.89)	1.57 (0.98,2.51)	1.23 (1.04,1.45)*
Seniors-ENRICA-2					
Cases/n	33/391	28/398	26/403	28/375	115/1567
Model 1[a]	Ref.	0.89 (0.53,1.52)	0.82 (0.48,1.40)	0.96 (0.56,1.65)	1.01 (0.81,1.26)
Model 2[b]	Ref.	0.89 (0.52,1.52)	0.81 (0.47,1.40)	0.99 (0.58,1.70)	1.02 (0.81,1.27)
	Vegetable protein intake				
	Quartile 1[d]	Quartile 2[d]	Quartile 3[d]	Quartile 4[d]	Per 0.25 g/kg/day
Pooled sample					
Cases/n	77/741	66/740	61/741	88/740	292/2962
Model 1[a]	Ref.	0.98 (0.68,1.39)	0.97 (0.67,1.39)	1.56 (1.09,2.24)*	1.61 (1.25,2.09)***
Model 2[b]	Ref.	1.01 (0.71,1.44)	1.01 (0.69,1.47)	1.71 (1.18,2.48)**	1.77 (1.35,2.32)***
Seniors-ENRICA-1					
Cases/n	53/404	43/339	30/318	51/334	177/1395
Model 1[a]	Ref.	1.05 (0.68,1.63)	0.79 (0.49,1.28)	1.63 (1.04,2.57)*	1.48 (1.08,2.03)*
Model 2[b]	Ref.	1.09 (0.70,1.70)	0.81 (0.49,1.33)	1.77 (1.12,2.81)*	1.61 (1.17,2.23)**
Seniors-ENRICA-2					
Cases/n	24/337	23/401	31/423	37/406	115/1567
Model 1[a]	Ref.	0.88 (0.48,1.59)	1.22 (0.70,2.15)	1.51 (0.87,2.62)	1.88 (1.26,2.82)**
Model 2[b]	Ref.	0.90 (0.49,1.65)	1.30 (0.74,2.30)	1.66 (0.95,2.92)	2.09 (1.38,3.16)***

*$p < 0.05$. **$p < 0.01$. ***$p < 0.001$.

[a] Model 1: Logistic regression model adjusted for cohort (pooled sample), sex, age, living conditions (make ends meet), living arrangements (alone, with a spouse/partner, or other), educational level (primary or less, secondary, or university), and energy intake (kcal/day) at baseline.

[b] Model 2: As Model 1 and additionally adjusted for smoking status (never, former, or current), alcohol consumption (never, former, or current), leisure-time physical activity (MET-hours/week), sedentary behavior (TV hours/week), diabetes, cardiovascular disease, chronic lung disease, musculoskeletal disease, cancer, depression, dependence in instrumental activities of daily living, fruit, vegetable, and sugar-sweetened beverage consumption (g/day).

[c] Animal protein intake: Quartile 1, 0.27 to 0.68 g/kg/day; Quartile 2, 0.68 to 0.81 g/kg/day; Quartile 3, 0.81 to 0.96 g/kg/day; Quartile 4, 0.96–2.39 g/kg/day.

[d] Vegetable protein intake: Quartile 1, 0.05 to 0.35 g/kg/day; Quartile 2, 0.35 to 0.41 g/kg/day; Quartile 3, 0.41 to 0.49 g/kg/day; Quartile 4, 0.50–1.44 g/kg/day.

3. Results

3.1. Descriptive data

Characteristics of the study participants are shown in Table 1. On one hand, higher animal protein intake was associated with being female, younger, having better living conditions, being more physically active and less sedentary, having higher energy intake, not having diabetes, eating more vegetables, and drinking fewer sugar-sweetened beverages. On the other hand, higher vegetable protein intake was correlated with being female, having less dependence in instrumental activities of daily living, being more physically active and less sedentary, having higher energy intake, not having diabetes or chronic lung disease, eating more fruits and vegetables, and drinking fewer sugar-sweetened beverages.

Animal protein intake comprised 65.7% of total protein. The main animal protein sources were, from largest to smallest, meat, dairy, fish, and eggs. The main vegetable protein sources were cereals, legumes, and nuts (Supplemental Table 3). The median study follow-up time was 2.6 years (interquartile range 2.3–2.8), and 292 participants (9.8%) improved their nutritional status over that period.

3.2. Main results

Higher animal and vegetable protein intake were associated with improvements in nutritional status in the pooled dataset. Fully adjusted odds ratios (95% confidence intervals) per 0.25 g/kg/day were 1.15 (1.00, 1.32) for animal protein and 1.77 (1.35, 2.32) for vegetable protein. The two cohorts showed a similar trend [Seniors-ENRICA 1 = 1.23 (1.04, 1.45) for animal and 1.61 (1.17, 2.23) for vegetable protein; Seniors-ENRICA 2 = 1.02 (0.81, 1.27) for animal and 2.09 (1.38, 3.16) for vegetable protein; p for interaction = 0.17 for animal and 0.30 for vegetable protein] (Table 2). Clear dose-response relationships were observed when plotting animal and -particularly- vegetable protein intake as restricted cubic splines (Supplemental Fig. 2).

Cereals were the vegetable protein source that showed the strongest association with improvements in nutritional status. Fully adjusted odds

Table 3
Odds Ratios (95% confidence interval) for the association of protein intake from animal and vegetable sources with improvements in nutritional status over 2.6 years in the pooled sample.

