Dietary proteins and protein sources and risk of death: the Kuopio Ischaemic Heart Disease Risk Factor Study

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ABSTRACT

Background: Previous studies investigating protein intake in relation to mortality have provided conflicting results.

Objective: We investigated the associations of dietary protein and protein sources with risk of disease death in the prospective, population-based Kuopio Ischaemic Heart Disease Risk Factor Study.

Methods: The study population consisted of 2641 Finnish men, aged 42–60 y at baseline in 1984–1989. We estimated protein intakes with 4-d dietary records at baseline and collected data on disease deaths from the national Causes of Death Register. Cox proportional hazards regression models were used to estimate HRs and 95% CIs.

Results: During the average follow-up of 22.3 y, we observed 1225 deaths due to disease. Higher intakes of total protein and animal protein had borderline statistically significant associations with increased mortality risk: multivariable-adjusted HR (95% CI) in the highest compared with the lowest quartile for total protein intake = 1.17 (0.99, 1.39; *P*-trend across quartiles = 0.07) and for animal protein intake = 1.13 (0.95, 1.35; *P*-trend = 0.04). Higher animal-to-plant protein ratio (extreme-quartile HR = 1.23; 95% CI: 1.02, 1.49; *P*-trend = 0.01) and higher meat intake (extreme-quartile HR = 1.23; 95% CI: 1.04, 1.47; *P*-trend = 0.01) were associated with increased mortality. When evaluated based on disease history at baseline, the association of total protein with mortality appeared more evident among those with a history of type 2 diabetes, cardiovascular disease, or cancer (n = 1094) compared with those without disease history (n = 1547) (*P*-interaction = 0.05 or 0.07, depending on the model). Intakes of fish, eggs, dairy, or plant protein sources were not associated with mortality.

Conclusions: Higher ratio of animal to plant protein in diet and higher meat intake were associated with increased mortality risk. Higher total protein intake appeared to be associated with mortality mainly among those with a predisposing disease. This trial was registered at clinicaltrials.gov as NCT03221127. *Am J Clin Nutr* 2019;0:1–10.

Keywords: dietary protein, animal protein, dairy, meat, plant protein, mortality, prospective study, men

Introduction

Optimal macronutrient composition of a diet for supporting longevity remains unclear. Especially the amount and quality of protein have aroused much interest during the past several years. Short-term interventions aiming to increase protein intake have provided some promising effects on health; for example, higher protein intake has supported weight loss and lowered blood pressure (1). However, long-term epidemiological studies investigating the association between protein intake and mortality have provided divergent conclusions. Whereas some studies have suggested that higher protein intake or high-protein, low-carbohydrate diets are associated with decreased all-cause mortality (2, 3), some studies have indicated the opposite (4–6), and some have not found an association (7–10). One study also revealed that higher protein intake was associated with higher mortality in those aged ≤ 65 y but appeared to be protective in older individuals (11).

Higher protein diets can be constituted in several ways, and the typical protein sources may vary between different populations and cohorts. Thus, the protein sources likely modify the associations observed between protein intake and mortality. Higher animal protein intake has been associated with increased all-cause mortality risk (4, 11, 12), whereas higher plant protein intake has indicated either no association (7, 9) or has been related to decreased risk (8, 12). Furthermore, animal and plant protein

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Supplemental Figure 1 and Supplemental Tables 1–6 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

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Abbreviations used: CVD, cardiovascular disease; ICD, International Classification of Diseases; KIHD, Kuopio Ischaemic Heart Disease Risk Factor Study; PREDIMED, PREvención con DIeta MEDiterránea.

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groups combine protein from a wide variety of protein sources that may also have differential associations with mortality risk. For example, intake of red or processed meat has been associated with increased risk of total mortality (13–15), whereas nut intake has had an inverse association (13), and the findings with fish (13, 16), dairy (13, 17, 18), and egg intakes (13, 19) have been inconclusive.

Overall, the previously cited studies have provided contradictory conclusions, and only a few of them have considered both the intake of protein and the protein sources in the same context. Therefore, to obtain a more comprehensive picture of the roles of dietary protein and the central protein sources in relation to mortality risk, we examined whether protein intake or different protein sources are associated with risk of disease mortality in middle-aged and older men from eastern Finland. Most of the previous studies excluded subjects who had major diseases at study baseline. To determine whether the baseline disease status has an effect on the associations, we assessed interactions by history of major chronic diseases [type 2 diabetes, cardiovascular disease (CVD) or cancer].

Methods

Study population

The Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD) was designed to investigate risk factors for CVD, atherosclerosis, and related outcomes in a population-based, randomly selected sample of men from eastern Finland (20). Therefore, the non-CVD outcomes can be regarded as secondary endpoints. The baseline examinations were carried out in 1984–1989 on a total of 2682 men (83% of those eligible) who were 42, 48, 54, or 60 y old. The KIHD protocol was approved by the Research Ethics Committee of the University of Kuopio. All subjects gave written informed consent. Subjects with missing data on dietary intakes ($n = 41$) were excluded, which left 2641 men for the analyses (**Supplemental Figure 1**). Of these men, 1094 had a history of type 2 diabetes, CVD, or cancer at baseline, and 1547 were free of these diseases.

