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Association of Animal and Plant Protein Intake With All-Cause and Cause-Specific Mortality

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IMPORTANCE Epidemiological evidence regarding the long-term effects of higher dietary protein intake on mortality outcomes in the general population is not clear.

OBJECTIVE To evaluate the associations between animal and plant protein intake and all-cause and cause-specific mortality.

DESIGN, SETTING, AND PARTICIPANTS This prospective cohort study included 70 696 participants in the Japan Public Health Center–based Prospective Cohort who were aged 45 to 74 years and had no history of cancer, cerebrovascular disease, or ischemic heart disease at study baseline. Data were collected from January 1, 1995, through December 31, 1999, with follow-up completed December 31, 2016, during which 12 381 total deaths were documented. Dietary intake information was collected through a validated food frequency questionnaire and used to estimate protein intake in all participants. Participants were grouped into quintile categories based on their protein intake, expressed as a percentage of total energy. Data were analyzed from July 18, 2017, through April 10, 2019.

MAIN OUTCOMES AND MEASURES Hazard ratios (HRs) and 95% CIs for all-cause and cause-specific mortality were estimated using Cox proportional hazards regression models with adjustment for potential confounding factors.

RESULTS Among the 70 696 participants, 32 201 (45.5%) were men (mean [SD] age, 55.6 [7.6] years) and 38 495 (54.5%) were women (mean [SD] age, 55.8 [7.7] years). Intake of animal protein showed no clear association with total or cause-specific mortality. In contrast, intake of plant protein was associated with lower total mortality, with multivariable-adjusted HRs of 0.89 (95% CI, 0.83-0.95) for quintile 2; 0.88 (95% CI, 0.82-0.95) for quintile 3; 0.84 (95% CI, 0.77-0.92) for quintile 4; and 0.87 (95% CI, 0.78-0.96) for quintile 5, with quintile 1 as the reference category (P = .01 for trend). For cause-specific mortality, this association with plant protein intake was evident for cardiovascular disease (CVD)–related mortality (HRs, 0.84 [95% CI, 0.73-0.96] to 0.70 [95% CI, 0.59-0.83]; P = .002 for trend). Isocaloric substitution of 3% energy from plant protein for red meat protein was associated with lower total (HR, 0.66; 95% CI, 0.55-0.80), cancer-related (HR, 0.61; 95% CI, 0.45-0.82), and CVD-related (HR, 0.58; 95% CI, 0.39-0.86) mortality; substitution for processed meat protein was associated with lower total (HR, 0.54; 95% CI, 0.38-0.75) and cancer-related (HR, 0.50; 95% CI, 0.30-0.85) mortality.

CONCLUSIONS AND RELEVANCE In this large prospective study, higher plant protein intake was associated with lower total and CVD-related mortality. Although animal protein intake was not associated with mortality outcomes, replacement of red meat protein or processed meat protein with plant protein was associated with lower total, cancer-related, and CVD-related mortality.

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Supplemental content

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Exploration of the health effects of a high-protein diet have attracted substantial interest during recent years.[1-4] In short-term trials, consumption of high-protein diets have been shown to produce greater weight loss, loss of fat mass, and preservation of lean mass compared with the consumption of normal-protein diets.[5,6] These favorable effects of a high-protein diet in body weight management may be due to the modulation of amino acids in appetitive signaling, leading to increased satiety and hence reduced energy intake.[7,8] High-protein diets are also linked to improvements in cardiovascular risk factors, including blood pressure, blood lipid and lipoprotein profiles, and glycemic regulation.[9,10] Despite these benefits, the health effects of adherence to high dietary protein intake on long-term health and mortality remain to be clarified. Importantly, high consumption of dietary protein is unavoidably linked to a decrease in other foods, usually carbohydrates, and a decrease in carbohydrate foods with high fiber and other micronutrients may have a negative effect. Furthermore, among protein sources, proteins originating from animal and plant sources have different amino acid combinations,[11] and the choices of dietary protein source also necessarily influence other macronutrients, micronutrients, and polyphenols in the diet,[12] potentially leading to differential health effects. Thus, clarifying the association between high dietary protein intake and long-term health outcomes is essential; in particular, clarifying the association between specific sources of protein and mortality may help individuals increase longevity by substituting one type of protein for another.

Earlier ecologic studies[13,14] reported a positive correlation between overall animal protein intake and mortality due to cardiovascular disease (CVD) and cancer. However, only a few epidemiologic studies[15-17] have evaluated the association between protein intake in association with mortality outcomes. An analysis using the US National Health and Nutrition Examination Survey (NHANES) reported a significant increase in the risk of death due to all causes associated with higher protein intake.[15] However, that study was based on relatively few deaths. Two other US studies[16,17] did not replicate this positive association of overall mortality with animal protein but did report a reduced risk of CVD-related mortality associated with higher plant protein. Moreover, no reports on this issue have yet appeared from an Asian population, despite the differences in dietary habits between Asian and western populations. The higher fish and soy product consumption in Japan than in western populations suggests that their sources of animal and plant proteins may differ. Herein, we evaluated the association between animal and plant protein intake and all-cause and cause-specific mortality in a Japanese population within the Japan Public Health Center–based Prospective Cohort (JPHC) Study.

Key Points

Question What is the long-term association between dietary protein intake and all-cause or cause-specific mortality in the Japanese population?

Findings In this cohort study of 70 696 Japanese adults followed up for a mean of 18 years, higher intake of plant protein was associated with lower total mortality. Moreover, substitution of plant protein for animal protein, mainly for red or processed meat protein, was associated with lower risk of total, cancer-related, and cardiovascular disease–related mortality.