	Protein intake from animal sources				
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	Per 0.25 g/kg/day
Dairy[c]					
Cases/n	61/741	65/740	75/741	91/740	292/2962
Model 1[a]	Ref.	1.11 (0.77,1.62)	1.20 (0.84,1.73)	1.47 (1.03,2.10)*	1.28 (0.99,1.64)
Model 2[b]	Ref.	1.13 (0.78,1.65)	1.23 (0.85,1.77)	1.49 (1.04,2.14)*	1.28 (0.99,1.65)
Meat[d]					
Cases/n	92/741	62/740	56/741	82/740	292/2962
Model 1[a]	Ref.	0.75 (0.53,1.06)	0.68 (0.48,0.98)*	1.09 (0.78,1.51)	1.07 (0.88,1.32)
Model 2[b]	Ref.	0.76 (0.54,1.08)	0.69 (0.48,0.99)*	1.09 (0.78,1.53)	1.08 (0.88,1.32)
Eggs[e]					
Cases/n	74/741	68/740	69/741	81/740	292/2962
Model 1[a]	Ref.	0.98 (0.69,1.39)	1.03 (0.73,1.47)	1.20 (0.85,1.69)	2.56 (0.92,7.15)
Model 2[b]	Ref.	1.02 (0.72,1.46)	1.08 (0.76,1.54)	1.27 (0.90,1.80)	2.85 (1.01,8.01)*
Fish[f]					
Cases/n	81/741	67/740	72/741	72/740	292/2962
Model 1[a]	Ref.	0.90 (0.64,1.27)	0.98 (0.69,1.37)	0.98 (0.70,1.38)	1.06 (0.82,1.38)
Model 2[b]	Ref.	0.88 (0.62,1.25)	0.95 (0.67,1.34)	1.00 (0.70,1.42)	1.08 (0.82,1.41)
	Protein intake from vegetable sources				
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	Per 0.25 g/kg/day
Cereals[g]					
Cases/n	69/741	69/740	62/741	92/740	292/2962
Model 1[a]	Ref.	1.15 (0.80,1.64)	1.08 (0.75,1.57)	1.74 (1.22,2.48)**	1.91 (1.35,2.69)***
Model 2[b]	Ref.	1.18 (0.82,1.70)	1.10 (0.75,1.61)	1.79 (1.24,2.59)**	2.07 (1.44,2.98)***
Legumes[h]					
Cases/n	75/741	75/740	63/741	79/740	292/2962
Model 1[a]	Ref.	1.14 (0.81,1.61)	0.99 (0.69,1.42)	1.29 (0.91,1.81)	1.52 (0.73,3.14)
Model 2[b]	Ref.	1.14 (0.80,1.61)	1.00 (0.69,1.44)	1.29 (0.91,1.83)	1.55 (0.74,3.25)
Nuts[i]					
Cases/n	131/1263	59/566	53/567	49/566	292/2962
Model 1[a]	Ref.	1.08 (0.78,1.50)	1.01 (0.71,1.42)	0.95 (0.67,1.36)	0.90 (0.43,1.91)
Model 2[b]	Ref.	1.07 (0.77,1.50)	1.04 (0.73,1.47)	0.96 (0.67,1.38)	0.94 (0.44,2.01)

*$p < 0.05$. **$p < 0.01$. ***$p < 0.001$.

[a] Model 1: Logistic regression model adjusted for cohort, sex, age, living conditions (make ends meet), living arrangements (alone, with a spouse/partner, or other), educational level (primary or less, secondary, or university), and energy intake (kcal/day) at baseline.

[b] Model 2: As Model 1 and additionally adjusted for smoking status (never, former, or current), alcohol consumption (never, former, or current), leisure-time physical activity (MET-hours/week), sedentary behavior (TV hours/week), diabetes, cardiovascular disease, chronic lung disease, musculoskeletal disease, cancer, depression, dependence in instrumental activities of daily living, fruit, vegetable, and sugar-sweetened beverage consumption (g/day).

[c] Dairy protein intake: Quartile 1, 0 to 0.15 g/kg/day; Quartile 2, 0.15 to 0.21 g/kg/day; Quartile 3, 0.21 to 0.29 g/kg/day; Quartile 4, 0.29–1.13 g/kg/day.

[d] Meat protein intake: Quartile 1, 0 to 0.24 g/kg/day; Quartile 2, 0.24 to 0.32 g/kg/day; Quartile 3, 0.32 to 0.42 g/kg/day; Quartile 4, 0.42–1.24 g/kg/day.

[e] Egg protein intake: Quartile 1, 0 to 0.021 g/kg/day; Quartile 2, 0.021 to 0.036 g/kg/day; Quartile 3, 0.036 to 0.056 g/kg/day; Quartile 4, 0.056 to 0.26 g/kg/day.

[f] Fish protein intake: Quartile 1, 0 to 0.13 g/kg/day; Quartile 2, 0.13 to 0.19 g/kg/day; Quartile 3, 0.19 to 0.27 g/kg/day; Quartile 4, 0.27 to 0.93 g/kg/day.

[g] Cereal protein intake: Quartile 1, 0 to 0.18 g/kg/day; Quartile 2, 0.18 to 0.23 g/kg/day; Quartile 3, 0.23 to 0.29 g/kg/day; Quartile 4, 0.29–1.01 g/kg/day.

[h] Legume protein intake: Quartile 1, 0 to 0.025 g/kg/day; Quartile 2, 0.025 to 0.039 g/kg/day; Quartile 3, 0.039 to 0.061 g/kg/day; Quartile 4, 0.061 to 0.38 g/kg/day.