Baseline measurements

Fasting venous blood samples were collected between 0800 and 1000 at the baseline examinations. Subjects were instructed to abstain from ingesting alcohol for 3 d and from smoking and eating for 12 h before providing the sample. Detailed descriptions of the determination of serum lipids and lipoproteins (21), serum ferritin (21) and blood pressure (21), and the assessment of medical history and medications (21), family history of diseases (21), smoking (21), alcohol consumption (21), and leisure-time physical activity (22) at baseline have been published. Subjects were classified as having hypertension if they had systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg or if they used medication for hypertension. Type 2 diabetes was defined as a self-reported physician diagnosis of type 2 diabetes and/or fasting plasma glucose ≥7.0 mmol/L and/or use of medication for diabetes. CVD and cancer diagnoses at baseline were self-reported physician diagnoses. Number of years of education, annual income, marital status, and dietary supplement use were obtained from self-administered questionnaires. Family history of diabetes, CVD, or cancer was defined as positive if a first-degree relative of the participant had a history of these diseases. BMI was computed as the ratio of weight in kilograms to the square of height in meters. Serum C-reactive protein was measured with an immunometric assay (Immulite High Sensitivity CRP Assay, Diagnostics Products Corporation). Serum creatinine was measured with the clinical chemistry analyzer Kone Specific (KONE Instruments Oy) using Jaffe reaction, and estimated glomerular filtration rate was calculated by the Chronic Kidney Disease Epidemiology Collaboration formula (23).

Assessment of dietary intakes

The consumption of foods at baseline was assessed with an instructed food record of 4 d, 1 of which was a weekend day, by using household measures. A picture book of foods and dishes was used to help in the estimation of portion sizes. The book contained 126 common foods and drinks consumed in Finland during the 1980s. For each food item, the participant could choose 3–5 commonly used portion sizes or describe the portion size in relation to those shown in the book. To further improve accuracy, instructions were given and completed food records were checked by a nutritionist together with the participant. Nutrient intakes were estimated using NUTRICA 2.5 software (Social Insurance Institution). The software's databank is mainly based on Finnish values of nutrient composition of foods.

We calculated the intakes of total, animal, and plant protein and the intakes of different protein sources (**Supplemental Table 1**). In addition, we calculated the ratio between intakes of animal and plant protein in the diet, with a higher ratio indicating a higher animal-based diet. Total meat included red meat, white meat, and offal. Processed red meat included all red meat that had undergone any industrial processing—for example, by adding salt or preservatives. Participants did not use processed white meat. For the major plant protein sources, we combined the most protein-rich foods of the plant protein category—that is, grain products, legumes, nuts, and seeds. Each nutrient was energy-adjusted by the residual method (24).

Ascertainment of follow-up events

Deaths were ascertained by a computer linkage to the national Causes of Death Register with the use of the Finnish personal identification code (social security number). All deaths were coded according to the International Classification of Diseases (ICD), 10th revision, codes. All disease deaths that occurred from the study entry to 31 December, 2014, were included. Deaths due to accidents or suicides (ICD codes S00–T98) were not included.

Statistical analysis

Intakes of protein sources and energy-adjusted proteins were expressed as g/d in the analyses. The univariate associations of total, animal, and plant protein intakes with baseline characteristics were assessed by means and linear regression for continuous variables or by chi-square tests for categorical variables. Differences in baseline characteristics between those with and without disease history were compared with independent samples *t* test

for continuous variables or with chi-square test for categorical variables.

Person-years of follow-up were calculated from the baseline to the date of death or the end of follow-up. Cox proportional hazards regression models were used to estimate HRs in exposure quartiles, with the lowest category as the reference. Schoenfeld residuals did not indicate significant evidence of violation of the proportional hazards assumption.

We selected covariates based on the literature of identified and potential risk factors for mortality, previously published associations in the KIHD, or on associations with exposures and outcomes in the present analysis. Model 1 included age (years), examination year, and energy intake (kilocalories per day). Model 2 included the variables in model 1 plus education years; income (euros per year); marital status (married/unmarried); pack-years of smoking (cigarette packs smoked per day × years smoked); alcohol intake (grams per week); leisure-time physical activity (kilocalories per day); BMI (in kg/m^2); and diagnosis of type 2 diabetes, CVD, cancer, or hypertension at baseline or use of cardiac, hypercholesterolemia, hypertension, or diabetes medications (yes/no). Model 3 included model 2 plus intakes of the following nutrients: fiber (grams per day) and saturated, monounsaturated, polyunsaturated, and *trans* fatty acids (all grams per day). When using protein sources as exposure, nutrients were considered primarily as mediators; thus, the main model in those analyses was model 2. When using proteins as exposure, nutrients were considered primarily as confounders; thus, model 3 was the main model. Animal and plant proteins were also mutually adjusted for in model 3; thus, for the proteins, model 3 can be interpreted as isocaloric replacement of carbohydrates with the protein of interest. The following were not included in the models because they did not appreciably affect the associations (change in estimates <5%): use of dietary supplements; family history of diabetes, CVD, or cancer; and intake of fruits, berries, and vegetables.

All quantitative variables were entered in the models as continuous variables. The cohort mean was used to replace missing values in covariates (<2.0%). Tests of linear trend were conducted by assigning the median values for each category of exposure variable and treating those as a single continuous variable. The statistical significance of the interactions with baseline disease history (type 2 diabetes, CVD, or cancer) was assessed by likelihood ratio tests with the use of a cross-product term. The associations based on baseline disease history are presented only in cases in which the interaction was statistically significant. All P values were 2-tailed ($\alpha = 0.05$). Data were analyzed by using SPSS 25.0 for Windows (IBM Corporation).

Results

Baseline characteristics

The mean protein intake was 93.2 g/d, which comprised 15.8% of energy intake (E%) and corresponds to 1.2 g protein/kg/d [1.4 g/kg of ideal body weight, with ideal body weight defined as BMI 22 (25)]. Of the total protein intake, 70.0% was from animal sources and 27.7% from plant sources, whereas 2.3% was from mixed sources (e.g., dry ready meals and chocolate) and was not included in either of the categories. Dairy (44.2% of the animal protein intake), meat (37.7%), and fish (12.5%) were the most abundant animal protein sources, whereas grain products comprised 79.4% of the plant protein intake.

