**Animal protein intake is inversely associated with mortality in community-dwelling older adults: the InCHIANTI study**

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**Key points**

**Question:** What is the long-term association between both animal and plant protein intakes and all-cause and cause-specific mortality in a prospective cohort of community-dwelling older adults?

**Findings:** In the InCHIANTI cohort of 1,139 older adults (mean age: 75 years) followed up to 20 years, animal protein intake showed an inverse association with all-cause and cardiovascular mortality independently of confounders and diet quality. However, plant protein intake was not related to any of the mortality outcomes.

**Meaning:** In older adults, differences in long-term associations between animal or plant protein intake and mortality should be considered in future dietary recommendations.

**Abstract**

**Importance.** The long-term association between animal or plant protein intake and all-cause and cause-specific mortality among older adults is not clear.

**Objective.** To evaluate the long-term associations of animal and plant protein intake with all-cause and cause-specific mortality.

**Design, settings, and participants.** A prospective cohort study including 1,139 community-dwelling older adults (mean age 75 years, 56% women) living in Tuscany, Italy, followed for 20 years (InCHIANTI study) was conducted. Dietary intake by food frequency questionnaires and clinical information were assessed five times during the follow-up (baseline, 3, 6, 9 and 15 years). Protein intakes were expressed as percentages of total energy.

**Main outcomes and measures.** Hazard ratios (HRs) and 95% confidence intervals (CIs) for all-cause and cause-specific mortality were estimated using time-dependent Cox regression models adjusted for confounders, including diet quality.

**Results.** Animal protein intake was inversely associated with all-cause (HR per 1% increase, 95%CI: 0.96, 0.93-0.99) and cardiovascular mortality (HR per 1% increase, 95%CI: 0.93, 0.87-0.98). Plant protein intake showed no association with any of the mortality outcomes. Among participants without hypertension, there was a marginally significant, inverse association between plant protein and CVD mortality (HR per 1% increase, 95%CI: 0.79, 0.62-1.01).

**Conclusions and relevance.** Higher intake of animal protein was associated with lower risk for all-cause and cardiovascular mortality in older adults. The differences in long-term associations between animal or plant protein intake and mortality in older adults should be considered in future dietary recommendations.

**Keywords: protein, diet, mortality, longevity, older adults, cohort study.**

## Introduction

Total protein requirements in older adults are higher than in middle-aged adults.[1] However, debate still exists due to the heterogeneity of the older adult population.[2] According to the current recommendation of protein intake in older adults of 1.2 g/kg per day, inadequately low protein intake is frequent among older adults.[3] In general, the relationship between protein intake and health outcomes, such as all-cause or cause-specific mortality, depends on the source of dietary protein.[4–9] In middle-age adults higher animal protein intake was associated with an increased cardiovascular mortality, while plant protein exhibited an inverse relationship.[4,5,7–11] Studies carried out in older adults (≥65 years) observed an inverse association between total protein intake and both all-cause and cardiovascular disease (CVD) mortality, but they did not evaluate differences between protein sources.[12,13] Only Chan *et al.*[14] studied a cohort of community-dwelling older adults in Hong Kong and observed an inverse association between both total and plant protein intake and all-cause mortality among women but not in men. Among men, animal protein intake was associated with lower mortality[14], contrary to US and European studies carried out in middle-age adults.[4–6]

To date, no study has evaluated the association between plant and animal protein and all-cause and cause-specific mortality in a cohort of individuals aged ≥65 years in a Mediterranean country. The main aim of the present study was to evaluate the association of animal and plant protein intake as well as of total protein intake with all-cause, CVD, and cancer mortality after 20-years of follow-up in the InCHIANTI study. Our hypothesis is that total and plant protein, but not animal protein, would be a protective factor against mortality.