[i] Nut protein intake: Quartile 1, 0 g/kg/day; Quartile 2, 0.00026 to 0.023 g/kg/day; Quartile 3, 0.023 to 0.054 g/kg/day; Quartile 4, 0.054 to 0.43 g/kg/day.

ratios (95% confidence intervals) per 0.25 g/kg/day were 2.07 (1.44, 2.98), 1.55 (0.74, 3.25), and 0.94 (0.44, 2.01) for cereal, legume, and nut protein intake, respectively. With regards to animal sources, egg protein was associated with improvements in nutritional status, and dairy protein intake showed a positive trend. Odds ratios were 2.85 (1.01, 8.01), 1.28 (0.99, 1.65), 1.08 (0.88, 1.32), and 1.08 (0.82, 1.41) for egg, dairy, meat, and fish protein intake, respectively (Table 3).

Replacing 0.25 g/kg/day of total animal protein, meat, or fish protein -but not dairy or egg protein- with vegetable protein was associated with improvements in nutritional status [1.54 (1.13, 2.09), 1.70 (1.20, 2.41), 1.77 (1.18, 2.64), 1.31 (0.92, 1.87), and 0.84 (0.28, 2.56), respectively] (Fig. 1).

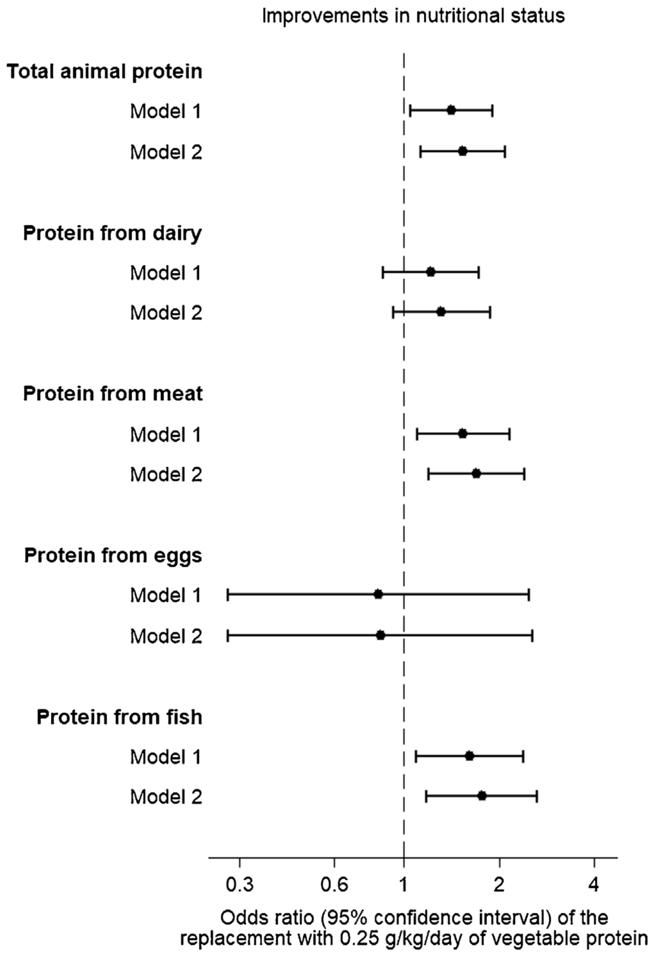

Fig. 1. Odds Ratios (95% confidence interval) for the association between the replacement of animal protein with vegetable protein and improvements in nutritional status over 2.6 years in the pooled sample.
Substitution models are logistic regression models including protein intake from all animal sources and vegetable protein intake. Substitution coefficients are calculated by subtracting the coefficient for the corresponding animal source of protein from that for total vegetable protein intake. Odds ratios below unity favor animal sources of protein, whereas those above one favor vegetable protein.
Model 1: Logistic regression model adjusted for cohort, sex, age, living conditions (make ends meet), living arrangements (alone, with a spouse/partner, or other), educational level (primary or less, secondary, or university), and energy intake (kcal/day) at baseline.
Model 2: As Model 1 and additionally adjusted for smoking status (never, former, or current), alcohol consumption (never, former, or current), leisure-time physical activity (MET-hours/week), sedentary behavior (TV hours/week), diabetes, cardiovascular disease, chronic lung disease, musculoskeletal disease, cancer, depression, dependence in instrumental activities of daily living, fruit, vegetable, and sugar-sweetened beverage consumption (g/day).

3.3. Ancillary results

When stratifying the analyses by etiologic criteria for the diagnosis of malnutrition, both vegetable and animal protein intake were favorably associated with malnutrition due to reduced food consumption or nutrient assimilation, but only vegetable protein was linked to inflammation-related improvements in nutritional status (Supplemental Table 4).

Associations between the theoretical replacement of animal protein with cereal protein and improvements in nutritional status are shown in Supplemental Figure 3 and were in line with the main results. When examining protein intake from animal subgroups, only protein from milk was significantly associated with improvements in nutritional status (Supplemental Table 5). The replacement of protein from most animal subgroups (yogurt, poultry, processed meat, oily fish, and white fish) with vegetable protein was associated with improvements in nutritional status, while the replacement of protein from cheese, milk, or red meat was not (Fig. 2).