Table 1 presents the baseline characteristics of the study population according to total protein intake, and **Supplemental Tables 2** and **3** present the characteristics according to animal and plant protein. Compared to men with the lowest protein intake, men with higher total protein intake were more likely to be married and to have higher education and income levels, but they also had higher BMI and were more likely to have type 2 diabetes. Higher total protein intake was also associated with higher intake of fiber, polyunsaturated fatty acids, and all animal protein sources. Men with higher animal protein intake had a more favorable socioeconomic factor profile but also had higher BMI and were more likely to smoke and have type 2 diabetes. They had lower intake of fiber but higher intake of polyunsaturated fatty acids than those in the lowest animal protein intake category. Higher plant protein intake was generally associated with healthier lifestyle and dietary factors (Table 1).

Associations of dietary proteins with risk of disease mortality

We recorded 1225 deaths due to disease during the mean follow-up of 22.31 y (SD = 7.89 y; range = 0.02–30.76 y). Of these, 618 were due to CVD, 347 were due to cancer, and 260 were due to other causes. Total protein intake was not associated with mortality risk in the model adjusted for age, examination year, and energy intake (model 1; **Table 2**), but those in the highest compared with the lowest intake quartile had a borderline statistically significant 17% increased risk of mortality in the multivariable model 3 (95% CI: −1, 39%; P-trend across quartiles = 0.07). Those in the highest compared with the lowest intake quartile of animal protein also had a trend toward 13% increased mortality risk (95% CI: −5, 35%; P-trend = 0.04). When assessed continuously, each 5 g/d higher intake of either total protein or animal protein was associated with a 3% (95% CI: 1, 5%; $P = 0.01$) higher mortality risk. Moreover, those in the highest quartile of animal-to-plant protein ratio had 23% (95% CI: 2, 49%; P-trend = 0.01) increased risk of mortality compared with those in the lowest quartile. Plant protein intake was not associated with mortality risk in the multivariable models (Table 2). The association for total protein appeared to be stronger among those with disease history (HR: 1.04; 95% CI: 1.01, 1.07; per 5 g/d increase in intake in model 3) than among those without (HR: 1.01; 95% CI: 0.98, 1.04; P-interaction = 0.05 in model 2 and P-interaction = 0.07 in model 3). Other interactions were not statistically significant (P-interactions ≥0.14).

Associations of dietary protein sources with risk of disease mortality

Those in the highest meat intake quartile had 23% (95% CI: 4, 47%; P-trend = 0.01) higher risk of mortality than those in the lowest quartile in multivariable-adjusted model 2 (**Table 3**). The adjustments for nutrient intakes further strengthened the association between meat and mortality (model 3). Parallel but weaker and statistically nonsignificant associations were observed with total red meat and unprocessed red meat intake.

TABLE 1 Baseline characteristics according to total protein intake among 2641 men from the Kuopio Ischaemic Heart Disease Risk Factor Study