## Methods

*Study design*

The InCHIANTI (Invecchiare in Chianti, aging in the Chianti area) cohort study included community-dwelling older adults living in the Chianti geographic area (Tuscany, Italy) and study details have been previously reported.[15]

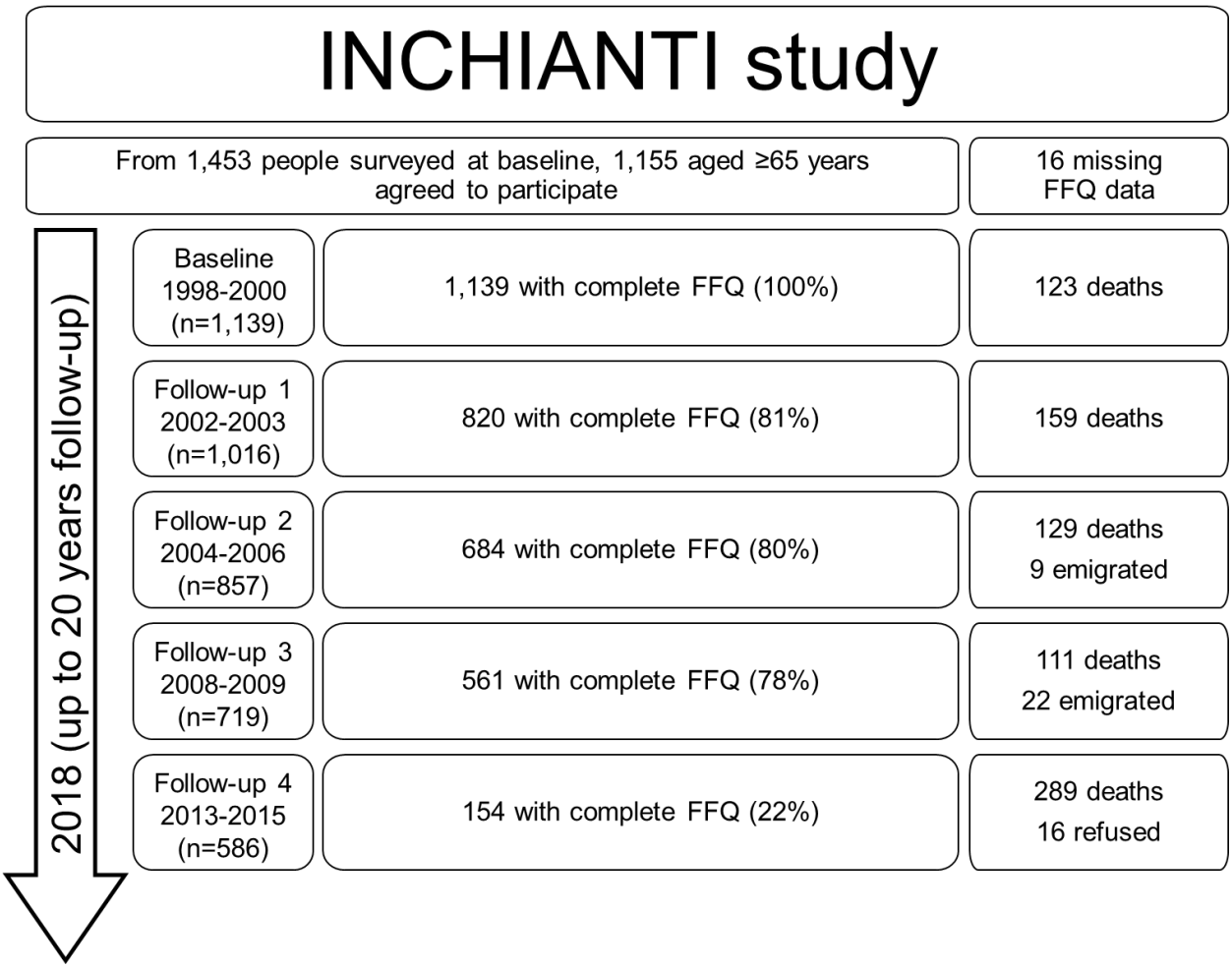
The Italian National Institute of Research and Care of Aging Institutional Review and Medstar Research Institute (Baltimore, MD, USA) approved the study protocol, and all participants signed an informed consent.

The current study was conducted and reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology-Nutritional Epidemiology (STROBE-NUT) guidelines (Supplementary Table 1).[16]

*Study population*

1,453 community-dwelling adults were randomly selected from the population registries of two Italian communities: *Greve in Chianti* and *Bagno a Ripoli*, with the use of a multistage, stratified sampling method. Baseline participation rate was 91.7%, and 1,155 participants aged ≥ 65 years agreed to participate. Sixteen participants had missing data on dietary questionnaires and the final studied population included 1,139 participants. Participants and data included in the analysis are shown in Figure 1. Clinical data was available in >95% of the cases during all the follow-up evaluations, except for the Follow-up 4 in which 80% had available data. In total, 4,317 observations from 1,139 participants were included in the analysis.