Study associations remained when adjusting the models for the frailty phenotype, Mediterranean Diet Adherence Screener, and energy intake using the residual method (Supplemental Table 6). Increasing animal and vegetable protein intake over the years showed a trend toward improvements in nutritional status in the Seniors-ENRICA 1 cohort [odds ratios per 0.25 g/kg/day increment were 1.11 (0.97, 1.26) and 1.19 (0.90, 1.56), respectively].

When assessing interactions, vegetable protein intake only seemed to confer benefits on malnutrition to the subjects whose total protein intake was higher, while the association between animal protein intake and improvements in nutritional status was only evident in those who were in good oral health. Other tested interactions were not significant (Supplemental Figure 4).

4. Discussion

In a pooled sample of community-dwelling older adults, higher animal and -especially- vegetable protein intake were associated with improvements in nutritional status. Cereal protein intake drove most of the latter association. Replacing total animal protein, meat, or fish protein with vegetable protein was associated with improvements in nutritional status.

4.1. Interpretation

There are several mechanisms potentially linking individual amino acids to improvements in nutritional status. To cite a few, sulfur-containing amino acids, such as methionine and cysteine, play a role in the immune system and in peroxidative protection mechanisms in the muscle, nervous, and cardiovascular systems. Branched-chain amino acids (leucine, isoleucine, and valine) promote protein biosynthesis. Lysine is needed for bone calcification, liver activities, and blood and muscle synthesis. Valine participates in the coordination of motor cells, while aspartate and glutamate are essential for hormone regulation and immunological stimulation, respectively [24].

4.1.1. Vegetable and animal protein intake and malnutrition

Not all dietary proteins are created equal, however. Their quality is related to the presence and amount of specific amino acids (biological value), protein digestibility, the food matrix, and food processing [7]. It is often acknowledged that plant-based proteins may have lower essential amino acid content (especially leucine, sulfur amino acids, and lysine) and lower digestibility than their animal counterparts [8]. Antinutrients are naturally produced by plants and could further interfere with the absorption, digestion, and utilization of protein [24]. Moreover, plant-based foods are sources of fiber, which may enhance satiety and reduce appetite in individuals at risk of malnutrition, such as older adults [5].

Nevertheless, there are growing concerns about the methodologies used to calculate the biological value of proteins, as it has been argued

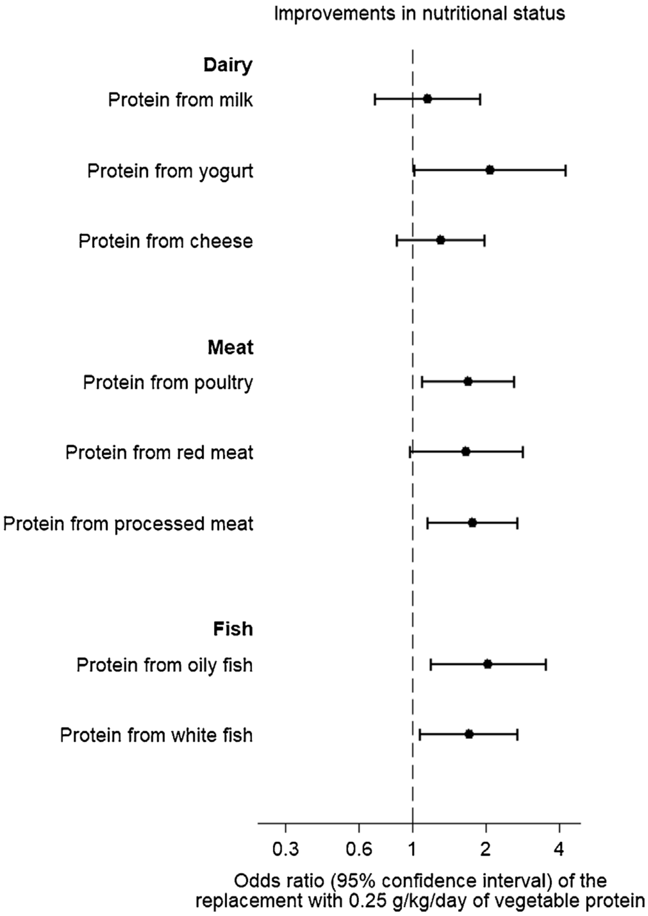

Fig. 2. Odds Ratios (95% confidence interval) for the association between the replacement of protein from animal subgroups with vegetable protein and improvements in nutritional status over 2.6 years in the pooled sample.
Substitution models are logistic regression models including protein intake from all animal subgroups and vegetable protein intake. Substitution coefficients are calculated by subtracting the coefficient for the corresponding animal source of protein from that for total vegetable protein intake. Odds ratios below unity favor animal sources of protein, whereas those above one favor vegetable protein.
Models are adjusted as Model 2 in Fig. 1: cohort, sex, age, living conditions (make ends meet), living arrangements (alone, with a spouse/partner, or other), educational level (primary or less, secondary, or university), energy intake (kcal/day), smoking status (never, former, or current), alcohol consumption (never, former, or current), leisure-time physical activity (MET-hours/week), sedentary behavior (TV hours/week), diabetes, cardiovascular disease, chronic lung disease, musculoskeletal disease, cancer, depression, dependence in instrumental activities of daily living, fruit, vegetable, and sugar-sweetened beverage consumption (g/day).

that they may be biased towards animal-based sources, especially if total protein intake is above recommendations [25]. Given the median protein intake of our study subjects (1.23 g/kg/day), their essential amino acid requirements (set at 0.214 g/kg/day by the Institute of Medicine) should be around 17% of total protein [25,26]. Protein in plant foods is above this threshold in almost every case, as the lowest value among common foods is oats (21% of its protein is essential amino acids), while other cereals and legumes have higher values [25].