	Total protein intake			
Characteristic	Quartile 1 (< 83.9 g/d)	Quartile 2 (83.9–92.1 g/d)	Quartile 3 (92.2–101.5 g/d)	Quartile 4 (> 101.5 g/d)
Subjects, *n*	660	660	661	660
Demographic and lifestyle factors				
Age, y	53.7 ± 4.6[2]	53.2 ± 4.9	52.7 ± 5.2	52.7 ± 5.6[3]
Education, y	8.2 ± 3.1	8.5 ± 3.4	8.8 ± 3.5	9.0 ± 3.7[3]
Income, 1000€	11.6 ± 7.7	12.9 ± 8.4	13.6 ± 9.3	14.4 ± 9.5[3]
Married, %	83.5	85.3	88.8	89.1[3]
Current smoker, %	33.5	32.9	30.9	30.0
Regular use of dietary supplements, %	6.1	6.4	8.8	8.5[3]
Alcohol intake, g/wk	102 ± 214	64 ± 93	66 ± 99	68 ± 87[3]
Leisure-time physical activity, kcal/d	135 ± 177	134 ± 160	148 ± 176	147 ± 184
BMI, kg/m2	26.5 ± 3.4	26.6 ± 3.5	26.8 ± 3.6	27.6 ± 3.7[3]
Health and disease status				
Serum total cholesterol to HDL ratio	4.77 ± 1.48	4.89 ± 1.54	4.88 ± 1.56	4.76 ± 1.40
Serum triglycerides, mmol/L	1.25 ± 0.74	1.33 ± 0.84	1.31 ± 0.84	1.37 ± 0.85[3]
Serum C-reactive protein, mg/L	2.60 ± 5.35	2.28 ± 3.87	2.46 ± 3.72	2.42 ± 3.47
Serum ferritin, μg/L	155 ± 162	163 ± 149	163 ± 135	193 ± 160[3]
Estimated glomerular filtration rate, mL/min	84.9 ± 13.4	84.8 ± 12.5	85.4 ± 12.2	85.6 ± 13.1
Family history of diabetes, %	27.6	26.5	28.4	29.5
Family history of CVD[1], %	82.7	83.8	79.0	83.9
Family history of cancer, %	25.3	24.8	24.2	22.9
Hypertension, %	61.4	58.8	59.5	61.7
Hypertension medication, %	20.2	24.8	22.2	23.3
Diabetes, %	3.8	4.7	7.3	8.0[3]
Diabetes medication, %	0.8	0.6	1.4	2.0[3]
CVD[1], %	40.2	36.5	38.3	36.1
Cardiac medication, %	2.4	3.2	2.6	3.2
Hypercholesterolemia medication, %	0.2	0.8	0.6	0.9
Cancer, %	2.7	1.5	1.8	1.7
Dietary intakes				
Energy, kcal/d	2532 ± 671	2336 ± 577	2360 ± 577	2534 ± 630
Protein, g/d	76.4 ± 7.3	88.0 ± 2.4	96.6 ± 2.7	111.8 ± 9.7[3]
Protein, E%[4]	12.9 ± 1.1	14.9 ± 0.7	16.5 ± 1.0	18.8 ± 2.1[3]
Animal protein, g/d	49.0 ± 8.9	59.5 ± 6.4	68.7 ± 6.1	83.6 ± 11.8[3]
Animal protein, E%[4]	8.2 ± 1.4	10.1 ± 1.2	11.7 ± 1.3	14.1 ± 2.3[3]
Plant protein, g/d	25.2 ± 6.4	26.3 ± 5.7	25.7 ± 5.4	26.1 ± 6.5[3]
Plant protein, E%[4]	4.2 ± 1.0	4.5 ± 1.1	4.4 ± 1.0	4.4 ± 1.0
Fat, E%[4]	39.9 ± 6.5	38.6 ± 5.6	38.7 ± 5.6	37.4 ± 5.9[3]
Saturated fatty acids, E%[4]	19.5 ± 4.5	18.3 ± 3.8	18.1 ± 3.8	16.9 ± 3.8[3]
Polyunsaturated fatty acids, E%[4]	4.2 ± 1.5	4.5 ± 1.4	4.6 ± 1.4	4.7 ± 1.3[3]
Monounsaturated fatty acids, E%[4]	11.7 ± 2.4	11.6 ± 2.1	11.8 ± 2.2	11.7 ± 2.3
trans Fatty acids, E%[4]	1.1 ± 0.4	1.1 ± 0.4	1.0 ± 0.4	1.0 ± 0.4[3]
Carbohydrates, E%[4]	43.6 ± 7.2	48.3 ± 6.1	42.2 ± 5.8	41.2 ± 6.4[3]
Fiber, g/d	24.4 ± 7.6	25.0 ± 6.5	24.9 ± 6.4	26.0 ± 8.1[3]
Fruits, berries, and vegetables,[5] g/d	231 ± 164	247 ± 154	247 ± 149	279 ± 159[3]
Whole grain products, g/d	159 ± 77	153 ± 72	153 ± 68	173 ± 84[3]
Unprocessed red meat, g/d	58 ± 40	66 ± 46	76 ± 45	97 ± 60[3]
Processed red meat, g/d	69 ± 61	62 ± 52	69 ± 56	79 ± 693
Fish, g/d	27 ± 33	35 ± 37	46 ± 46	76 ± 75[3]
Egg, g/d	31 ± 25	30 ± 24	31 ± 23	35 ± 29[3]
Nonfermented dairy, g/d	486 ± 308	504 ± 305	543 ± 347	564 ± 366[3]
Fermented dairy g/d	113 ± 143	165 ± 191	195 ± 211	273 ± 273[3]

[1]CVD, cardiovascular disease.
[2]Mean ± SD (all such values).
[3]*P* for trend across quartiles < 0.05. *P*-trend was assessed with linear regression (continuous variables) or with chi-square test (categorical variables).
[4]E%, percentage of energy intake.
[5]Excluding potatoes.

TABLE 2 Risk of disease death according to protein intake among 2641 men from the Kuopio Ischaemic Heart Disease Risk Factor Study[1]

	Intake quartile					Per 5 g/d increase			
	1 ($n = 660$)	2 ($n = 660$)	3 ($n = 661$)	4 ($n = 660$)	*P*-trend	All ($n = 2641$)	No disease history ($n = 1547$)	Disease history ($n = 1094$)	*P*-interaction
Total protein									
Median intake, g/d	78.5	87.9	96.3	109.1	—	—	—	—	—
No. of events	340	299	289	297	—	—	—	—	—
Incidence rate/1000 PY	23.39	20.07	19.29	20.46	—	—	—	—	—
Model 1	1	0.89	0.87	1.00 (0.85, 1.17)	0.95	1.00 (0.98, 1.02)	0.98 (0.95, 1.01)	1.02 (0.99, 1.04)	0.07
Model 2	1	1.00	1.01	1.14 (0.97, 1.33)	0.12	1.03 (1.01, 1.05)	1.01 (0.98, 1.04)	1.04 (1.01, 1.06)	0.05
Model 3	1	1.02	1.03	1.17 (0.99, 1.39)	0.07	1.03 (1.01, 1.05)	1.01 (0.98, 1.04)	1.04 (1.01, 1.07)	0.07
Animal protein									
Median intake, g/d	49.2	59.8	68.8	82.1	—	—	—	—	—
No. of events	325	287	300	313	—	—	—	—	—
Incidence rate/1000 PY	21.98	19.03	20.40	21.80	—	—	—	—	—
Model 1	1	0.89	0.99	1.15 (0.98, 1.34)	0.04	1.02 (1.00, 1.04)	—	—	0.18
Model 2	1	0.84	1.01	1.11 (0.95, 1.30)	0.06	1.02 (1.01, 1.04)	—	—	0.14
Model 3	1	0.86	1.02	1.13 (0.95, 1.35)	0.04	1.03 (1.01, 1.05)	—	—	0.19
Plant protein									
Median intake, g/d	19.6	23.8	27.2	32.2	—	—	—	—	—
No. of events	350	300	301	274	—	—	—	—	—
Incidence rate/1000 PY	24.92	20.53	19.98	18.02	—	—	—	—	—
Model 1	1	0.75	0.69	0.65 (0.55, 0.76)	<0.001	0.87 (0.83, 0.92)	—	—	0.32
Model 2	1	0.87	0.91	0.92 (0.77, 1.09)	0.43	0.99 (0.93, 1.04)	—	—	0.15
Model 3	1	0.89	0.95	0.98 (0.76, 1.26)	0.99	1.03 (0.93, 1.13)	—	—	0.17
Animal-to-plant protein ratio									
Median ratio	1.7	2.3	2.8	3.8	—	—	—	—	—
No. of events	301	286	297	341	—	—	—	—	—
Incidence rate/1000 PY	19.92	19.00	19.96	24.56	—	—	—	—	—
Model 1	1	0.94	1.01	1.42 (1.21, 1.65)	<0.001	1.12 (1.07, 1.17)[2]	—	—	0.44
Model 2	1	0.89	0.95	1.15 (0.98, 1.36)	0.02	1.05 (1.01, 1.10)[2]	—	—	0.67
Model 3	1	0.92	0.99	1.23 (1.02, 1.49)	0.01	1.07 (1.01, 1.13)[2]	—	—	0.55