Meaning A higher intake of plant-based proteins may contribute to long-term health and longevity.

Methods

Participants

The JPHC Study commenced January 1, 1990, with the enrollment of 61 595 registered residents aged 40 to 59 years from 5 public health center (PHC) areas across Japan (cohort 1). A further 78 825 individuals aged 40 to 69 years from another 6 PHC areas were added starting January 1, 1993 (cohort 2). At baseline, enrolled participants were provided with self-administered survey questionnaires to assess diet and lifestyle factors. Completion of these questionnaires was considered to indicate consent to participate in the study. Follow-up survey questionnaires were readministered at 5-year intervals after the baseline survey. Because the questionnaire in the 5-year follow-up survey contained more comprehensive information on food intake than that in the baseline survey, we used the 5-year follow-up survey data collected from January 1, 1995, through December 31, 1999, as baseline data for this analysis. Informed consent was obtained from all participants per the study protocol, which was approved by the institution review board of the National Cancer Center, Tokyo, Japan (approval number: 2001-021, 2004-059). Although written informed consent was not required, all eligible individuals were given an explanation of the aim and design of the study when invited to participate. This study followed the American Association for Public Opinion Research (AAPOR) reporting guideline.

After exclusion of 275 ineligible participants and 4803 participants who died or moved out of the study area before the 5-year follow-up survey, 103 428 eligible participants completed and returned questionnaires on demographics, medical and treatment history, and lifestyle and dietary habits. We further excluded 14 226 participants who reported a history of cancer, stroke, ischemic heart disease, or renal disease in the baseline or second survey; 5383 who reported extreme energy intake (<1001 or >4201 kcal/d for men or <844 or >3688 kcal/d for women); 172 in the top 0.1 percentile of protein intake variables; and 12 951 with missing covariate information (as detailed in eMethods in the Supplement). Finally, 70 696 participants were included in the analytic cohort.

Dietary Assessment

A semiquantitative food frequency questionnaire was used to assess usual intake of 138 food and beverage items during the previous year. For most foods, intake frequency ranged from rarely (<1 time per month) to at least 7 times per day in 9 categories. A standard portion size was prespecified for each food, and participants were asked to report their usual portion size relative to the standard portion size using 3 options (<0.5 times, standard, or >1.5 times). Daily food intake was calculated by multiplying intake frequency by standard portion and relative size for each food item in the food frequency questionnaire. The daily food intake data, with *Standard Tables of Food Composition in Japan* (Fifth Revised and Enlarged Edition),[18] was then used to estimate nutrient intake. The dietary intake information estimated from the food frequency questionnaire was previously compared with dietary intake estimates from 14- or 28-day dietary records (validity) and with intake estimates computed from subsequent questionnaires administered 1 year apart (reproducibility) in a cohort subsample. Spearman correlation coefficients were 0.31 in men and 0.33 in women for validity and 0.57 in men and 0.54 in women for reproducibility.[19,20] For the present analysis, we also estimated protein intake from animal and plant sources separately. Sources of animal protein were fish and shellfish, meat and processed meat, eggs, milk, and dairy products; sources of plant protein included foods other than animal foods. We expressed protein intake as a percentage of total energy consumption. Spearman correlation coefficients for validity for animal protein and plant protein were 0.21 and 0.59, respectively, in men and 0.26 and 0.49, respectively, in women.[21] Corresponding values for reproducibility were 0.49 for animal protein and 0.60 for plant protein in men and 0.48 for animal protein and 0.58 for plant protein in women.

Mortality Ascertainment

Residential and vital statuses of cohort participants during follow-up were determined annually through the residential registry. Causes of death, coded according to the *International Statistical Classification of Diseases and Related Health Problems, Tenth Revision*, were obtained from death certificates with permission of the Ministry of Health, Labour and Welfare. For this analysis, we assessed all-cause mortality and deaths due to cancer (codes C00-C99), CVD (codes I00-I99), heart disease (codes I20-I52), and cerebrovascular disease (codes I60-I69).

Statistical Analysis

Data were analyzed from July 18, 2017, through April 10, 2019. Person-years of follow-up for each participant were calculated from the date of response to the 5-year survey questionnaire until the date of death, move out of Japan, or end of the follow-up period, whichever came first. The end of the follow-up period was December 31, 2016, for all PHCs except Tokyo and Osaka, which concluded on December 31, 2009, and December 31, 2012, respectively. Individuals lost to follow-up were censored at the last confirmed date of presence in the study area. Cox proportional hazards regression models were used to evaluate the association between protein intake and mortality outcomes (eMethods in the Supplement). We adjusted for covariates in 2 models: the first adjusted for age, sex, and percentage of energy from saturated, monounsaturated, polyunsaturated, and other fats, whereas the second further adjusted for body mass index, smoking status, alcohol intake, total physical activity, coffee consumption, green tea consumption, and total calorie intake while leaving out the percentage of energy from carbohydrates. Mutual adjustment for animal protein and plant protein in the respective analyses was performed. The latter model assumes isocaloric substitution interpretation, wherein the coefficient for protein represents the substitution effect of an equal amount of energy from protein for carbohydrates.[22,23] Tests for trend were based on a Wald test for linear contrast of the model coefficients corresponding to variable categories. We conducted stratified analysis by lifestyle factors and tested the significance of interaction by the likelihood ratio test (eMethods in the Supplement). Next, we evaluated the isoenergic substitution effect within the protein group by protein food sources, wherein we estimated the hazard ratios (HRs) for replacement of 3% of energy from one source for the equivalent amount of energy from other sources. We used SAS, version 9.3 (SAS Institute, Inc), and R statistical software, version 3.5.3 (R Development Core Team, 2019) for analyses. All statistical tests were 2 sided, and $P < .05$ was considered statistically significant.