This claim is supported fourfold. First, a meta-analysis of nitrogen balance studies found no differences in protein requirements between subjects with animal-based, vegetable-based, and mixed diets [25]. Second, a meta-analysis of randomized trials demonstrated that, among older adults whose protein intakes were generally above recommendations, consumption of animal versus vegetable protein sources did not affect changes in lean mass or muscle strength [27]. Third, it has been shown that a 70/30% mix of plant proteins/whey protein could be as effective as whey protein alone in muscle anabolism stimulation in old rats if protein intake per meal is increased by 25% [7]. Fourth, the results of our interaction analyses suggest that higher total protein intake (i.e., above the study median) could be needed to see the benefits of plant protein on malnutrition.

With regards to protein digestibility, recent data from gold-standard oro-ileal nitrogen studies in humans only revealed small differences between vegetable and animal protein sources. For example, pea, wheat, and lupin flours had 89–92% digestibility, compared with 90–95 % for eggs, meat, and milk proteins [25].

Animal protein sources may also contribute more than plant proteins to metabolic derangements (e.g., metabolic acidosis, gut-derived uremic toxin production) that cause protein-energy wasting in patients with chronic illnesses, such as chronic kidney disease [8,28]. Conversely, higher consumption of plant foods (e.g., whole grains and nuts) and the vegetable-to-animal protein ratio are associated with lower levels of inflammatory biomarkers, and inflammation contributes to malnutrition through anorexia as well as altered metabolism [1,29,30]. Accordingly, while we observed that both vegetable and animal protein intake were favorably associated with malnutrition due to reduced food consumption or assimilation, only vegetable protein was linked to inflammation-related improvements in nutritional status.

Although antinutrients may be at odds with the use of plant proteins in malnutrition, they have also shown beneficial effects over risk factors of malnutrition-predictive health conditions (e.g., cardiovascular disease, cancer, microbial infections) [24]. Plant foods are also a source of fermentable fibers, which could exert a collateral influence on muscle mass via the gut microbiota, for specific bacteria have been associated with increased protein synthesis in hosts [31,32]. On the contrary, some meat proteins have been associated with altered gut microbiota, which could be detrimental to muscle-protein synthesis [32]. Regardless of fiber content, several studies did not find a deleterious impact on satiety of protein intake from plant sources in normal-weight adults [5].

In addition, raw foods are commonly used for biological value calculations, whereas most protein-rich plant foods undergo heating, processing, or both before consumption. Common cooking techniques modify protein use (e.g., by removing most antinutrients), and therefore heat-treated plant-based proteins have higher digestibility than unprocessed proteins [24,25]. For instance, the fermentation of grain, together with heating or other cooking techniques, as in the case of bread, can increase the digestibility of its protein to meat-like standards [25].

These issues may explain why, despite their presumed higher biological value, some animal-based proteins do not seem to increase muscle protein synthesis more than isonitrogenous plant-based proteins (e.g., milk protein vs corn protein, casein vs soy protein) [25]. Even if animals were superior sources of protein to plants, the blend of plant foods with different limiting amino acids -such as rice (low in lysine) and lentils (low in methionine)- or the combination of plant and animal-based protein sources -as in traditional legume-based recipes- could result in similar biological values to animal proteins alone [8,28]. Of note is that these practices are common in the Seniors-ENRICA 1 cohort [33], and that the association of vegetable protein intake with improvements in nutritional status was rather stronger in the subjects who had higher animal protein intake.

4.1.2. Vegetable and animal protein sources

Any explanation of the different associations of individual vegetable and animal protein sources with malnutrition must be conjectural. Since our study comprised older adults, tooth loss and reductions in masticatory capacity and chewing muscle mass could have led to a differential reduction in protein digestibility across food groups [34]. The rupture strain of meat and nuts is almost 6-fold that of bread or rice, and more than 16 times that of cheese, meaning that, in the elderly, the former foods may reach the stomach without proper breakdown [35]. Together with gastrointestinal tract aging (e.g., atrophic gastritis), this could lead to an even greater absorption reduction in the protein coming from meat and nuts in older adults [34]. Accordingly, a study assessing amino acid bioavailability from meat showed that denture wearers had a delayed absorption of proteins, lower amino acid concentrations in blood during the post-meal period, as well as lower anabolic capacity when compared to fully dented individuals [7]. Aside from that, since gastric expansion suppresses the release of key appetite-inducing hormones, consumption of energy-dense vegetable protein sources may be especially important in older adults, as many suffer from impairment of taste and smell, decreased appetite, and early satiety [6,28]. While legumes and animal foods often have similar energy densities, they may be less than half of cereals' [28]. Consistent with this set of hypotheses, we observed a beneficial association of cereal protein intake with improvements in nutritional status, but not of legume, nut, or meat protein intake. Moreover, the association of animal protein intake (and by extension of meat) with improvements in nutritional status was only apparent among the subjects in good oral health.