[1] Values are HRs (95% CIs) derived from Cox proportional hazards regression models. The significance of the interactions on a multiplicative scale was assessed by stratified analysis and likelihood ratio tests by using a cross-product term. Subgroup analyses are shown only when *P* value for interaction is ≤0.05. Model 1 adjusted for age, examination year, and energy intake (kilocalories per day). Model 2 adjusted for model 1 and income (euros per year); education years; marital status (married/unmarried); leisure-time physical activity (kilocalories per day); pack-years of smoking (packs smoked per day × years smoked); alcohol intake (grams per week); BMI (kg/m^2); and diagnosis of type 2 diabetes, cardiovascular disease, cancer, or hypertension or use of cardiac, hypercholesterolemia, hypertension, or diabetes medications (yes/no). Model 3 adjusted for model 2 and intakes of fiber (grams per day) and saturated, monounsaturated, polyunsaturated, and *trans* fatty acids (all grams per day). For animal and plant proteins, mutual adjustment was conducted in model 3. PY, person-years.

[2] For animal-to-plant protein ratio, presented per 1 unit increase in the ratio.

Nonfermented dairy and milk intakes indicated associations with higher mortality risk in the minimally adjusted model 1, but these associations were attenuated after multivariable adjustments (models 2 and 3; Table 3). Intakes of total and fermented dairy, cheese, fish, eggs, and major plant protein sources were not associated with mortality risk in the multivariable-adjusted models. Although the associations of nonfermented dairy and milk indicated statistically significant interactions by disease history (*P*-interactions = 0.05), the subgroup analyses did not reveal significant differences in the associations (Table 3).

Sensitivity analyses

Because the association of total protein appeared slightly stronger among those with disease history than among those without, we explored the baseline characteristics according to the baseline disease history (**Supplemental Table 4**). There were no significant differences in protein intake between these groups. However, those with a disease history had slightly lower intakes of unprocessed red meat; eggs; and fruits, berries, and vegetables (Supplemental Table 4).

Meat is an important source of bioavailable heme iron, and we have previously reported that high iron stores are associated with higher risk of coronary artery disease (21) and impaired glucose metabolism (26). Thus, we tested whether adjusting further for serum ferritin affects the associations observed with meat and animal protein intakes in model 3. This adjustment had only minor impact on the associations; for example, HR (95% CI) per 100 g/d higher meat intake was 1.11 (1.00, 1.24; $P = 0.06$), and HR per 5 g/d higher animal protein intake was 1.03 (1.01, 1.05; $P = 0.01$).

Because we assessed dietary intakes only at the study baseline, and the follow-up time was long, we repeated the analyses using shorter follow-up time (mean: 11.2 y; $n = 348$ cases). In general, the results were similar to those with the longer follow-up (**Supplemental Tables 5** and **6**). However, diverging from the results with the complete follow-up, higher fish intake

TABLE 3 Risk of disease death according to the major protein sources among 2641 men from the Kuopio Ischaemic Heart Disease Risk Factor Study[1]

