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

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IMPORTANCE Defining what represents a macronutritionally balanced diet remains an open question and a high priority in nutrition research. Although the amount of protein may have specific effects, from a broader dietary perspective, the choice of protein sources will inevitably influence other components of diet and may be a critical determinant for the health outcome.

OBJECTIVE To examine the associations of animal and plant protein intake with the risk for mortality.

DESIGN, SETTING, AND PARTICIPANTS This prospective cohort study of US health care professionals included 131 342 participants from the Nurses' Health Study (1980 to end of follow-up on June 1, 2012) and Health Professionals Follow-up Study (1986 to end of follow-up on January 31, 2012). Animal and plant protein intake was assessed by regularly updated validated food frequency questionnaires. Data were analyzed from June 20, 2014, to January 18, 2016.

MAIN OUTCOMES AND MEASURES Hazard ratios (HRs) for all-cause and cause-specific mortality.

RESULTS Of the 131 342 participants, 85 013 were women (64.7%) and 46 329 were men (35.3%) (mean [SD] age, 49 [9] years). The median protein intake, as assessed by percentage of energy, was 14% for animal protein (5th-95th percentile, 9%-22%) and 4% for plant protein (5th-95th percentile, 2%-6%). After adjusting for major lifestyle and dietary risk factors, animal protein intake was not associated with all-cause mortality (HR, 1.02 per 10% energy increment; 95% CI, 0.98-1.05; *P* for trend = .33) but was associated with higher cardiovascular mortality (HR, 1.08 per 10% energy increment; 95% CI, 1.01-1.16; *P* for trend = .04). Plant protein was associated with lower all-cause mortality (HR, 0.90 per 3% energy increment; 95% CI, 0.86-0.95; *P* for trend < .001) and cardiovascular mortality (HR, 0.88 per 3% energy increment; 95% CI, 0.80-0.97; *P* for trend = .007). These associations were confined to participants with at least 1 unhealthy lifestyle factor based on smoking, heavy alcohol intake, overweight or obesity, and physical inactivity, but not evident among those without any of these risk factors. Replacing animal protein of various origins with plant protein was associated with lower mortality. In particular, the HRs for all-cause mortality were 0.66 (95% CI, 0.59-0.75) when 3% of energy from plant protein was substituted for an equivalent amount of protein from processed red meat, 0.88 (95% CI, 0.84-0.92) from unprocessed red meat, and 0.81 (95% CI, 0.75-0.88) from egg.

CONCLUSIONS AND RELEVANCE High animal protein intake was positively associated with cardiovascular mortality and high plant protein intake was inversely associated with all-cause and cardiovascular mortality, especially among individuals with at least 1 lifestyle risk factor. Substitution of plant protein for animal protein, especially that from processed red meat, was associated with lower mortality, suggesting the importance of protein source.

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Defining what represents a macronutritionally balanced diet remains an open question and a high priority in nutrition research.[1,2] In short-term randomized clinical trials, substitution of protein for carbohydrate has been shown to favor weight management, decrease blood pressure, and improve cardiometabolic biomarkers, including blood lipid and lipoprotein profiles and glycemic regulation.[3-5] These beneficial effects are partly dependent on weight loss and possibly owing to the enhanced postprandial satiety and energy expenditure when exchanging protein for carbohydrate.[6] Therefore, high-protein and low-carbohydrate diets have been promoted for weight loss and health improvement. Although the amount and type of protein may have specific effects,[7] such as insulinlike growth factor 1 levels,[8] from a broader dietary perspective, the choice of protein sources will inevitably influence other components of diet, including macronutrients, micronutrients, and phytochemicals, that can in turn influence health outcomes. Therefore, taking into account food sources is critical to better understand the health effect of protein intake and fine-tune dietary recommendations.