Dairy protein showed in our study a somewhat stronger association with improvements in nutritional status than protein coming from meat and fish. This is consistent with extensive evidence on dairy protein and malnutrition-related outcomes. A meta-analysis on dairy protein supplements demonstrated an increase in body weight and lean body mass, especially in frail/prefrail older adults [36]. Several macro- and micronutrients that are found in dairy products more than in any other food group (e.g., lactose, calcium, phosphorus, vitamin D) have demonstrated potential growth-stimulating effects and/or a positive impact on muscle mass and strength [37,38]. With regards to dairy subgroups, the theoretical replacement of protein from yogurt with vegetable protein was associated with improvements in nutritional status, while that of protein from cheese or milk was not. On one hand, trials using cheese and milk protein supplementation have shown improvements in fat-free mass and muscle quality, especially in people with geriatric syndromes [39,40]. On the other hand, some yogurts are a source of added sugars, and these are linked to inflammation, oxidative stress, and insulin resistance, which are responsible for the increased muscle anabolic threshold in older adults [7]. Accordingly, intake of added sugars has already been associated with frailty in the Seniors-ENRICA 1 cohort [41].

4.2. *Generalizability*

As discussed previously, the effect of plant proteins on malnutrition could differ at different levels of animal and total protein intake. On one hand, only 34% of protein intake in our study came from vegetable sources, in line with data from older British adults (where animal proteins contributed to nearly two-thirds of total protein intake) [5]. On the other hand, the mean protein intake in our study sample was rather high in absolute terms and somewhat higher than that of older adults from other North American and European countries, which ranged from 0.94 (USA) to 1.17 g/kg/day (Italy) [5,9]. Together with the interaction analyses that we conducted, this may raise concerns about the generalizability of our findings to lower and middle-income countries, where plant-based sources dominate the protein supply and total protein intake is often lower [24]

4.3. *Limitations*

Several limitations should be acknowledged. Since malnutrition estimates depend on the screening tool, the use of the GLIM criteria or other instruments in the analyses could have rendered different results -note that up to 22 of such tools have demonstrated acceptable validity for older adults [4]. In addition, malnutrition can be transient, and therefore some short or mid-term changes in nutritional status were likely overlooked by our 2.6-year follow-up time, which also came with somewhat high loss to follow-up rates. Moreover, objective and complete measurements on every criterion for the diagnosis of malnutrition were not conducted at every time point and cohort. For instance, weight loss in the previous three and twelve months was self-reported, while supportive laboratory measures of inflammation were lacking in the Seniors-ENRICA 1 cohort.

Even though particular attention has been given to the timing of protein intake, we did not take it into account in our analysis. Nevertheless, in the Seniors-ENRICA 1 cohort, dietary protein distribution across meals was not associated with impaired lower-extremity function, regardless of protein source and amount [42]. In this cohort, we found that increasing vegetable and animal protein intake showed a trend toward improvements in nutritional status. Unfortunately, longitudinal dietary information was not collected in the Seniors-ENRICA 2 cohort, and hence we could not account for protein intake changes in the pooled analysis. It should be noted that we did not collect data on protein supplements, although their use may not be frequent among community-dwelling older adults [17,43]. Finally, as in any observational study, we could not entirely disentangle protein intake from other nutrients nor rule out residual confounding.

Conclusions

In a pooled sample of community-dwelling older adults, higher animal and -especially- vegetable protein intake were associated with improvements in nutritional status, as well as the theoretical replacement of animal protein with vegetable protein. Implications of favoring vegetable sources of protein over animal protein in malnutrition management could range from promoting gut microbiota eubiosis, lowering the levels of inflammatory biomarkers, and reducing the risk of all-cause mortality, to making diets more affordable and environmentally sustainable [5,10,12,24,29,31,32].

Still, animal protein intake did show an association with improvements in nutritional status, and most of our subjects included foods of both plant and animal origin in their diet. The extent to which our findings apply to settings where protein intake is lower, or vegetable foods are the main protein source could be subject of future research. More evidence from intervention studies targeting the replacement of dietary animal protein with vegetable protein (or the comparison between animal and vegetable protein supplements) would also be desirable.

Authors' contributions

Adrián Carballo-Casla: methodology, formal analysis, data curation, writing - original draft, visualization.

Mercedes Sotos-Prieto: writing - review & editing, funding acquisition.

Esther García-Esquinas: writing - review & editing, funding acquisition.

Ellen A Struijk: writing - review & editing.

Francisco Félix Caballero: writing - review & editing.

Amaia Calderón-Larrañaga: writing - review & editing.

Esther Lopez-Garcia: writing - review & editing, funding acquisition.

Fernando Rodríguez-Artalejo: conceptualization, resources, writing - review & editing, supervision, project administration, funding acquisition.

Rosario Ortolá: data curation, writing - review & editing, supervision.

Funding

This work was supported by FIS grants 19/319 and 22/1111 (funded by the Institute of Health Carlos III; the Secretary of R + D + I; and the European Regional Development Fund/European Social Fund); PLEC2022-009352 grant (funded by MCIN/AEI/10.13039/501100011033 and the European Union “NextGenerationEU/PRTR”); FACINGLCOVID-CM project (funded by Comunidad de Madrid and European Regional Development Fund “Funding REACT EU Program”). Adrián Carballo-Casla was supported by Universidad Autónoma de Madrid (FPI fellowship). The funding agencies had no role in study

design, data collection and analysis, interpretation of results, manuscript preparation or the decision to submit this manuscript for publication.

Competing interests

The authors disclose no conflicts of interest related to the content of this manuscript.

Ethics approval and consent to participate

The Clinical Research Ethics Committee of the “La Paz” University Hospital in Madrid approved the research protocol of each cohort, and all participants gave written informed consent.