	Intake quartile					Per 100 g/d increase[2]			
	1 ($n = 660$)	2 ($n = 660$)	3 ($n = 661$)	4 ($n = 660$)	*P*-trend	All ($n = 2641$)	No disease history ($n = 1547$)	Disease history ($n = 1094$)	*P*-interaction
Total meat[3]									
Median intake, g/d	76	125	171	251	—	—	—	—	—
No. of events	322	309	304	290	—	—	—	—	—
Incidence rate/1000 PY	22.40	20.88	20.49	19.44	—	—	—	—	—
Model 1	1	1.06	1.11	1.29 (1.09, 1.53)[2]	0.003	1.10 (1.02, 1.19)	—	—	0.57
Model 2	1	1.04	1.12	1.23 (1.04, 1.47)	0.01	1.08 (1.00, 1.17)	—	—	0.54
Model 3	1	1.08	1.19	1.36 (1.09, 1.70)	0.01	1.13 (1.01, 1.26)	—	—	0.64
Total red meat									
Median intake, g/d	65	113	156	230	—	—	—	—	—
No. of events	322	300	322	281	—	—	—	—	—
Incidence rate/1000 PY	22.49	20.17	22.02	18.59	—	—	—	—	—
Model 1	1	0.99	1.16	1.19 (1.00, 1.41)	0.02	1.11 (1.02, 1.21)	—	—	0.42
Model 2	1	1.03	1.16	1.13 (0.95, 1.35)	0.09	1.07 (0.98, 1.16)	—	—	0.37
Model 3	1	1.04	1.19	1.17 (0.94, 1.46)	0.09	1.09 (0.97, 1.22)	—	—	0.47
Processed red meat									
Median intake, g/d	10	40	76	138	—	—	—	—	—
No. of events	314	303	311	297	—	—	—	—	—
Incidence rate/1000 PY	21.29	21.27	20.84	19.78	—	—	—	—	—
Model 1	1	1.06	1.16	1.24 (1.05, 1.47)	0.01	1.12 (1.01, 1.24)	—	—	0.99
Model 2	1	1.06	1.07	1.09 (0.92, 1.28)	0.37	1.03 (0.93, 1.14)	—	—	0.75
Model 3	1	1.06	1.06	1.07 (0.88, 1.30)	0.56	1.01 (0.89, 1.15)	—	—	0.82
Unprocessed red meat									
Median intake, g/d	21	53	80	132	—	—	—	—	—
No. of events	322	301	305	297	—	—	—	—	—
Incidence rate/1000 PY	22.29	20.09	20.84	19.99	—	—	—	—	—
Model 1	1	0.91	1.02	1.06 (0.90, 1.25)	0.29	1.06 (0.94, 1.19)	—	—	0.23
Model 2	1	0.92	1.05	1.14 (0.97, 1.35)	0.04	1.09 (0.97, 1.22)	—	—	0.35
Model 3	1	0.93	1.06	1.16 (0.98, 1.37)	0.04	1.09 (0.97, 1.22)	—	—	0.40
Fish									
Median intake, g/d	0	18	48	102	—	—	—	—	—
No. of events	314	291	297	323	—	—	—	—	—
Incidence rate/1000 PY	21.41	19.30	19.81	22.76	—	—	—	—	—
Model 1	1	0.85	0.86	1.01 (0.86, 1.18)	0.45	1.06 (0.96, 1.18)	—	—	0.69
Model 2	1	0.82	0.88	0.90 (0.77, 1.06)	0.66	0.99 (0.89, 1.10)	—	—	0.36
Model 3	1	0.82	0.88	0.91 (0.78, 1.08)	0.82	1.00 (0.90, 1.12)	—	—	0.37
Egg									
Median intake, g/d	8	20	34	59	—	—	—	—	—
No. of events	359	281	278	307	—	—	—	—	—
Incidence rate/1000 PY	26.62	18.72	18.25	20.21	—	—	—	—	—
Model 1	1	0.70	0.67	0.74 (0.63, 0.87)	0.01	0.89 (0.79, 1.01)[2]	—	—	0.27
Model 2	1	0.86	0.83	0.91 (0.77, 1.06)	0.37	0.98 (0.87, 1.10)[2]	—	—	0.28
Model 3	1	0.85	0.83	0.89 (0.75, 1.05)	0.29	0.97 (0.86, 1.10)[2]	—	—	0.22
Dairy									
Median intake, g/d	291	578	801	1120	—	—	—	—	—
No. of events	281	302	325	317	—	—	—	—	—
Incidence rate/1000 PY	18.91	20.47	22.14	21.66	—	—	—	—	—
Model 1	1	1.06	1.11	1.19 (0.99, 1.43)	0.05	1.03 (1.01, 1.05)	—	—	0.23
Model 2	1	0.99	1.00	1.08 (0.89, 1.30)	0.44	1.02 (1.00, 1.04)	—	—	0.21
Model 3	1	0.99	1.02	1.12 (0.92, 1.37)	0.26	1.02 (1.00, 1.05)	—	—	0.19
Nonfermented dairy									
Median intake, g/d	158	372	586	913	—	—	—	—	—
No. of events	285	302	305	333	—	—	—	—	—
Incidence rate/1000 PY	19.10	20.46	20.54	23.13	—	—	—	—	—
Model 1	1	1.05	1.06	1.33 (1.12, 1.59)	0.001	1.03 (1.01, 1.05)	1.04 (1.01, 1.06)	1.02 (0.99, 1.05)	0.12
Model 2	1	1.01	0.93	1.14 (0.96, 1.37)	0.21	1.01 (0.99, 1.03)	1.02 (0.99, 1.05)	1.00 (0.97, 1.03)	0.05
Model 3	1	1.02	0.94	1.17 (0.97, 1.41)	0.15	1.01 (0.99, 1.04)	1.01 (0.98, 1.04)	1.01 (0.98, 1.04)	0.05
Milk									
Median intake, g/d	143	352	565	880	—	—	—	—	—
No. of events	285	297	313	330	—	—	—	—	—
Incidence rate/1000 PY	19.06	20.07	21.08	23.03	—	—	—	—	—

(*Continued*)

TABLE 3 (*Continued*)