**Figure 1. InCHIANTI study flowchart of participants across follow-up assessments**




*Dietary intake*

Habitual dietary intake was assessed by trained interviewers using the Italian version of the food frequency questionnaire (FFQ) developed and validated in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Italy study.[17] Daily intake of energy, macronutrients and micronutrients were estimated from food intake by a specific software developed for the EPIC-Italy study.[18] For this analysis, dietary data at baseline, 3-, 6-, 9- and 15-years of follow-up were considered (Figure 1). In the 15-years follow-up, the reason for the low number of complete FFQ was that only participants from one site, *Bagno a Ripoli*, underwent the dietary assessment.

*Mediterranean diet adherence score*

Adherence to Mediterranean diet was computed using an 9-point linear scale as described by Trichopoulou *et al.* [19]. The overall adherence to Mediterranean diet was calculated as the sum of each component resulting in a score between 0 (no adherence) and 9 (high adherence).

*Covariates*

Covariates were selected on the basis of existent literature and previous associations with mortality in the InCHIANTI study.[4,11,20,21] Age, sex, years of education, disability in activities of daily life (disabled ADL ≥1 or not), and physical activity (categorized in 3 levels) were assessed through questionnaires. Smoking habits were self-reported, and participants were classified into never smokers, former smokers or current smokers. Height and weight were measured, and BMI was calculated in $kg/m^2$. Global cognitive performance was assessed with the Mini-Mental State Examination (MMSE). Comorbidities considered were hypertension, diabetes, impaired renal function [three categories, normal: glomerular filtration rate (GFR) >60 ml/min, mild: GFR<60 ml/min but ≥30 ml/min and severe: GFR<30 ml/min], chronic obstructive pulmonary disease (COPD), ischemic heart disease (IHD, angina pectoris or myocardial infarction), cerebrovascular disease (stroke or transient ischemic attack), peripheral artery disease, congestive heart failure (CHF), Parkinson's disease, dementia, cognitive impairment (MMSE<24 points), and cancer. They were defined using standard clinical definitions by algorithms combining information from self-reported physician diagnoses, pharmacological treatments, medical history, clinical examinations and blood tests. The presence of comorbidities was updated in every follow-up evaluation. In addition, cognitive decline was defined as ≥3 points decline in MMSE between follow-up evaluations.[22]

*Outcome assessment*

Mortality data on 20-years was collected using the Mortality General Registry from the Tuscany Region, as well as death certificates delivered after the decease of participants to the municipal registry office. Mortality records were coded by the 9$^{th}$ and 10$^{th}$ Revision of the International Classification of Diseases (ICD-9 or -10).

*Statistical analysis*

**Data preparation.** Energy equivalents of 9, 4 and 7 kcal/g were used for fat (and fat subtypes), for carbohydrates and protein, and for alcohol, respectively, to express macronutrients as percentage of energy. Total, animal, and plant protein as percentage of energy were categorized into quintiles for descriptive statistics. Missing data at the baseline evaluation were found in BMI and renal function of 166 participants (15%) and values were imputed by the median. During the follow-up evaluations, missing values in clinical information (n=206 observations, 4.7%) and in dietary data (n=959 observations, 22.2%, Figure 1) were imputed by Last Observation Carried Forward (LOCF). Dummy variables were created to identify participants with imputed values at baseline and observations with LOCF imputation.

**Descriptive analysis.** Data for continuous variables are shown as means (SD) or medians (interquartile range). Categorical variables are expressed as percentages. Differences in general characteristics across quintiles of total, animal, or plant protein intake and in participants with and without hypertension were assessed using generalized linear models adjusted for age and sex. To evaluate differences in dietary data across the follow-up assessments linear mixed models using site- and individual-specific random effects were used, and intraclass correlation coefficients were calculated.