Results

Of the 70 696 participants, 32 201 (45.5%) were men (mean [SD] age, 55.6 [7.6] years) and 38 495 (54.5%) were women (mean [SD] age, 55.8 [7.7] years). During a mean follow-up of 18 years, we documented 12 381 deaths due to all causes, including 5055 due to cancer, 3025 due to CVD, 1528 due to heart disease, and 1198 due to cerebrovascular disease. Mean (SD) intakes, expressed as percentage of total energy, were 7.7% (2.7%) for animal protein and 6.7% (1.4%) for plant protein. Fish and seafood products (47.1%), red meats (19.4%), milk or dairy products (16.7%), and eggs (9.5%) were the major sources of animal protein intake compared with cereals (50.3%), pulses (24.1%), vegetables (7.8%), and fruits (3.8%) for plant protein intake (eFigure in the Supplement). Participants with higher intake of protein from animal and plant sources were less likely to be men (34.1% and 30.5%, respectively), less likely to smoke (23.5% and 21.5%, respectively) and consume alcohol (33.3% and 21.9%, respectively), and more likely to regularly drink green tea (50.7% and 57.2%, respectively) than participants with lower protein intake (**Table 1**). Compared with participants with lower intake, those with higher animal protein intake tended to consume more total energy (mean [SE], 2287 [4.9] kcal/d) and fat (mean [SE], 32.0% [0.04%]) but less carbohydrates (mean [SE], 47.1% [0.1%]), whereas those with higher plant protein intake tended to consume less total energy (mean [SE], 1914 [5.0] kcal/d) and fat (mean [SE], 22.9% [0.1%]) but more carbohydrates (mean [SE], 60.0% [0.1%]). As expected, compared with those in the lowest quintile of plant protein intake, participants in the highest quintile had higher intakes of soy foods (mean [SE], 144 [0.5] g/d), fruits (mean [SE], 237 [1.3] g/d), and vegetables (mean [SE], 253 [1.0] g/d) but lower intake of meat (mean [SE], 33.0 [0.3] g/d). Compared with those in the lowest quintile of animal protein intake, those in the highest quintiles had higher intake of meats (mean [SE], 60.6 [0.3] g/d) but lower intake of fruits (mean [SE], 180.8 [1.3] g/d), vegetables (mean [SE], 198.0 [1.0] g/d), and soy foods (mean [SE], 78.0 [0.6] g/d).

Table 1. Distribution of Baseline Characteristic of Participants According to Quintile Category of Total, Animal, and Plant Protein Intake Expressed as Percentage of Total Energy