Availability of data and material

The datasets used and/or analysed during the current study available from the corresponding authors on reasonable request.

Consent for publication

Not applicable.

Acknowledgements

Not applicable.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jnha.2023.100002.

References

[1] Cederholm T, Jensen GL, Correia MITD, Gonzalez MC, Fukushima R, Higashiguchi T, et al. GLIM criteria for the diagnosis of malnutrition - A consensus report from the global clinical nutrition community. Clin Nutr. 2019;38:1–9.

[2] Nakahara S, Takasaki M, Abe S, Kakitani C, Nishioka S, Wakabayashi H, et al. Aggressive nutrition therapy in malnutrition and sarcopenia. Nutrition. 2021;84.

[3] Gomes F, Baumgartner A, Bounoure L, Bally M, Deutz NE, Greenwald JL, et al. Association of nutritional support with clinical outcomes among medical inpatients who are malnourished or at nutritional risk: an updated systematic review and meta-analysis. JAMA Netw Open. 2019;2.

[4] Leij-Halfwerk S, Verwijs MH, van Houdt S, Borkent JW, Guaitoli PR, Pelgrim T, et al. Prevalence of protein-energy malnutrition risk in European older adults in community, residential and hospital settings, according to 22 malnutrition screening tools validated for use in adults ≥65 years: a systematic review and meta-analysis. Maturitas 2019;126:80–9.

[5] Lonnie M, Hooker E, Brunstrom JM, Corfe BM, Green MA, Watson AW, et al. Protein for life: review of optimal protein intake, sustainable dietary sources and the effect on appetite in ageing adults. Nutrients. 2018;10.

[6] Perna S, Rondanelli M, Spadaccini D, Lenzi A, Donini LM, Poggiogalle E. Are the therapeutic strategies in anorexia of ageing effective on nutritional status? A systematic review with meta-analysis. J Hum Nutr Diet. 2019;32:128–38.

[7] Dardevet D, Mosoni L, Savary-Auzeloux I, Peyron MA, Polakof S, Remond D. Important determinants to take into account to optimize protein nutrition in the elderly: solutions to a complex equation. Proc Nutr Soc. 2021;80:207–20.

[8] Berrazaga I, Micard V, Gueugneau M, Walrand S. The role of the anabolic properties of plant- versus animal-based protein sources in supporting muscle mass maintenance: a critical review. Nutrients. 2019;11.

[9] Hengeveld LM, Boer JMA, Gaudreau P, Heymans MW, Jagger C, Mendonça N, et al. Prevalence of protein intake below recommended in community-dwelling older adults: a meta-analysis across cohorts from the PROMISS consortium. J Cachexia Sarcopenia Muscle. 2020;11:1212–22.

[10] Chen Z, Glisic M, Song M, Aliahmad HA, Zhang X, Moumdjian AC, et al. Dietary protein intake and all-cause and cause-specific mortality: results from the Rotterdam Study and a meta-analysis of prospective cohort studies. Eur J Epidemiol. 2020;35:411–29.

[11] Kitada M, Ogura Y, Monno I, Koya D. The impact of dietary protein intake on longevity and metabolic health. EBioMedicine. 2019;43:632–40.

[12] Seidelmann SB, Claggett B, Cheng S, Henglin M, Shah A, Steffen LM, et al. Dietary carbohydrate intake and mortality: a prospective cohort study and meta-analysis. Lancet Public Health. 2018;3:e419–28.

[13] Rodríguez-Artalejo F, Graciani A, Guallar-Castillón P, León-Muñoz LM, Zuluaga MC, López-García E, et al. Rationale and methods of the study on nutrition and cardiovascular risk in Spain (ENRICA). Rev Esp Cardiol. 2011;64:876–82.

[14] Ortolá R, García-Esquinas E, Sotos-Prieto M, Struijk EA, Caballero FF, Lopez-Garcia E, et al. Mediterranean diet and changes in frequency, severity, and localization of pain in older adults: the seniors-ENRICA cohorts. J Gerontol A Biol Sci Med Sci. 2022;77:122–30.

[15] Ortolá R, García-Esquinas E, Cabanas-Sánchez V, Migueles JH, Martínez-Gómez D, Rodríguez-Artalejo F. Association of physical activity, sedentary behavior, and sleep with unhealthy aging: consistent results for device-measured and self-reported behaviors using isotemporal substitution models. J Gerontol A Biol Sci Med Sci. 2021;76:85–94.

[16] Ortolá R, García-Esquinas E, García-Esquinas G, Struijk EA, Rodríguez-Artalejo F, López-García E. Influence of changes in diet quality on unhealthy aging: the seniors-ENRICA cohort. Am J Med. 2019;132:1091–102 e9.

[17] Guallar-Castillón P, Sagardui-Villamor J, Balboa-Castillo T, Sala-Vila A, Ariza Astolfi MJ, Sarrión Pelous MD, et al. Validity and reproducibility of a spanish dietary history. PLoS One. 2014;9:e86074.

[18] Cruz-Jentoft AJ, Bahat G, Bauer J, Boirie Y, Bruyère O, Cederholm T, et al. Sarcopenia: revised European consensus on definition and diagnosis. Age Ageing. 2019;48:16–31.

[19] Lawton MP, Brody EM. Assessment of older people: self-maintaining and instrumental activities of daily living. Gerontologist. 1969;9:179–86 3_Part_1.