**Survival analysis.** A multivariable nutrient density model was used for Cox Regression models with adjustment for total energy and percentage of energy from subtypes of fats, total or animal and plant protein, and alcohol as described previously.[4,11,23] For the main analyses, we used two time-dependent Cox models stratified by site: model 1 included total or animal and plant protein (continuous) adjusted for age, sex, total energy and percentage of energy from SFA, MUFA, PUFA, and alcohol. Model 2 was further adjusted for BMI, years of education, smoking, ADL disability, physical activity, impaired renal function, diabetes, COPD, hypertension, IHD, cerebrovascular disease, peripheral artery disease, CHF, Parkinson's disease, dementia, cognitive impairment, cognitive decline, cancer, and Mediterranean diet score (continuous). In all Cox models, visual inspection of scaled Schoenfeld residuals were used to check for the proportional hazard assumption and they were mostly satisfied. Presence of hypertension was the only variable that showed a major violation of the proportional hazard assumption. In

consequence, Cox regression models also included hypertension as stratification variable.

We compared the models with and without restricted cubic splines with 3 knots by the likelihood ratio test to assess for non-linear relationships between exposure and outcomes. Cox models were centered to the median intake of total, animal, or plant protein for better visualization.

Interactions between total, animal or plant protein intake and age, sex, physical activity, BMI categories, diabetes, smoking, ADL disability, hypertension, IHD, cerebrovascular disease, CHF, cognitive impairment, and cancer were tested using likelihood ratio tests.

Sensitivity analysis were done after the exclusion of participants with imputed values, subjects who died within the first 2 years of follow-up and subjects with baseline cancer, severe impaired kidney function or cognitive impairment. Last, we created a dummy variable to identify the different periods between follow-up evaluations (from 0 to 4) and included an interaction terms between it and total or animal and plant protein in Cox regression models. Significant interactions in these analyses represent a difference of effect across the different periods of the follow-up. The same approach was used to test for difference of effect between study sites in participants with and without imputations, considering that FFQs of the last follow-up were only available in one site of the study.

For the statistical analyses SPSS version 25.0 (IBM, USA) and R 3.6.2 (R foundation, Vienna, Austria) were used.

## Results

### Baseline characteristics

The studied population consisted in 1,139 participants (56% women) with a mean age of 75±8 years at baseline. Mean (SD) intake of total protein was 74 (21) g/day, and the normalized value by weight was 1.1 (0.3) g/kg of body weight per day. Overall, a 63 (1) % of total protein intake was animal protein. Sources of animal protein were: 26% for dairy products, 26% for processed meat products, 20% for red meat, 7.7% for fish and seafood, 6.3% for chicken, 2.7% for eggs, and the rest from rabbit, game and offals. Mean contribution from different sources of plant protein were: 73% for cereals, 11.4% for vegetables, 9.0% for fruits and nuts, and 5.3% for legumes.

According to quintiles of total protein intake, participants in the highest compared to the lowest quintile were more likely to be women, less educated, and to present diabetes at baseline (Table 1). There were no differences in plant protein as percentage of energy across the quintiles of total protein intake. Participants in the highest quintile of total protein intake tended to consume more meat and dairy products, fish and seafood, as well as less fruits, cereals, and alcohol, and showed a lower Mediterranean diet score compared to those in the lowest quintile (Supplementary Table 2).

Across quintiles of animal protein intake, sociodemographic, clinical characteristics and dietary intake data are similar to the results across total protein intake quintiles (Table 1 and Supplementary Table 2). Participants in the highest compared to the lowest quintile of animal protein intake were older and showed a lower plant protein intake.

Participants in the highest quintile of plant protein intake were more likely to be men, less educated, and to present higher prevalence of hypertension and diabetes than those in the lowest quintile (Table 1). Total energy and total protein intakes were not different across plant protein quintiles. Moreover, the consumption of vegetables and cereals was higher and the consumption of meat and dairy products, fish and seafood, and alcohol were lower in the highest compared to the lowest plant protein quintile (Supplementary Table 2).