	Intake quartile					Per 100 g/d increase[2]			
	1 ($n = 660$)	2 ($n = 660$)	3 ($n = 661$)	4 ($n = 660$)	*P*-trend	All ($n = 2641$)	No disease history ($n = 1547$)	Disease history ($n = 1094$)	*P*-interaction
Model 1	1	1.03	1.08	1.35 (1.13, 1.61)	<0.001	1.03 (1.01, 1.05)	1.04 (1.01, 1.07)	1.02 (0.99, 1.05)	0.11
Model 2	1	1.00	0.95	1.14 (0.96, 1.37)	0.19	1.01 (0.99, 1.03)	1.02 (0.99, 1.05)	1.00 (0.97, 1.03)	0.05
Model 3	1	1.01	0.96	1.17 (0.97, 1.41)	0.14	1.01 (0.99, 1.04)	1.01 (0.98, 1.05)	1.01 (0.98, 1.04)	0.05
Fermented dairy									
Median intake, g/d	3	55	180	437	—	—	—	—	—
No. of events	348	281	271	325	—	—	—	—	—
Incidence rate/1000 PY	24.96	18.45	17.86	22.29	—	—	—	—	—
Model 1	1	0.78	0.70	0.86 (0.74, 1.00)	0.36	1.00 (0.97, 1.03)	—	—	0.67
Model 2	1	0.95	0.84	1.01 (0.87, 1.18)	0.72	1.01 (0.99, 1.04)	—	—	0.39
Model 3	1	0.96	0.85	1.04 (0.89, 1.21)	0.53	1.02 (0.99, 1.04)	—	—	0.44
Cheese									
Median intake, g/d	0	9	24	50	—	—	—	—	—
No. of events	429	317	250	229	—	—	—	—	—
Incidence rate/1000 PY[4]	27.95	20.56	17.74	16.28	—	—	—	—	—
Model 1	1	0.71	0.64	0.71 (0.60, 0.83)	<0.001	0.80 (0.70, 0.91)[2]	—	—	0.13
Model 2	1	0.88	0.83	0.99 (0.83, 1.17)	0.93	1.03 (0.90, 1.17)[2]	—	—	0.08
Model 3	1	0.88	0.83	0.99 (0.83, 1.17)	0.91	1.02 (0.89, 1.16)[2]	—	—	0.10
Major plant protein sources[5]									
Median intake, g/d	163	220	276	366	—	—	—	—	—
No. of events	368	310	279	268	—	—	—	—	—
Incidence rate/1000 PY	26.83	21.26	18.28	17.43	—	—	—	—	—
Model 1	1	0.78	0.64	0.63 (0.52, 0.76)	<0.001	0.85 (0.78, 0.92)	—	—	0.28
Model 2	1	0.93	0.78	0.87 (0.71, 1.06)	0.09	0.97 (0.89, 1.06)	—	—	0.31
Model 3	1	0.91	0.75	0.80 (0.63, 1.03)	0.04	0.96 (0.85, 1.08)	—	—	0.34

[1]Values are HRs (95% CIs) derived from Cox proportional hazards regression models. The significance of the interactions on a multiplicative scale was assessed by stratified analysis and likelihood ratio tests by using a cross-product term. Subgroup analyses are shown only when *P* value for interaction is ≤0.05. Model 1 adjusted for age, examination year, and energy intake (kilocalories per day). Model 2 adjusted for model 1 and income (euros per year); education years; marital status (married/unmarried); leisure-time physical activity (kilocalories per day); pack-years of smoking (packs smoked per day × years smoked); alcohol intake (grams per week); BMI (kg/m^2); and diagnosis of type 2 diabetes, cardiovascular disease, cancer, or hypertension or use of cardiac, hypercholesterolemia, hypertension, or diabetes medications (yes/no). Model 3 adjusted for model 2 and intakes of fiber (grams per day) and saturated, monounsaturated, polyunsaturated, and *trans* fatty acids (all grams per day). PY, person-years.

[2]For egg and cheese intakes, the HRs (95% CIs) in the continuous models are presented per 50 g/d increase in intake because of the low average intake compared with other food groups.

[3]Total meat includes red meat, white meat, and offal.

[4]Number of subjects in quartiles 1–4: 750 (zero intake), 682, 603, and 606, respectively.

[5]Major plant protein sources include grain products, legumes, nuts, and seeds.

was associated with increased mortality among those who had a disease history, and higher cheese intake was associated with lower mortality among those who were free of diseases (Supplemental Table 6).

We also tested the exclusion of deaths that occurred during the first 2 years of the follow-up ($n = 38$) and during the first 5 years of the follow-up ($n = 108$), but the results were not markedly changed (data not shown).

Discussion

In this cohort of eastern Finnish middle-aged and older men, a higher ratio of animal to plant protein in the diet and higher meat intake were related to increased mortality risk. Furthermore, higher total protein intake appeared to be associated with increased mortality risk mainly among those with a disease history at baseline. Apart from meat intake, other protein sources—that is, dairy, fish, egg, and plant protein sources—were not associated with mortality risk.

In line with results from a previous study (7), protein intake did not appear to be associated with mortality among those without disease history. However, some studies have observed that higher protein intake, especially when combined with lower carbohydrate intake, is associated with mortality also in subjects free of major disease (5, 6). In addition, a meta-analysis (27) and a review (28) suggested that high-protein, low-carbohydrate diets are associated with higher all-cause mortality, whereas the role of protein per se remains inconclusive (28). More evident associations among those with diabetes have also been reported (12), but conflicting evidence also exists (5, 9). Interestingly, some studies have linked higher protein intake to a lower mortality risk (2, 3) or lower protein intake to a higher risk (29). Different intake scales of protein and other macronutrients, as well as the age of the population, could partly explain differential findings. For example, in our study, low protein intakes were rare: <3.1% of the subjects had lower daily protein intake than is usually recommended for healthy adults—that is, 0.83 g/kg of ideal body weight (30). In contrast, in a French study, daily protein intake in the lowest intake tertile was <0.7 g/kg

of ideal body weight (3), which might have affected the conclusion that higher protein intake was protective against mortality. Likewise, the results of the PREvención con DIeta MEDiterránea (PREDIMED) study support the importance of protein intake scale: The association between protein intake and total mortality was U-shaped—both the subjects with low protein intakes (<1 g/kg body weight) and the subjects high protein intakes (>1.5 g/kg body weight) had an increased mortality risk (4).