To date, data examining protein sources in relation to mortality are sparse. Although no association was found between animal or plant protein and all-cause mortality in a cohort of postmenopausal women, substitution of plant protein for animal protein was associated with lower mortality due to cardiovascular disease (CVD).[9] A positive association between animal protein and mortality was also found in the other study using the National Health and Nutrition Examination Survey.[8] Nevertheless, these data are far from conclusive owing to several limitations, including the relatively small sample size, single assessment of diet at baseline, and lack of data on detailed food sources of animal and plant protein. Therefore, we used data from 2 large US cohort studies with repeated measures of diet and up to 32 years of follow-up to prospectively examine animal protein vs plant protein in relation to the risk for all-cause and cause-specific mortality and to perform an isocaloric substitution analysis for a variety of food sources of protein.

Key Points

Question What is the association of the source of protein intake with mortality in US adults?

Findings In this cohort study, high intake of animal protein was positively associated with mortality, with the inverse true for high intake of plant protein, especially among individuals with at least 1 lifestyle risk factor. Replacement of animal protein with plant protein was associated with lower mortality, suggesting the importance of protein source.

Meaning Public health recommendations should focus on improvement of protein sources.

Methods

Study Population

The Nurses' Health Study (NHS)[10] included 121 700 US registered female nurses who were aged 30 to 55 years in 1976; for this study, data were collected from 1980 to June 1, 2012. The Health Professionals Follow-up Study (HPFS)[11] included 51 529 US male health care professionals who were aged 40 to 75 years in 1986; data were collected from 1986 to January 31, 2012. Details of the 2 cohorts have been described elsewhere.[10,11] Briefly, follow-up questionnaires were administered at baseline enrollment and every 2 years thereafter to collect lifestyle and medical information. Dietary intake was assessed by the food frequency questionnaires (FFQs) every 4 years. The follow-up rates were 95.4% in the NHS and 95.9% in the HPFS. The study protocol was approved by the institutional review board at the Brigham and Women's Hospital and the Harvard T. H. Chan School of Public Health. All participants provided written informed consent.

Among participants who returned baseline questionnaires, we excluded those who had a history of cancer (except nonmelanoma skin cancer), CVD, or diabetes at baseline, left more than 10 items blank on the baseline FFQ in the NHS and more than 70 items blank in the HPFS, or reported implausible energy intake levels (<500 or >3500 kcal/d for women, or <800 or >4200 kcal/d for men). After exclusions, 85 013 women and 46 329 men were available for the analysis.

Dietary Assessment

In each FFQ, participants were asked how often, on average, they consumed a standardized portion size of each food during the previous year. The mean daily nutrient intake was calculated by multiplying the consumption frequency of each food item by its nutrient content and then summing across all foods. Animal and plant protein intake was expressed as a percentage of total energy consumption. Major sources of animal protein included processed and unprocessed red meat, poultry, dairy products, fish, and egg. Major food contributors to plant protein included bread, cereals, pasta, nuts, beans, and legumes. We derived protein intake from processed red meat by summing the products between intake frequency (servings per day) and the protein content (grams per serving) for various processed red meats (ie, bacon, beef or pork hot dogs, salami, bologna or other processed meat sandwiches, other processed meats [eg, sausage, kielbasa]). Similar calculations were performed for protein intake from unprocessed red meat, poultry, fish, egg, and dairy. Food frequency questionnaires have demonstrated good validity in assessing protein intake. The Spearman correlation coefficient of intake assessed by the FFQs and 7-day dietary record was 0.56 for animal protein and 0.66 for plant protein,[12] as detailed in eMethods in the Supplement.

Ascertainment of Death

We identified deaths from state statistics records, the National Death Index, next of kin, and the postal system. Using these methods, we were able to ascertain more than 96% of the deaths in each cohort.[13] Cause of death was identified from death certificates or review of medical records by physicians. For this analysis, we assessed all-cause mortality and deaths due to CVD (*International Classification of Diseases, Eighth Revision*, codes 390-458), cancer (*International Classification of Diseases, Eighth Revision*, codes 140 to 207), and other causes.