Characteristic	Protein Source[a]								
	Total			Animal			Plant		
	Quintile 1 (n = 13 568)	Quintile 3 (n = 14 526)	Quintile 5 (n = 13 820)	Quintile 1 (n = 13 431)	Quintile 3 (n = 14 537)	Quintile 5 (n = 13 781)	Quintile 1 (n = 13 725)	Quintile 3 (n = 14 381)	Quintile 5 (n = 13 857)
Male, %	72.8	41.5	27.7	59.2	44.7	34.1	63.7	44.3	30.5
Age, mean (SD), y	55.1 (7.8)	55.6 (7.6)	56.7 (7.4)	55.8 (7.9)	55.5 (7.6)	56.2 (7.5)	54.9 (7.7)	55.6 (7.6)	56.9 (7.5)
BMI	23.4 (0.03)	23.4 (0.02)	23.6 (0.03)	23.4 (0.03)	23.4 (0.02)	23.6 (0.03)	23.6 (0.03)	23.4 (0.03)	23.6 (0.03)
Physical activity, METS-h/d	32.6 (0.1)	32.5 (0.1)	32.4 (0.1)	32.5 (0.1)	32.5 (0.1)	32.4 (0.1)	32.4 (0.1)	32.5 (0.1)	32.4 (0.1)
Current smoker, %	29.4	22.3	21.9	26.7	23.5	23.5	28.8	23.4	21.5
Current alcohol consumption, %	49.8	32.4	29.0	35.5	35.4	33.3	53.2	33.5	21.9
Coffee intake ≥1 cup/d, %	19.3	18.8	13.7	16.9	18.7	15.7	20.3	17.7	13.1
Green tea intake ≥1 cup/d, %	38.0	50.6	56.1	44.4	50.4	50.7	36.5	50.8	57.2
Agriculture/forestry/fishery occupation, %	23.7	21.0	21.8	24.6	20.3	21.8	20.5	21.9	22.6
Dietary intake									
Total energy, kcal/d	1859 (5.1)	2004 (4.8)	2244 (5.0)	1831 (4.9)	1996 (4.7)	2287 (4.9)	2208 (5.0)	2004 (4.8)	1914 (5.0)
Total protein, % of energy	11.0 (0.01)	14.3 (0.01)	17.9 (0.01)	11.6 (0.01)	14.2 (0.01)	17.5 (0.01)	14.4 (0.02)	14.3 (0.02)	14.6 (0.02)
Animal protein, % of energy	4.5 (0.01)	7.5 (0.01)	11.3 (0.01)	4.1 (0.01)	7.5 (0.01)	11.7 (0.01)	9.5 (0.02)	7.7 (0.02)	6.0 (0.02)
Plant protein, % of energy	6.4 (0.01)	6.8 (0.01)	6.6 (0.01)	7.5 (0.01)	6.8 (0.01)	5.8 (0.01)	4.9 (0.004)	6.6 (0.004)	8.6 (0.004)
Total fat, % of energy	18.9 (0.05)	26.0 (0.05)	31.0 (0.05)	18.6 (0.04)	25.8 (0.04)	32.0 (0.04)	29.3 (0.1)	25.1 (0.1)	22.9 (0.1)
Saturated fat, % of energy	5.6 (0.02)	7.9 (0.02)	9.2 (0.02)	5.2 (0.02)	7.7 (0.02)	9.9 (0.02)	9.6 (0.02)	7.5 (0.02)	6.1 (0.02)
Monounsaturated fat, % of energy	6.5 (0.02)	9.0 (0.02)	10.7 (0.02)	6.3 (0.02)	8.9 (0.02)	11.2 (0.02)	10.4 (0.02)	8.7 (0.02)	7.5 (0.02)
Polyunsaturated fat, % of energy	4.4 (0.01)	5.9 (0.01)	7.1 (0.01)	4.8 (0.01)	5.9 (0.01)	6.8 (0.01)	5.7 (0.01)	5.7 (0.01)	6.3 (0.01)
Other fat, % of energy	2.3 (0.01)	3.3 (0.01)	4.1 (0.01)	2.3 (0.005)	3.2 (0.005)	4.1 (0.005)	3.6 (0.01)	3.2 (0.01)	2.9 (0.01)
Total carbohydrates, % of energy	60.0 (0.1)	56.4 (0.1)	48.3 (0.1)	63.1 (0.1)	55.8 (0.1)	47.1 (0.1)	46.7 (0.1)	57.1 (0.1)	60.0 (0.1)
Refined carbohydrates, % of energy	40.5 (0.1)	36.2 (0.1)	28.6 (0.1)	43.2 (0.1)	35.7 (0.1)	27.7 (0.1)	27.9 (0.1)	37.0 (0.1)	39.5 (0.1)
Other carbohydrates, % of energy	19.4 (0.05)	20.3 (0.05)	19.7 (0.05)	19.9 (0.05)	20.1 (0.05)	19.4 (0.05)	18.8 (0.05)	20.1 (0.05)	20.5 (0.05)
Soy food, g/d	60.8 (0.6)	83.0 (0.5)	107.3 (0.5)	88.7 (0.6)	83.5 (0.5)	78.0 (0.6)	49.3 (0.5)	73.1 (0.5)	144.0 (0.5)
Vegetables, g/d	182.8 (1.1)	215.5 (1.0)	217.9 (1.0)	208.3 (1.1)	213.4 (1.0)	198.0 (1.0)	165.1 (1.0)	208.0 (1.0)	253.0 (1.0)
Fruits, g/d	202.4 (1.4)	218.9 (1.3)	192.7 (1.3)	222.2 (1.4)	215.1 (1.3)	180.8 (1.3)	158.3 (1.3)	219.0 (1.3)	237.0 (1.3)
Fish, g/d	48.5 (0.3)	79.0 (0.3)	135.9 (0.3)	49.0 (0.3)	81.4 (0.3)	129.8 (0.3)	86.5 (0.4)	87.2 (0.4)	78.3 (0.4)
Red and processed meat, g/d	40.3 (0.3)	52.0 (0.3)	52.9 (0.3)	33.2 (0.3)	51.7 (0.3)	60.6 (0.3)	66.5 (0.3)	48.9 (0.3)	33.0 (0.3)
Chicken, g/d	8.8 (0.1)	10.8 (0.1)	11.4 (0.1)	8.3 (0.1)	10.8 (0.1)	11.9 (0.1)	11.3 (0.1)	10.7 (0.1)	8.6 (0.1)

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by square of height in meters); METS, metabolic equivalents.

[a] Unless otherwise indicated, data are expressed as mean (SE). All variables are adjusted for age and sex (other than age and sex themselves).

Higher total and animal protein intake was not associated with risk of overall mortality or cause-specific mortality (**Table 2**). In contrast, plant protein intake was significantly inversely associated with the risk of overall mortality (HR for quintile 2, 0.89 [95% CI, 0.83-0.95]; HR for quintile 3, 0.88 [95% CI, 0.82-0.95]; HR for quintile 4, 0.84 [95% CI, 0.77-0.92]; HR for quintile 5, 0.87 [95% CI, 0.78-0.96]; with quintile 1 as the reference category) (*P* = .01 for trend). Among cause-specific mortality, this inverse association with plant protein intake was evident for CVD mortality (HR for quintile 5, 0.73; 95% CI, 0.59-0.91) and its subdivisions of heart disease (HR for quintile 5, 0.72; 95% CI, 0.54-0.97) and cerebrovascular disease mortality (HR for quintile 5, 0.72; 95% CI, 0.51-1.00), but not with cancer mortality (overall or site-specific) (Table 2 and eTable 1 in the Supplement). Repetition of the above analysis by including observations with missing information for covariates using multiple imputation produced similar HRs to that of complete case analysis (eTable 2 in the Supplement). In other sensitivity analyses, although we further adjusted for diet quality score[24] and other dietary variables, including intake of vegetables, fruits, pulses or soy foods, red and processed meats, fish, polyunsaturated fatty acids, folate, fiber, and sodium, the observed results were not substantially changed. The observed association did not substantially change after further adjustment for history of hypertension, dyslipidemia, or type 2 diabetes or after exclusion of deaths occurring during the first 5 years of follow-up (n = 1644). In subgroup analysis, the association between plant protein and total mortality appeared to be stronger for participants who never smoked (HR for quintile 5, 0.80; 95% CI, 0.69-0.93), with regular alcohol consumption (HR for quintile 5, 0.84; 95% CI, 0.72-0.98), with lean body mass (HR for quintile 5, 0.86; 95% CI, 0.76-0.97), and less physically active (HR for quintile 5, 0.82; 95% CI, 0.70-0.96), although the interaction was significant only for alcohol use (*P* = .01) and body mass index (*P* = .02) and appeared not to differ by age (eTable 3 in the Supplement). Total and animal