**Table 1. Baseline characteristics of the population according to quintiles of intake of total, animal or plant protein.**

| (n= 1,139) | *Total protein* | | | *Animal protein* | | | *Plant protein* | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Q1 (227)** | **Q3 (228)** | **Q5 (227)** | **Q1 (228)** | **Q3 (229)** | **Q5 (228)** | **Q1 (227)** | **Q3 (227)** | **Q5 (227)** |
| **Clinical characteristics** | | | | | | | | | |
| Age (years) | 75±7 | 75±8 | 76±7 | 74±7 | 75±8 | 76±8† | 77±8 | 76±8 | 75±7 |
| Women (%) | 42 | 52 | 70* | 38 | 55 | 72* | 64 | 59 | 51* |
| BMI (kg/m²)# | 27±4 | 27±4 | 27±4 | 27±4 | 27±4 | 27±4 | 27±4 | 27±4 | 27±4 |
| Education (years) | 6±3 | 5±3 | 5±3† | 6±3 | 5±3 | 5±3 | 6±4 | 5±3 | 5±3* |
| Current smoking (%) | 33 | 27 | 21 | 35 | 25 | 24 | 31 | 24 | 31 |
| ADL disability (%) | 6 | 11 | 12 | 3 | 11 | 15† | 13 | 12 | 6‡ |
| Physical activity (n,%) | | | | | | | | | |
| Sedentary | 21 | 23 | 30 | 20 | 20 | 31 | 27 | 29 | 20 |
| Light | 37 | 42 | 44 | 37 | 45 | 43 | 41 | 40 | 45 |
| Mod- High | 41 | 35 | 26 | 43 | 35 | 25 | 30 | 31 | 35 |
| Hypertension (%) | 55 | 56 | 56 | 56 | 59 | 56 | 52 | 56 | 57‡ |
| Diabetes (%) | 7 | 15 | 22* | 6 | 13 | 18* | 8 | 16 | 16† |
| IRF (%)# | | | | | | | | | |
| Mild | 27 | 32 | 33 | 25 | 33 | 33 | 34 | 33 | 27 |
| Severe | 3 | 1 | 2 | 3 | 3 | 0 | 2 | 1 | 3 |
| IHD (%) | 6 | 7 | 11‡ | 5 | 7 | 9 | 5 | 8 | 8 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Cerebrovascular disease (%) | 5 | 8 | 6 | 6 | 8 | 7 | 5 | 7 | 8 |
| PAD (%) | 11 | 11 | 8 | 10 | 10 | 9 | 10 | 12 | 12 |
| CHF (%) | 4 | 4 | 7‡ | 4 | 6 | 6 | 2 | 8 | 6 |
| COPD (%) | 11 | 7 | 7 | 11 | 7 | 7 | 7 | 11 | 6 |
| Cancer (%) | 5 | 3 | 7 | 4 | 7 | 8 | 5 | 5 | 3 |
| Dementia (%) | 5 | 7 | 7 | 4 | 4 | 10 | 7 | 9 | 5 |
| Cognitive impairment (%) | 23 | 31 | 38† | 21 | 31 | 40† | 35 | 32 | 31 |
| Parkinson's disease (%) | 1 | 1 | 1 | 1 | 2 | 2 | 1 | 2 | 2 |
| **Dietary characteristics** | | | | | | | | | |
| Energy ($10^3$ kcal/d) | 2.1±0.6 | 1.9±0.5 | 1.6±0.4* | 2.1±0.7 | 1.9±0.5 | 1.7±0.5* | 1.8±0.6 | 1.8±0.6 | 1.9±0.6 |
| Total protein (% E) | 13±1 | 16±1 | 19±1* | 13±1 | 16±1 | 19±1* | 16±2 | 16±2 | 16±2 |
| Animal protein (% E) | 7±1 | 10±1 | 13±2* | 7±1 | 10±1 | 14±1* | 12±2 | 10±2 | 8±2* |
| Plant protein (% E) | 5.6±1.0 | 5.9±1.0 | 5.7±1.0 | 6.4±1.0 | 5.8±0.8 | 5.1±0.9* | 4.3±0.5 | 5.8±0.1 | 7.2±0.5* |
| SFA (% E) | 10±2 | 10±2 | 11±2* | 9±2 | 10±2 | 12±2* | 12±2 | 11±2 | 8±2* |
| MUFA (% E) | 14±3 | 15±3 | 16±3* | 14±3 | 15±3 | 17±3* | 16±3 | 15±3 | 14±3* |
| PUFA (% E) | 3.1±0.7 | 3.3±0.7 | 3.6±0.6* | 3.0±0.7 | 3.3±0.6 | 3.7±0.6* | 3.4±0.6 | 3.4±0.7 | 3.3±0.7 |
| Carbohydrates | 52±7 | 52±6 | 47±6* | 54±7 | 51±5 | 46±6* | 46±7 | 50±5 | 56±5* |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| (% E) | | | | | | | | | |
| Alcohol (% E) | 6 (10) | 3 (5) | 0 (3)* | 5 (10) | 3 (7) | 0 (5)* | 5 (10) | 2 (7) | 1 (4)* |