Statistical Analysis

Data were analyzed from June 20, 2014, to January 18, 2016. We calculated person-time of follow-up for each participant from the age in months at the return date of the baseline FFQ (1980 for the NHS and 1986 for the HPFS) until the age in months at the date of death, loss to follow-up, or end of follow-up (June 1, 2012, for the NHS and January 31, 2012, for the HPFS), whichever came first. We used time-varying Cox proportional hazards regression models with age as the time scale to estimate the hazard ratio (HR) and 95% CI for mortality associated with animal and plant protein intake.

To reduce random within-person variation and to best represent long-term dietary intake, we calculated the cumulative mean protein intake from our repeated FFQs.[14] We stopped updating dietary information when a participant reported a diagnosis of cancer (except nonmelanoma skin cancer), diabetes, stroke, coronary heart disease, or angina, because these conditions may lead to dietary change.[15]

We used a nutrient density model with adjustment for total energy intake and the percentage of energy from various fats (saturated, polyunsaturated, monounsaturated, and *trans*-fat).[16] Thus, the coefficient for animal and plant protein reflects the substitution effect of an equal amount of energy from protein for carbohydrate. In the multivariable analysis, we further adjusted for several potential dietary and lifestyle confounding factors, including multivitamin use, smoking status, pack-years of smoking, body mass index, physical activity, alcohol consumption, history of hypertension diagnosis, glycemic index, and intake of whole grains, total fiber, fruits, and vegetables. To address the possibility of residual confounding, we further adjusted for a propensity score that reflected associations of protein consumption with potential confounding covariates.[17] Details about covariate assessment and propensity score analysis are provided in the eMethods in the Supplement.

We performed stratified analyses by age and lifestyle factors and evaluated the interaction via a likelihood ratio test. To minimize the confounding effect and test for potential modification by an overall lifestyle pattern, we further performed a stratified analysis according to a priori–defined healthy lifestyle pattern, as characterized by never smoking or ever smoking for fewer than 5 pack-years, never or moderate alcohol intake (<14 g/d in women and <28 g/d in men), body mass index (calculated as weight in kilograms divided by height in meters squared) of at least 18.5 and less than 25.0, and physical activity of at least 150 min/wk at a moderate level or at least 75 min/wk at a vigorous level (equivalent to ≥7.5 metabolic equivalent h/wk) as recommended.[18] Likewise, given the previous report that protein intake was associated with a higher risk for diabetes-related mortality,[8] we examined the protein-mortality association according to the history of diabetes.

Finally, we estimated the effect of substituting 3% of energy from plant protein for an equivalent amount of animal protein from various sources, including processed and unprocessed red meat, poultry, fish, egg, and dairy, by simultaneously including these protein items as continuous variables in the multivariable model. The HRs and 95% CIs for the isoprotein substitution effect were derived from the difference between the regression coefficients, variance, and covariance.[19]

The analyses were first conducted in each cohort separately, and because no appreciable difference was detected by cohort (eTable 1 in the Supplement), we then conducted the pooled analysis using the sex-stratified Cox proportional hazards regression model in the combined data set. More details about statistical analysis are provided in the eMethods in the Supplement.

Results

In the 2 cohorts with 3 540 791 person-years of follow-up, we documented 36 115 deaths, of which 8851 were due to CVD, 13 159 were due to cancer, and 14 105 were due to other causes. Participants' median intake, as assessed by percentage of energy, was 14% (5th-95th percentile, 9%-22%) for animal protein and 4% (5th-95th percentile, 2%-6%) for plant protein. Animal protein intake decreased, whereas plant protein intake increased over time throughout follow-up (eFigure 1 in the Supplement). **Table 1** shows the basic characteristics of participants according to protein intake. Compared with participants who consumed no more than 10% of energy from animal protein, those consuming more than 18% were slightly heavier and less physically active and consumed more fats (especially saturated fat) and less fiber and plant foods. In contrast, those with higher plant protein intake demonstrated a clustering of positive health behaviors and had a substantially healthier diet than those with lower plant protein consumption.