Table 2. Hazards for All-Cause and Cause-Specific Mortality According to Percentage of Energy From Total, Animal, and Plant Protein Intake

Cause of Mortality	Quintile Category of Intake, HR (95% CI)					*P* Value for Trend
	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	
Total Protein Intake[a]						
All						
No. of deaths	2888	2517	2361	2218	2397	NA
Model 1[b]	1 [Reference]	0.91 (0.86-0.96)	0.90 (0.84-0.96)	0.88 (0.82-0.95)	0.99 (0.91-1.08)	.61
Model 2[c]	1 [Reference]	0.95 (0.89-1.01)	0.93 (0.86-1.00)	0.92 (0.84-0.99)	0.99 (0.90-1.09)	.63
Cancer						
No. of deaths	1180	1016	988	931	940	NA
Model 1[b]	1 [Reference]	0.90 (0.82-0.98)	0.92 (0.83-1.02)	0.91 (0.81-1.02)	0.97 (0.85-1.11)	.79
Model 2[c]	1 [Reference]	0.93 (0.84-1.03)	0.96 (0.86-1.08)	0.94 (0.83-1.06)	1.00 (0.86-1.16)	.96
CVD						
No. of deaths	707	606	554	542	616	NA
Model 1[b]	1 [Reference]	0.89 (0.79-1.00)	0.84 (0.74-0.96)	0.84 (0.73-0.98)	0.97 (0.81-1.15)	.56
Model 2[c]	1 [Reference]	0.91 (0.80-1.04)	0.84 (0.72-0.98)	0.90 (0.76-1.06)	0.97 (0.80-1.18)	.75
Heart disease						
No. of deaths	363	317	277	270	301	NA
Model 1[b]	1 [Reference]	0.94 (0.79-1.10)	0.87 (0.72-1.05)	0.89 (0.72-1.09)	1.01 (0.79-1.29)	.91
Model 2[c]	1 [Reference]	0.92 (0.76-1.11)	0.82 (0.66-1.01)	0.85 (0.68-1.08)	0.95 (0.72-1.24)	.57
Cerebrovascular disease						
No. of deaths	285	225	221	215	252	NA
Model 1[b]	1 [Reference]	0.77 (0.64-0.93)	0.75 (0.61-0.93)	0.73 (0.57-0.92)	0.83 (0.63-1.09)	.19
Model 2[c]	1 [Reference]	0.85 (0.69-1.05)	0.82 (0.64-1.03)	0.90 (0.69-1.17)	0.95 (0.70-1.29)	.91
Animal Protein Intake[d]						
All						
No. of deaths	2730	2447	2444	2348	2412	NA
Model 1[b]	1 [Reference]	0.91 (0.86-0.96)	0.94 (0.88-1.01)	0.95 (0.88-1.02)	1.00 (0.91-1.10)	.68
Model 2[c]	1 [Reference]	0.91 (0.85-0.97)	0.95 (0.88-1.02)	0.97 (0.89-1.05)	0.98 (0.88-1.08)	.93
Cancer						
No. of deaths	1097	997	1017	980	964	NA
Model 1[b]	1 [Reference]	0.90 (0.82-0.98)	0.94 (0.85-1.05)	0.95 (0.85-1.07)	0.97 (0.84-1.12)	.98
Model 2[c]	1 [Reference]	0.91 (0.83-1.01)	0.95 (0.85-1.06)	0.97 (0.86-1.10)	0.97 (0.83-1.14)	.96
CVD						
No. of deaths	672	607	563	577	606	NA
Model 1[b]	1 [Reference]	0.93 (0.83-1.05)	0.90 (0.79-1.03)	0.97 (0.83-1.12)	1.02 (0.85-1.22)	.77
Model 2[c]	1 [Reference]	0.90 (0.79-1.03)	0.89 (0.77-1.03)	0.99 (0.84-1.17)	0.97 (0.79-1.19)	.87
Heart disease						
No. of deaths	338	315	283	293	299	NA
Model 1[b]	1 [Reference]	1.00 (0.85-1.18)	0.96 (0.79-1.16)	1.05 (0.85-1.30)	1.09 (0.84-1.42)	.46
Model 2[c]	1 [Reference]	0.93 (0.78-1.12)	0.91 (0.74-1.11)	0.97 (0.77-1.23)	0.97 (0.73-1.30)	.96
Cerebrovascular disease						
No. of deaths	278	235	214	229	242	NA
Model 1[b]	1 [Reference]	0.82 (0.68-0.99)	0.75 (0.61-0.93)	0.81 (0.64-1.03)	0.83 (0.62-1.10)	.25
Model 2[c]	1 [Reference]	0.86 (0.70-1.06)	0.79 (0.62-0.99)	0.95 (0.73-1.23)	0.89 (0.65-1.23)	.73
Plant Protein Intake[e]						
All						
No. of deaths	2738	2475	2427	2320	2421	NA
Model 1[b]	1 [Reference]	0.86 (0.81-0.91)	0.82 (0.78-0.88)	0.76 (0.71-0.82)	0.81 (0.74-0.88)	<.001
Model 2[c]	1 [Reference]	0.89 (0.83-0.95)	0.88 (0.82-0.95)	0.84 (0.77-0.92)	0.87 (0.78-0.96)	.01
Cancer						
No. of deaths	1096	1066	970	991	932	NA
Model 1[b]	1 [Reference]	0.95 (0.87-1.04)	0.87 (0.79-0.96)	0.89 (0.80-0.99)	0.89 (0.78-1.01)	.05
Model 2[c]	1 [Reference]	1.02 (0.92-1.13)	0.99 (0.88-1.11)	1.04 (0.91-1.19)	1.04 (0.88-1.23)	.59