BMI, body mass index; ADL, activities of daily life; PA, physical activity; IHD, ischemic heart disease; PAD, peripheral artery disease; CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. Data for continuous variables are shown as mean ± S.D. or median (interquartile range). Cut-off points of intake category (percentage of total energy): for total protein were: quintile 1, <14.1%; quintile 2, 14.1% to <15.2%; quintile 3, 15.2% to <16.3%; quintile 4, 16.3% to <17.6%; and quintile 5, ≥17.6%. For animal protein were: quintile 1, <8.1%; quintile 2, 8.1% to <9.3%; quintile 3, 9.3% to <10.5%; quintile 4, 10.5% to <12.0%; and quintile 5, ≥12.0%. For plant protein were: quintile 1, <4.9%; quintile 2, 4.9% to <5.5%); quintile 3, 5.5% to <6.0%; quintile 4, 6.0% to < 6.6%; and quintile 5, ≥6.6%.

[#]Data available in 973 subjects.

*p for trend<0.001, † p for trend<0.01, ‡ p for trend<0.05 using age- and sex-adjusted generalized linear models.

The prevalence of chronic diseases increased across the follow-up assessments being hypertension, mild impaired renal function (both around 80%) and cognitive impairment (67%) the most frequent conditions at the last evaluation (Supplementary Figure 1). Small changes were observed in dietary intake data across follow-up assessments (within the ±8% of baseline values for almost all the macronutrients except for PUFA intake which varied within ±12%). Intraclass correlation coefficients were within the range of 0.23-0.50.

**Association between dietary protein sources and mortality**

During the 20-years of follow up (mean: 12±6 years), 811 deaths occurred (292 of CVD and 151 of cancer-related causes). Associations between total, animal, and plant protein intake and mortality outcomes are shown in Figure 2 and Supplementary Table 3. In the fully adjusted model, a statistically significant inverse association was observed between total protein and CVD mortality. Animal protein, but not plant protein intake, was inversely associated with both all-cause and CVD mortality. Neither total, nor animal, nor plant protein intakes were associated with cancer mortality (Figure 2). The associations between protein intake and mortality were linear in all cases, and non-linear terms did not improve the models fit.

**Figure 2. Association between total, animal and plant protein intake and all-cause, cardiovascular and cancer mortality**