As shown in **Table 2**, higher intake of animal protein was associated with higher CVD mortality. After adjusting for major lifestyle and dietary risk factors, the HR per 10% increment of animal protein intake from total energy intake was 1.02 (95% CI, 0.98-1.05; *P* for trend = .33) for all-cause mortality and 1.08 (95% CI, 1.01-1.16; *P* for trend = .04) for CVD mortality. In contrast, a higher level of plant protein intake was associated with lower mortality, with the multivariable HR per 3% increment of total energy intake of 0.90 (95% CI, 0.86-0.95; *P* for trend < .001) for all-cause mortality and 0.88 (95% CI, 0.80-0.97; *P* for trend = .007) for CVD mortality. The associations did not differ by duration of follow-up (eTable 2 in the Supplement). We did not detect any statistically significant nonlinear relationship between protein intake and mortality by spline analysis (data not shown). The results remained largely unchanged when we adjusted for a propensity score that predicted protein intake levels (eTable 3 in the Supplement).

The increased mortality associated with higher animal protein intake was more pronounced among obese participants (*P* for interaction = .008) and those with heavy alcohol intake (*P* for interaction = .06) (eFigure 2 in the Supplement). The association between higher plant protein intake and lower mortality was stronger among participants who were 65 years or younger or older than 80 years, currently smoked, consumed at least 14 g/d of alcohol, were overweight or obese, and were physically inactive (*P* for interaction ≤.02 for all).

Because most of the statistically significant associations were seen among participants with an unhealthy lifestyle, we further divided participants into healthy- and unhealthy-lifestyle groups according to a priori–defined criteria. **Table 3** shows the basic characteristics of the 2 groups. Participants in

Table 1. Age- and Sex-Standardized Characteristics of Study Participants According to Percentage of Energy From Protein Intake

Characteristic	Type of Protein Intake, % of Total Energy[a]					
	Animal			Plant		
	≤10	>12 to ≤15	>18	≤3	>4 to ≤5	>6
Male sex, %	52	29	12	13	35	70
Mean age, y	63.4	60.7	60.8	60.3	61.2	64.4
Mean BMI	25.5	25.7	26.9	26.3	25.9	25.3
Physical activity, mean MET h/wk	21.6	20.4	20.0	17.2	20.5	25.2
Current smoking, %	13	12	11	13	12	9
Mean pack-years of smoking[b]	25.0	23.9	23.5	24.9	23.8	21.2
Current multivitamin use, %	50	52	53	47	51	58
History of hypertension, %	37	39	42	39	39	36
Postmenopausal women, %[c]	87	87	87	87	87	87
Current hormone use, %[d]	14	18	18	17	17	18
Mean dietary intake[e]						
Alcohol, g/d	9.1	7.6	5.9	7.9	7.8	5.6
Total protein, g/d	59.2	76.5	103.6	79.0	79.2	78.2
Animal protein, g/d	36.0	58.0	88.1	68.8	60.0	48.1
Plant protein, g/d	23.2	18.6	15.6	10.2	19.1	30.1
Carbohydrate, g/d	226.6	188.0	155.5	143.6	189.7	217.9
Glycemic index	53.5	52.8	51.1	51.8	52.9	52.8
Total fat, g/d	60.2	68.9	72.7	75.9	67.5	59.3
Ratio of polyunsaturated to saturated fat	0.7	0.6	0.6	0.5	0.6	0.8
Fiber, g/d	21.0	19.7	19.8	16.6	19.7	25.4
Fruit, No. of servings/wk	18.2	16.7	15.6	14.7	17.0	18.8
Vegetable, No. of servings/wk	22.3	22.3	23.7	20.3	22.5	28.3
Legume, No. of servings/wk	1.9	1.8	1.6	1.3	1.7	2.8
Nut, No. of servings/wk	2.2	1.5	1.1	1.0	1.4	3.0
Bean, No. of servings/wk	0.9	0.7	0.7	0.5	0.7	1.3
Whole grain, g/d	27.1	24.5	24.4	19.9	23.6	35.3
Unprocessed red meat, No. of servings/wk	2.9	4.0	4.0	4.4	3.9	2.5
Processed red meat, No. of servings/wk	1.8	2.1	1.7	2.1	2.0	1.1
Chicken, No. of servings/wk	1.9	2.5	3.4	2.4	2.6	2.5
Egg, No. of servings/wk	1.6	2.0	2.1	2.0	2.0	1.6
Fish, No. of servings/wk	1.4	1.8	2.6	1.6	1.9	2.0
High-fat dairy product, No. of servings/wk	8.4	8.3	6.3	8.9	8.1	5.8
Low-fat dairy product, No. of servings/wk	5.9	8.3	9.9	8.2	8.4	7.6

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); MET, metabolic equivalent.