(continued)

Table 2. Hazards for All-Cause and Cause-Specific Mortality According to Percentage of Energy From Total, Animal, and Plant Protein Intake (continued)

Cause of Mortality	Quintile Category of Intake, HR (95% CI)					*P* Value for Trend
	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	
CVD						
No. of deaths	666	609	574	547	629	NA
Model 1[b]	1 [Reference]	0.82 (0.73-0.92)	0.73 (0.64-0.82)	0.64 (0.56-0.74)	0.69 (0.59-0.82)	<.001
Model 2[c]	1 [Reference]	0.84 (0.73-0.96)	0.77 (0.66-0.90)	0.70 (0.59-0.83)	0.73 (0.59-0.91)	.002
Heart disease						
No. of deaths	339	321	279	282	307	NA
Model 1[b]	1 [Reference]	0.86 (0.73-1.01)	0.72 (0.60-0.86)	0.68 (0.56-0.82)	0.70 (0.56-0.89)	<.001
Model 2[c]	1 [Reference]	0.84 (0.70-1.02)	0.73 (0.59-0.91)	0.72 (0.57-0.92)	0.72 (0.54-0.97)	.02
Cerebrovascular disease						
No. of deaths	269	216	238	210	265	NA
Model 1[b]	1 [Reference]	0.68 (0.56-0.82)	0.69 (0.57-0.84)	0.55 (0.44-0.68)	0.62 (0.47-0.80)	<.001
Model 2[c]	1 [Reference]	0.75 (0.60-0.93)	0.79 (0.62-1.00)	0.64 (0.49-0.85)	0.72 (0.51-1.00)	.04

Abbreviations: CVD, cardiovascular disease; HR, hazard ratio; NA, not applicable.

[a] Median (cutoff points) of intake category (percentage of total energy): quintile 1, 11.3% (<12.4%); quintile 2, 13.0% (12.3% to <13.7%); quintile 3, 14.3% (13.7% to <14.9%); quintile 4, 15.6% (14.9% to <16.3%); and quintile 5, 17.6% (≥16.3%).

[b] Adjusted for age (≤50, 51-55, 56-60, 61-65, 66-70, or >70 years), sex, and percentage of energy from saturated fat, monounsaturated fat, polyunsaturated fat, and other fat (all continuous).

[c] Further adjusted for body mass index (calculated as weight in kilograms divided by the height in meters squared; <22.5, 22.5 to <25.0, 25.0 to <27.5, or ≥27.5), smoking (never, past, current with ≤20 and >20 cigarettes/d), alcohol use (none/occasional or regular consumption of ethanol of <150, 150 to <300, or ≥300 g per day), physical activity (quartile category in metabolic equivalent hours per day), occupation status (agriculture/forestry/fishery, salaried/professional, self-employed, housework/unemployed, or other), intake of green tea (never, <1, 1, 2-3, or ≥4 cups per day) and coffee (never, <1, 1, or ≥2 cups per day), and total energy. Mutual adjustment was performed for animal protein and plant protein in the respective analysis.

[d] Median (cutoff points) of intake category (percentage of total energy): quintile 1, 4.3% (<5.4%); quintile 2, 6.1% (5.4% to <6.8%); quintile 3, 7.5% (6.8% to <8.2%); quintile 4, 8.9% (8.2% to <9.8%); and quintile 5, 11.2% (≥9.8%).

[e] Median (cutoff points) of intake category (percentage of total energy): quintile 1, 5.0% (<5.6%); quintile 2, 6.0% (5.6% to <6.3%); quintile 3, 6.6% (6.3% to <7.0%); quintile 4, 7.3% (7.0% to <7.7%); and quintile 5, 8.4% (≥7.7%).

protein, however, showed no clear association in subgroup analysis by these factors.

Next, we examined the association of substituting one protein source for another with the risk of mortality outcomes. In this analysis, isocaloric substitution of 3% energy from plant protein for red meat protein was associated with lower total (HR, 0.66; 95% CI, 0.55-0.80), cancer-related (HR, 0.61; 95% CI, 0.45-0.82), and CVD-related (HR, 0.58; 95% CI, 0.39-0.86) mortality, whereas substitution for processed meat was associated with lower total (HR, 0.54; 95% CI, 0.38-0.75) and cancer-related (HR, 0.50; 95% CI, 0.30-0.85) mortality (**Figure 1**). The estimated absolute risk reduction at 15 years for isocaloric substitution of 3% energy from plant protein for red meat protein was 3.60% (95% CI, 2.10%-4.86%) for total, 1.92% (95% CI, 0.87%-2.71%) for cancer-related, and 1.16% (95% CI, 0.39%-1.68%) for CVD-related mortality (eTable 4 in the Supplement). The corresponding value for substitution of plant protein for processed meat protein was 4.95% (95% CI, 2.62%-6.65%) for total and 2.45% (95% CI, 0.72%-3.48%) for cancer-related mortality, although the estimate was not significant for cardiovascular mortality. Among animal proteins, substitution of fish protein for red meat was associated with lower total (HR, 0.75; 95% CI, 0.65-0.87), cancer-related (HR, 0.67; 95% CI, 0.53-0.85), and CVD-related (HR, 0.67; 95% CI, 0.50-0.91) mortality; and substitution of fish protein for processed meat was associated with lower total (HR, 0.61; 95% CI, 0.44-0.84) and cancer-related (HR, 0.55; 95% CI, 0.34-0.91) mortality (**Figure 2**). Among plant proteins, no clear association was observed when vegetable and fruit protein were substituted for cereal or soy protein, which may indicate that all 3 sources are beneficial (eTable 5 in the Supplement).