In some previous studies, higher protein intake had a direct association with mortality in subjects ≤65 y old (11) but an inverse association among those >65 y old (2, 11). We were not able to conduct a similar comparison because in our cohort, the oldest subjects were 60 y old at baseline. Thus, our results should not be generalized to older age groups, who may need more protein to maintain muscle mass and avoid frailty (31, 32). Higher protein requirements of the elderly compared with younger adults are already highlighted in several recommendations (31–33).

The source of protein appears also to be a significant factor modifying the association between protein intake and mortality. In line with our finding, higher animal-to-plant protein ratio was associated with increased mortality in the PREDIMED study (4). Also, other studies suggest harm with higher animal protein intake (11, 12), whereas plant protein intake has generally had a neutral (7, 9) or an inverse association with mortality (8, 12).

Several mechanisms could explain the risks of higher-protein and animal-based diets. First, higher protein intake may be related to loss of kidney function and microalbuminuria in those with multiple diseases (e.g., diabetes and hypertension) (34, 35). Second, higher-protein, animal-based diets may increase potentially genotoxic metabolites, such as nitrogenous compounds, in the gut (36, 37), as well as accelerate insulin-like growth factor-1 secretion, which may promote cancer incidence and progression (11). Finally, it is likely that in addition to distinct amino acid compositions of animal and plant proteins, other nutrients such as minerals and bioactive compounds from protein sources partly explain their differential associations (38). Therefore, the whole dietary pattern that is high in animal protein sources and low in plant protein sources is likely more important than single nutrients in these sources.

Because meat was the only protein source that was associated with the mortality risk, the harms of animal-based diet seem to be partly due to higher meat intake. Accordingly, intake of red meat, especially processed red meat, has been previously associated with increased mortality risk (13–15). There is likely a cluster of factors explaining the harms of meat intake. In one study, heme iron and nitrate—substances with pro-oxidant capacity (15)—partly mediated the association of meat intake with mortality (14). However, in our analyses, adjusting for serum ferritin had little impact on the associations with meat, suggesting that iron load does not explain the increased mortality risk with higher meat intake. Unfortunately, we do not have information on nitrate intake. Branched-chain amino acids, sodium, fat quality, polycyclic aromatic hydrocarbons, advanced glycation end products, and heterocyclic aromatic amines are among other factors of meat that have been linked with diabetes, CVD, and/or cancer (15, 39).

Our findings with dairy partly support the previous observations. Although the totality of the evidence suggests that dairy intake is not associated with all-cause mortality (17, 18), one recent meta-analysis found a direct association with very high dairy intakes (>750 g/d) (13). Despite the considerably high dairy intake in our study population (median intake: 1120 g/d in the highest quartile), there was little evidence for increased mortality risk with higher dairy intake. Lifestyle factors associated with dairy intake may be one factor explaining the heterogeneity between the findings. For example, in our cohort, controlling for several potential confounders attenuated the associations of dairy intake with mortality.

Although higher fish intake was not associated with mortality with the complete follow-up, it was related to increased mortality among those with a disease history with a shorter follow-up. One previous meta-analysis reported an inverse association between fish intake and mortality risk (13), but another meta-analysis that compared Western and Asian studies revealed that in Western countries the relation was U-shaped: Risk was increased by intakes >20 g/d (16). Differences in the types of fish, in nutritional content due to fishing locations, and in preparation methods may explain the discrepancy in the results (16). Because of the numerous analyses, it is also possible that our finding occurred due to chance.

The absence of an association between egg intake and mortality in our population might seem conflicting with the meta-analysis reporting a direct association between egg intake and mortality (13). However, the meta-analysis indicated significant heterogeneity, and the quality of evidence was rated very low (13). Our finding with egg is not surprising because unlike reported elsewhere (19), in our cohort egg intake is not a marker of a poor quality diet or unfavorable lifestyle (40), and it has not been related to increased disease risks (40–42).

The strengths of this study are the population-based cohort setting, virtually no loss to follow-up, and comprehensive information about possible confounding factors. We also recognize the limitations. First, due to the observational design, we cannot exclude the possibility of residual confounding. Second, we included several analyses in the current study. If conservative adjustment for multiple comparisons is used, no association would remain statistically significant. However, because our analyses are based on relevant a priori hypotheses, we did not consider these corrections as necessary. Third, because we assessed dietary intakes with a single 4-d food recording, we might not have captured the typical intakes of occasionally consumed foods, such as fish and processed meat. The single measurement of diet at the baseline accompanied with a long follow-up increases the risk for random error, which typically attenuates the associations. However, the results were generally comparable with a shorter follow-up. Finally, although there were no substantial differences in the mean dietary intakes between those with and those without disease history (Supplemental Table 4), the reverse causality cannot be fully ruled out because those with more severe forms of disease might have been more susceptible to dietary changes than those with less severe forms. Also, because those with the most aggressive CVD or cancer may have died soon after the diagnosis (i.e., before our study baseline), the results should not be generalized to the most severe forms of diseases.

In conclusion, our results strengthen the evidence that a highly animal-based diet might not be optimal for long-term health. These findings also suggest that those with predisposing

diseases might be more susceptible for harms of high protein intake.

The authors' responsibilities were as follows—HEKV, SV, TTK, JM, PK, T-PT, JTS, and JKV: acquired the data and designed and conducted the research; HEKV: analyzed the data and drafted the manuscript; JKV analyzed the data and had primary responsibility for final content; SV, TTK, JM, PK, MPTY, T-PT, JTS, and JKV: critically revised the manuscript for important intellectual content; and all authors: read and approved the final manuscript. JTS is the CEO of MAS–Metabolic Analytical Services Oy. The other authors report no conflicts of interest.

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