[a] Updated information throughout follow-up was used to calculate the mean for continuous variables and percentage for categorical variables. All variables are age and sex standardized except age and sex.

[b] Among ever smokers only.

[c] Among women only.

[d] Among postmenopausal women only.

[e] All dietary factors are energy adjusted.

the healthy-lifestyle group demonstrated slightly more homogeneous distributions in health behaviors than those in the unhealthy-lifestyle group. At similar amounts of protein intake, protein sources differed between the 2 groups. Compared with the healthy-lifestyle group, the unhealthy-lifestyle group with similar animal protein intake consumed more unprocessed and processed red meat, eggs, and high-fat dairy products, but less chicken, fish, and low-fat dairy products. At similar amounts of plant protein intake, the unhealthy-lifestyle group consumed less fiber, fruit, vegetables, and whole grains than the healthy-lifestyle group.

Table 4 shows the associations of protein intake and mortality in the 2 groups. The positive association with all-cause mortality for animal protein intake and the inverse association for plant protein intake were restricted to the unhealthy-lifestyle group (*P* for interaction <.001), although the association with animal protein intake did not reach statistical significance. In the unhealthy-lifestyle group, the multivariable HR per 10% increment of animal protein was 1.03 (95% CI, 0.99-1.07; *P* for trend = .16) and the HR per 3% increment of plant protein was 0.90 (95% CI, 0.85-0.95; *P* for trend < .001). Similar results were observed for CVD mortality. When stratified by history of diabetes, the positive association with all-cause mortality for animal protein intake and the inverse association for plant protein intake appeared to be stronger among participants with diabetes than those without diabetes (*P* for interaction = .06 and *P* for interaction .02, respectively; eTable 4 in the Supplement).