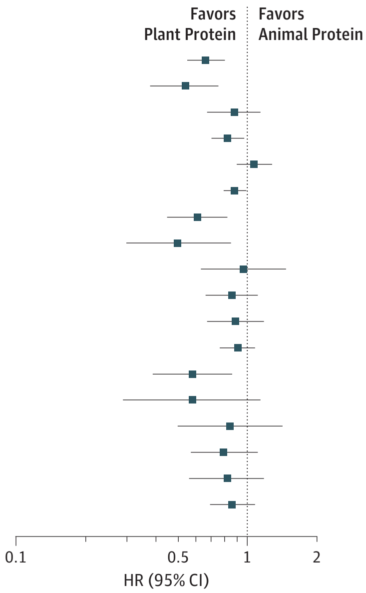
Discussion

In this large prospective study, plant protein intake was associated with lower risk of all-cause and CVD-related mortality. Moreover, substitution of plant protein for animal protein was associated with lower risk of total, cancer-related, and CVD-related mortality. Our study suggests that plant protein may provide beneficial health effects and that replacement of red and processed meat protein with plant or fish protein may increase longevity.

To date, only a few prospective studies[16,17] have evaluated animal and plant protein intake separately in association with the risk of overall or cause-specific mortality. In a study of postmenopausal women in Iowa,[16] higher vegetable protein intake was associated with 30% lower risk of coronary heart disease mortality compared with lower vegetable protein intake. More recently, in a combined analysis of the Nurses' Health Study and the Health Professionals Follow-up Study, Song et al[17] revealed a similar inverse association with plant protein intake, wherein a 3% increase in energy from plant protein was associated with 10% lower risk of overall mortality and 12% lower risk of cardiovascular mortality. We applied similar analysis methods to the above studies, and our findings for plant protein support their findings. Interestingly, the greatest change in HRs in our study was between the first and second quintiles, which might suggest the possibility that very low intake or deprivation of plant pro-

Figure 1. Hazard Ratios (HRs) for Mortality Associated With Isocaloric Substitution of 3% Energy From Plant Protein for Animal Protein From Various Sources

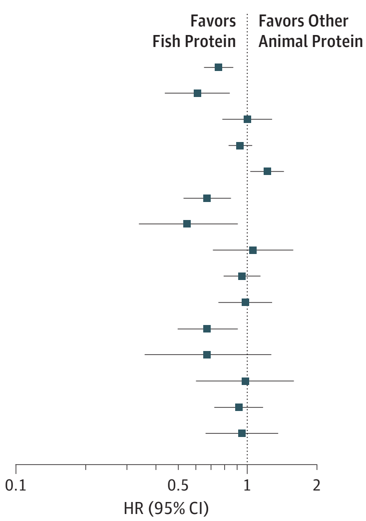
Cause of Death	Protein Source	HR (95% CI)
All-cause	Red meat	0.66 (0.55-0.80)
	Processed meat	0.54 (0.38-0.75)
	Chicken	0.88 (0.67-1.14)
	Egg	0.82 (0.70-0.97)
	Dairy	1.07 (0.90-1.28)
	Fish	0.88 (0.79-0.99)
Cancer	Red meat	0.61 (0.45-0.82)
	Processed meat	0.50 (0.30-0.85)
	Chicken	0.96 (0.63-1.47)
	Egg	0.86 (0.66-1.11)
	Dairy	0.89 (0.67-1.18)
	Fish	0.91 (0.76-1.08)
Cardiovascular disease	Red meat	0.58 (0.39-0.86)
	Processed meat	0.58 (0.29-1.14)
	Chicken	0.84 (0.50-1.42)
	Egg	0.79 (0.57-1.11)
	Dairy	0.82 (0.56-1.18)
	Fish	0.86 (0.69-1.08)



Model includes plant protein and the various sources of animal protein and is adjusted for total energy, percentage of energy from fats (saturated, monounsaturated, polyunsaturated, and other) and carbohydrates (all continuous), age (≤50, 51-55, 56-60, 61-65, 66-70, or >70 years), sex, body mass index (calculated as weight in kilograms divided by the height in meters squared; <22.5, 22.5 to <25.0, 25.0 to <27.5, or ≥27.5), smoking (never, past, or current with ≤20 or >20 cigarettes per day), alcohol use (none or occasional or regular ethanol consumption of <150, 150 to <300, or ≥300 g per day), physical activity (quartile category in metabolic equivalent hours per day), occupation status (agriculture/forestry/fishery, salaried/professional, self-employed, housework/unemployed, or other), and intake of green tea (never, <1, 1, 2-3, or ≥4 cups per day) and coffee (never, <1, 1, or ≥2 cups per day).