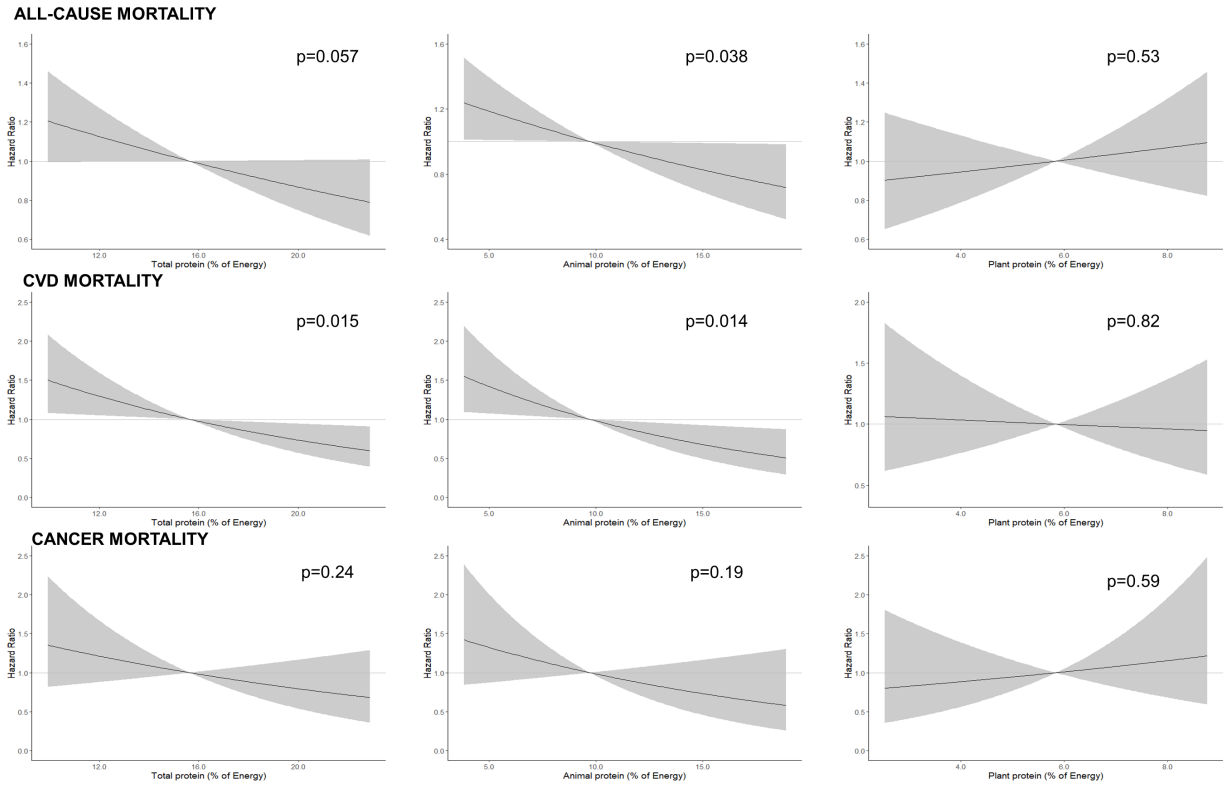



**Figure 2.** Cox regression stratified by site and baseline hypertension adjusted for age, sex, total energy, percentage of energy from saturated fatty acids (SFA), mono-unsaturated fatty acids (MUFA), poly-unsaturated fatty acids (PUFA), alcohol, total or plant and animal protein, BMI, years of education, smoking status (current *vs.* former and non-smoker), ADL disability, physical activity, impaired renal function, diabetes, ischemic heart disease, cerebrovascular disease, peripheral artery disease, congestive heart failure, chronic obstructive pulmonary disease, cancer, dementia, cognitive impairment, cognitive decline, Parkinson's disease and Mediterranean diet adherence score. Number of participants: 1,139. Number of observations = 4,317. Shaded areas are the 95% confidence intervals.

For all-cause mortality, there was a statistically significant interaction between plant protein and hypertension (Supplementary Figure 2.); for CVD mortality, between animal protein and IHD, and between plant protein and hypertension, and IHD; and for cancer mortality, between plant protein and ADL disability, and cognitive impairment. No other interaction was statistically significant for any of the mortality outcomes.

Hypertension had a major effect on the association between plant protein and all-cause and CVD mortality. Indeed, plant protein displayed an inverse, marginally significant association with CVD mortality among participants without baseline hypertension (HR, 95%CI: 0.79, 0.62-1.01, Supplementary Figure 2). Comparison of participants with and without baseline hypertension (n=644 *vs.* n=495), showed that subjects with hypertension were more likely to be women and showed higher age and sex-adjusted prevalence of obesity, impaired renal function, IHD, PAD, and CHF (Supplementary Table 4). Moreover, they showed a lower intake of SFA and a trend towards a higher plant protein intake than those without hypertension (p=0.057, Supplementary Table 4).

In sensitivity analyses, results were similar after the exclusion of subjects with imputed values, participants who died within the first 2 years of the follow-up, subjects with severe impaired renal function at baseline, baseline cancer, or with cognitive impairment, who may be inaccurate in reporting food intake (Supplementary Table 3). Across the follow-up period, the association between plant protein and CVD mortality was significantly reduced during the last follow-up periods of the study (p= 0.032). No other difference of effects was observed during the follow-up assessments with neither total protein nor animal protein intake for any mortality outcome. Likewise, there was no difference of effect between sites whether using the data with or without imputations for participants and observations (p>0.05).