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

	Protein Intake Category						
	1	2	3	4	5	HR (95% CI)[a]	*P* Value for Trend
Animal Protein							
Intake category, % of total energy	≤10	>10 to 12	>12 to 15	>15 to 18	>18	NA	NA
Median intake, % of energy	8.9	11	14	16	20	NA	NA
Person-years of follow-up	317 851	544 922	1 171 916	893 047	613 056	NA	NA
All-cause mortality							
No. of deaths	3770	6151	11 909	8401	5884	NA	NA
Age-adjusted HR (95% CI)[b]	1 [Reference]	0.97 (0.93-1.01)	0.97 (0.93-1.00)	0.98 (0.94-1.02)	1.01 (0.96-1.06)	1.03 (1.00-1.06)	.09
Multivariable-adjusted HR (95% CI)[c]	1 [Reference]	1.01 (0.97-1.05)	1.03 (0.99-1.07)	1.03 (0.98-1.07)	1.03 (0.98-1.08)	1.02 (0.98-1.05)	.33
CVD mortality							
No. of deaths	974	1527	2967	1987	1396	NA	NA
Age-adjusted HR (95% CI)[b]	1 [Reference]	0.94 (0.87-1.02)	1.01 (0.94-1.09)	1.07 (0.99-1.17)	1.19 (1.09-1.30)	1.20 (1.12-1.28)	<.001
Multivariable-adjusted HR (95% CI)[c]	1 [Reference]	0.98 (0.90-1.07)	1.05 (0.97-1.14)	1.06 (0.97-1.16)	1.09 (0.99-1.20)	1.08 (1.01-1.16)	.04
Cancer mortality							
No. of deaths	1322	2176	4325	3136	2200	NA	NA
Age-adjusted HR (95% CI)[b]	1 [Reference]	0.96 (0.89-1.02)	0.94 (0.88-1.00)	0.93 (0.87-1.00)	0.94 (0.87-1.02)	0.96 (0.91-1.01)	.15
Multivariable-adjusted HR (95% CI)[c]	1 [Reference]	1.00 (0.93-1.07)	1.01 (0.94-1.08)	1.01 (0.94-1.09)	1.02 (0.94-1.11)	1.00 (0.95-1.06)	.91
Other mortality							
No. of deaths	1474	2448	4617	3278	2288	NA	NA
Age-adjusted HR (95% CI)[b]	1 [Reference]	0.99 (0.92-1.05)	0.96 (0.90-1.02)	0.96 (0.90-1.02)	0.97 (0.90-1.05)	0.99 (0.95-1.04)	.80
Multivariable-adjusted HR (95% CI)[c]	1 [Reference]	1.04 (0.97-1.11)	1.02 (0.96-1.09)	1.01 (0.94-1.08)	0.99 (0.92-1.07)	0.99 (0.94-1.05)	.80
Plant Protein							
Intake category, % of total energy	≤3	>3 to 4	>4 to 5	>5 to 6	>6	NA	NA
Median intake, % of energy	2.6	3.5	4.5	5.4	6.6	NA	NA
Person-years of follow-up	710 592	1 060 873	929 193	550 015	290 118	NA	NA
All-cause mortality							
No. of deaths	6160	9661	10 235	6602	3457	NA	NA
Age-adjusted HR (95% CI)[b]	1 [Reference]	0.92 (0.89-0.96)	0.85 (0.82-0.89)	0.72 (0.69-0.76)	0.67 (0.63-0.70)	0.73 (0.70-0.75)	<.001
Multivariable-adjusted HR (95% CI)[c]	1 [Reference]	0.97 (0.94-1.01)	0.95 (0.91-0.99)	0.91 (0.86-0.96)	0.89 (0.84-0.96)	0.90 (0.86-0.95)	<.001

(continued)

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

	Protein Intake Category						
	1	2	3	4	5	HR (95% CI)[a]	*P* Value for Trend
CVD mortality							
No. of deaths	1260	2126	2638	1811	1016	NA	NA
Age-adjusted HR (95% CI)[b]	1 [Reference]	0.88 (0.82-0.95)	0.78 (0.72-0.85)	0.63 (0.57-0.69)	0.60 (0.53-0.67)	0.67 (0.62-0.72)	<.001
Multivariable-adjusted HR (95% CI)[c]	1 [Reference]	0.93 (0.86-1.01)	0.90 (0.82-0.99)	0.83 (0.74-0.93)	0.85 (0.74-0.97)	0.88 (0.80-0.97)	.007
Cancer mortality							
No. of deaths	2372	3678	3664	2330	1115	NA	NA
Age-adjusted HR (95% CI)[b]	1 [Reference]	0.98 (0.92-1.03)	0.93 (0.87-0.99)	0.83 (0.77-0.90)	0.72 (0.65-0.79)	0.80 (0.75-0.85)	<.001
Multivariable-adjusted HR (95% CI)[c]	1 [Reference]	1.02 (0.97-1.08)	1.02 (0.95-1.09)	1.01 (0.92-1.10)	0.92 (0.82-1.03)	0.97 (0.90-1.05)	.46
Other mortality							
No. of deaths	2528	3857	3933	2461	1326	NA	NA
Age-adjusted HR (95% CI)[b]	1 [Reference]	0.90 (0.85-0.95)	0.82 (0.78-0.88)	0.69 (0.64-0.74)	0.66 (0.60-0.72)	0.70 (0.66-0.74)	<.001
Multivariable-adjusted HR (95% CI)[c]	1 [Reference]	0.95 (0.90-1.00)	0.91 (0.86-0.98)	0.86 (0.79-0.94)	0.89 (0.80-0.99)	0.86 (0.79-0.92)	<.001

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

[a] Calculated per 10% increment for animal protein and per 3% increment for plant protein.