Figure 2. Hazard Ratios (HRs) for Mortality Associated With Isocaloric Substitution of 3% Energy From Fish Protein for Other Animal Protein Sources

Cause of Death	Protein Source	HR (95% CI)
All-cause	Red meat	0.75 (0.65-0.87)
	Processed meat	0.61 (0.44-0.84)
	Chicken	1.00 (0.78-1.28)
	Egg	0.93 (0.83-1.05)
	Dairy	1.22 (1.03-1.44)
Cancer	Red meat	0.67 (0.53-0.85)
	Processed meat	0.55 (0.34-0.91)
	Chicken	1.06 (0.71-1.58)
	Egg	0.95 (0.79-1.14)
	Dairy	0.98 (0.75-1.28)
Cardiovascular disease	Red meat	0.67 (0.50-0.91)
	Processed meat	0.67 (0.36-1.27)
	Chicken	0.98 (0.60-1.59)
	Egg	0.92 (0.72-1.17)
	Dairy	0.95 (0.66-1.36)



Model includes animal protein from various sources and plant protein and is adjusted for total energy, percentage of energy from fats (saturated, monounsaturated, polyunsaturated, and other) and carbohydrates (all continuous), age (≤50, 51-55, 56-60, 61-65, 66-70, or >70 years), sex, body mass index (calculated as weight in kilograms divided by the height in meters squared; <22.5, 22.5 to <25.0, 25.0 to <27.5, or ≥27.5), smoking (never, past, or current with ≤20 and >20 cigarettes per day), alcohol use (none or occasional or regular consumption of ethanol of <150, 150 to <300, or ≥300 g per day), physical activity (quartile category in metabolic equivalent hours per day), occupation status (agriculture/forestry/fishery, salaried/professional, self-employed, housework/unemployed, or other) and intake of green tea (never, <1, 1, 2-3, or ≥4 cups per day) and coffee (never, <1, 1, or ≥2 cups per day).

tein might also be a risk for increased mortality. In contrast to these findings for plant protein, our results for animal protein intake showed no clear association with mortality outcomes. This lack of association with animal protein does not accord with the above-mentioned US study,[17] wherein higher animal protein intake was positively associated with CVD-related mortality. In another report from the NHANES III study,[15] although higher total protein intake levels were linked with significantly increased risks of all-cause mortality (among participants younger than 65 years), this association was significantly attenuated when animal protein was controlled for, a finding that was also replicated in our study (eTable 6 in the Supplement), implicating the role of animal proteins in this association. This discrepancy in findings for animal protein between our present study and the US study[17] may be attributable to the difference in percentage of energy from animal protein, which was higher in the US study (median intake, as expressed in percentage of energy, of 14%) than in the present study (7.7%). The discrepancy might also be attributable to a difference in the main dietary source of animal protein, which was red and processed meat in the US study vs fish intake in the present study. Collectively, these findings suggest that proteins from animal and plant sources may have differing effects on long-term health and that a preference for plant-based foods in obtaining the required protein may provide long-term health benefits.

Indeed, intake of plant protein, but not animal protein, has been associated with favorable changes in blood pressure level, waist circumference, and weight.[25-28] Plant protein, unlike animal protein, was not significantly associated with higher insulinlike growth factor 1 levels.[29,30] A recent systematic review[27] found that consumption of plant protein (soy protein with isoflavones) was more strongly linked to lower levels of total and low-density lipoprotein cholesterol than the consumption of animal protein, whereas another study[31] found that substitution of nonmeat protein for meat protein was favorably associated with fasting insulin levels and insulin resistance. Other evidence suggests a null or even lower risk of type 2 diabetes associated with higher intake of plant protein vs increased risk associated with animal protein.[32,33] Moreover, intake of nuts and grains or legumes, a rich source of plant protein, was associated with lower CVD-related and all-cause mortality,[34,35] whereas higher intake of red or processed meat, major sources of animal protein, was associated with higher all-cause and CVD-related mortality, including cancer mortality.[36,37]

Cereals, pulses, vegetables, and fruits were the major sources of plant protein intake and carbohydrates. Because these foods are also often represented in healthy dietary patterns,[38,39] replacing them may have adverse health effects. Thus, we also conducted substitution analysis within protein groups by protein food source. In this analysis, substitution of plant protein for red and processed meat protein was associated with lower total mortality, a finding that accords with the US study.[17] Furthermore, our study showed that substitution of 3% energy of plant protein instead of red meat protein would result in an absolute risk reduction of overall mortality at 15 years of 3.60% (95% CI, 2.10%-4.86%). For an average person with 2000 kcal/d of energy intake, 3% of energy from plant protein would be approximately 260 g for a protein-rich food such as soy.

Strengths and Limitations

Strengths of this study include its population-based design, prospective collection of lifestyle data, use of validated food frequency questionnaires, large sample size, and long follow-up. Our study also had some limitations. The correlation coefficient for validity for protein intake was moderate to low. Dietary information was also based on a single assessment at baseline, and dietary habits might have changed during follow-up. However, any such misclassification in exposure assessment is likely to have been nondifferential among study participants and would likely have attenuated the risk estimates associated with mortality outcomes toward null. Nevertheless, we were still able to see differential associations with mortality outcomes in our study. Although we excluded participants with a history of chronic diseases, the presence of subclinical diseases might have led to changes in dietary habits. Plant protein intake may represent a healthy eating behavior; although adjustment for several lifestyle factors showed little difference in the overall results, the possibility of residual confounding in the association between plant protein and mortality remains.

Conclusions

We found that plant protein intake was associated with lower risk of all-cause and CVD-related mortality. Furthermore, replacement of red or processed meat protein with plant protein was associated with a decreased risk of total, cancer-related, and CVD-related mortality. Our study suggests that encouraging diets with higher plant-based protein intake may contribute to long-term health and longevity.

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