## Discussion

Higher intakes of animal protein were associated with lower risk for all-cause and CVD mortality in this study of community-dwelling older adults after a 20-years follow-up.

The present study is the first showing an inverse association between animal protein and mortality in older women and men from a Mediterranean country. Chan *et al.*[14] reported a similar association in older men, but not in women. The borderline statistically significant inverse association between total protein intake and overall mortality is also in agreement with other studies carried out in older adults.[12–14] In this study, total and animal protein intake displayed similar relationships with all-cause and CVD mortality, opposite to results from a recent meta-analysis.[7]

Increased animal protein intake may be inversely associated with mortality in older adults through its protective effects on muscle strength, frailty, sarcopenia or immune responses, all of which merit further studies.[24] Increased intake of total or animal protein was positively associated with muscle strength,[25] which in turn was inversely associated with all-cause mortality in a recent meta-analysis of studies in older adults.[26] Moreover, chronic or acute inflammatory conditions may impair the direct relationship between protein intake and muscle strength in older adults, increasing dietary protein requirements.[1,27] Further studies should determine if intrinsic characteristics of animal protein (i.e. its amino acid profile) and/or the overall levels of protein intake are behind its inverse association with mortality.

Recently, an analysis from the Rotterdam study showed a positive association between animal protein and all-cause mortality.[6] Besides the differences in age and comorbidities at baseline between their study and ours, there were also large differences in dietary intake. For instance, total fat (as percentage of total energy) was lower in our study (27% *vs.* 35%) and qualitatively different with a higher MUFA intake (14% *vs.* 12%) and lower SFA and PUFA intakes (10% *vs.* 14%, and 3.2% *vs.* 7.0%, respectively). In line with an effect of fatty acid composition of the consumed foods, they found that substitution of dairy or meat protein (correlated with SFA intake) for carbohydrates were the largest contributors to the positive association between animal protein and mortality.[6] These dietary particularities may have contributed to the differences between studies.

Importantly, we adjusted for overall diet quality, which has major implications shaping the relationship between intake of protein from different sources and mortality.

In the present cohort, plant protein was mostly coming from cereals, and this fact could be related to the lack of association between plant protein and mortality. Indeed, in Asian studies where an inverse association between plant protein and all-cause mortality was observed, legumes and pulses contributed to approximately 25% of total plant protein (*vs.* 5% in the present study).[11,14] Recently, Huang *et al.* reported an inverse association between plant protein and mortality in a US prospective cohort study.[8] In their analysis they observed that the inverse association between plant protein intake and mortality was decreased in the groups of participants aged ≥65y and with a follow-up>10 years. Thus, our results are not in disagreement with these results, but rather complimentary by extending the analysis to an older age segment of the population.

The strengths of the current study include a long follow-up in a well-established cohort of older adults, and the inclusion of repeated dietary assessments to reduce bias from measurement errors in dietary questionnaires. Our study also has limitations. The relatively small sample size and low incidence of cancer-related deaths could have compromised the statistical power. However, protein intake was not associated with cancer mortality in a meta-analysis.[7] Medical advice could have promoted a lower intake of carbohydrates and higher intake of proteins (from plant or animal sources to a different extent) potentially affecting the dietary choices. Indeed, higher plant protein intakes are encouraged within a DASH diet compared to a western diet.[28] Last, residual confounding may still remain, even though we adjusted the analyses by a Mediterranean diet adherence score.

In conclusion, refuting our initial hypothesis, higher intake of animal protein was associated with lower risk for all-cause and CVD mortality in older adults. Nonetheless, plant protein showed a borderline statistically significant association with CVD mortality, at least in participants without hypertension. In older adults, differences in long-term associations between animal or plant protein intake and mortality should be considered in future dietary recommendations to promote longevity.

## AUTHOR CONTRIBUTIONS

TM, RZR and CAL designed the research; NHL, MR, SB, LF, AC and CAL, conducted the research; TM, RZR, NHL and MR performed statistical analysis; TM, NHL and MR wrote the paper; RZR, SB, LF, MF, AC and CAL provided critical revision; and CAL had primary responsibility for the final content. All authors read and approved the final manuscript.

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**CONFLICT OF INTEREST**

The authors are not aware of any conflict of interest.

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