[20] Fried LP, Tangen CM, Walston J, Newman AB, Hirsch C, Gottdiener J, et al. Frailty in older adults: evidence for a phenotype. J Gerontol A Biol Sci Med Sci. 2001;56.

[21] Schröder H, Fitó M, Estruch R, Martínez-González MA, Corella D, Salas-Salvadó J, et al. A short screener is valid for assessing Mediterranean diet adherence among older Spanish men and women. J Nutr. 2011;141:1140–5.

[22] Thiébaut A, Kesse E, Com-Nougué C, Clavel-Chapelon F, Bénichou J. Adjustment for energy intake in the assessment of dietary risk factors. Rev Epidemiol Sante Publique. 2004;52:539–57.

[23] Ortolá R, Struijk EA, García-Esquinas E, Rodríguez-Artalejo F, Lopez-Garcia E. Changes in dietary intake of animal and vegetable protein and unhealthy aging. Am J Med. 2019;133:231–9.

[24] Langyan S, Yadava P, Khan FN, Dar ZA, Singh R, Kumar A. Sustaining protein nutrition through plant-based foods. Front Nutr. 2022;8.

[25] Craddock JC, Genoni A, Strutt EF, Goldman DM. Limitations with the Digestible Indispensable Amino Acid Score (DIAAS) with special attention to plant-based diets: a review. Curr Nutr Rep. 2021;10:93–8.

[26] Trumbo P, Schlicker S, Yates AA, Poos M. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. J Am Diet Assoc. 2002;102:1621–30.

[27] Lim MT, Pan BJ, Toh DWK, Sutanto CN, Kim JE. Animal protein versus plant protein in supporting lean mass and muscle strength: a systematic review and meta-analysis of randomized controlled trials. Nutrients. 2021;13:1–18.

[28] St-Jules DE, Goldfarb DS, Popp CJ, Lou Pompeii M, Liebman SE. Managing protein-energy wasting in hemodialysis patients: a comparison of animal- and plant-based protein foods. Semin Dial. 2019;32:41–6.

[29] Di Giosia P role of nutrition in inflammaging, Stamerra CA, Giorgini P, Jamialahamdi T, Butler AE, Sahebkar A. The role of nutrition in inflammaging. Ageing Res Rev. 2022;77.

[30] Miki A, Hashimoto Y, Matsumoto S, Ushigome E, Fukuda T, Sennmaru T, et al. Protein intake, especially vegetable protein intake, is associated with higher skeletal muscle mass in elderly patients with type 2 diabetes. J Diabetes Res. 20172017:.

[31] Liu C, Cheung WH, Li J, Chow SKH, Yu J, Wong SH, et al. Understanding the gut microbiota and sarcopenia: a systematic review. J Cachexia Sarcopenia Muscle. 2021;12:1393–407.

[32] Prokopidis K, Cervo MM, Gandham A, Scott D. Impact of protein intake in older adults with sarcopenia and obesity: a gut microbiota perspective. Nutrients. 2020;12:1–24.

[33] Caballero FF, Ballesteros JM, Garciá-Esquinas E, Struijk EA, Ortolá R, Rodríguez-Artalejo F, et al. Are legume-based recipes an appropriate source of nutrients for healthy ageing? A prospective cohort study. Br J Nutr. 2020;124:943–51.

[34] Lee S, Choi YS, Jo K, Yong HI, Jeong HG, Jung S. Improvement of meat protein digestibility in infants and the elderly. Food Chem. 2021;356.

[35] Kohyama K, Mioche L, Martin JF. Chewing patterns of various texture foods studied by electromyography in young and elderly populations. J Texture Stud. 2002;33:269–83.

[36] Dewansingh P, Melse-Boonstra A, Krijnen WP, van der Schans CP, Jager-Wittenaar H, van den Heuvel EGHM. Supplemental protein from dairy products increases body weight and vitamin D improves physical performance in older adults: a systematic review and meta-analysis. Nutr Res. 2018;49:1–22.

[37] Du Y, Oh C, No J. Advantage of dairy for improving aging muscle. J Obes Metab Syndr. 2019;28:167–74.

[38] Grenov B, Michaelsen KF. Growth components of cow’s milk: emphasis on effects in undernourished children. Food Nutr Bull. 2018;39(2_suppl):S45–53.

[39] Zanini B, Simonetto A, Zubani M, Castellano M, Gilioli G. The effects of cow-milk protein supplementation in elderly population: systematic review and narrative synthesis. Nutrients. 2020;12:1–26.

[40] Cuesta-Triana F, Verdejo-Bravo C, Fernández-Pérez C, Martín-Sánchez FJ. Effect of milk and other dairy products on the risk of frailty, sarcopenia, and cognitive performance decline in the elderly: a systematic review. Adv Nutr. 2019;10(suppl_2): S105–19.

[41] Laclaustra M, Rodriguez-Artalejo F, Guallar-Castillon P, Banegas JR, Graciani A, Garcia-Esquinas E, et al. Prospective association between added sugars and frailty in older adults. Am J Clin Nutr. 2018;107:772–9.

[42] Estrada-DeLeón DB, Struijk EA, Caballero F, Rodríguez-Artalejo F, Lopez-Garcia E. Distribution of daily protein intake across meals and lower extremity functioning in community-dwelling Spanish older adults: a prospective cohort study. Eur J Nutr. 2021;60:665–75.

[43] Kantor ED, Rehm CD, Du M, White E, Giovannucci EL. Trends in dietary supplement use among US adults from 1999-2012. JAMA. 2016;316:1464–74.