[b] Cox proportional hazards model with age as the time scale was stratified by sex and calendar time and adjusted for total caloric intake and percentage of energy from saturated fat, polyunsaturated fat, monounsaturated fat, and *trans*-fat (all continuous).

[c] Adjusted for variables in the age-adjusted model and multivitamin use (yes or no), smoking status (never, past, or current [1-14, and ≥15 cigarettes/d]), pack-years of smoking (in women, ≤15, 16-25, 26-45, and ≥46; in men, <10, 11-24, 25-44, and ≥45), body mass index (calculated as weight in kilograms divided by height in meters squared; <23.0, 23.0-24.9, 25.0-26.9, 27.0-29.9, 30.0-34.9, and ≥35), physical activity (quintiles), alcohol consumption (in women, 0, 0.1-5.0, 5.1-15.0, and >15.0 g/d; in men, 0, 0.1-10.0, 10.1-20.0, and >20.0 g/d), history of hypertension diagnosis (yes or no), glycemic index (in quintiles), and intake of whole grains, total fiber, fruits, and vegetables (all in quintiles). Mutual adjustment was conducted for animal protein and plant protein analysis.

Table 3. Age- and Sex-Standardized Characteristics of Study Participants According to Percentage of Energy From Animal and Plant Protein Intake in the Healthy- and Unhealthy-Lifestyle Groups

	Lifestyle Group by Type of Protein Intake, %[a]							
	Animal				Plant			
	Healthy Group (n = 19 647)		Unhealthy Group (n = 106 134)		Healthy Group (n = 19 647)		Unhealthy Group (n = 106 134)	
Variable	≤10	>18	≤10	>18	≤3	>6	≤3	>6
Male sex, %	68	12	51	12	16	75	13	68
Mean age, y	64.5	61.8	63.6	61.1	61.7	64.3	60.6	64.7
Mean BMI	22.8	23.1	26.2	27.6	23.1	22.7	26.9	26.3
Physical activity, mean MET h/wk	32.7	30.5	18.7	17.9	27.5	35.5	15.0	21.0
Current smoking, %	0	0	17	14	0	0	17	13
Mean pack-years of smoking[b]	2.9	2.8	26.8	25.1	2.8	2.8	26.2	23.5
Current multivitamin use, %	57	58	49	53	50	62	47	56
History of hypertension, %	30	31	41	45	29	28	42	40
Postmenopausal women, %[c]	91	91	89	89	91	91	87	87
Current hormone use, %[d]	15	18	14	17	17	16	17	18
Mean dietary intake[e]								
Alcohol, g/d	5.2	5.1	10.4	6.2	4.2	4.8	8.4	6.0
Total protein, g/d	62.2	107.1	58.7	103.8	79.3	79.0	78.5	77.9
Animal protein, g/d	35.6	89.6	36.2	88.3	68.9	47.3	68.3	48.4
Plant protein, g/d	26.6	17.6	22.5	15.5	10.3	31.7	10.2	29.6
Carbohydrate, g/d	245.6	173.8	222.1	154.4	149.8	237.4	140.9	211.2
Glycemic index	53.5	51.6	53.4	51.0	52.7	52.8	51.5	52.6
Total fat, g/d	59.6	70.4	60.5	73.0	77.0	57.8	75.7	59.9
Ratio of polyunsaturated to saturated fat	0.8	0.6	0.7	0.6	0.6	0.8	0.5	0.7
Fiber, g/d	24.3	21.6	20.2	19.6	18.6	27.5	16.5	24.5
Fruit, No. of servings/wk	22.1	17.4	17.2	15.3	17.7	21.7	14.4	17.7
Vegetable, No. of servings/wk	25.3	24.7	21.9	23.8	22.4	29.6	20.3	27.9
Legume, No. of servings/wk	2.4	1.8	1.8	1.6	1.5	3.1	1.3	2.6
Nut, No. of servings/wk	2.7	1.4	2.1	1.1	1.3	3.2	1.0	2.9
Bean, No. of servings/wk	1.1	0.8	0.8	0.7	0.6	1.4	0.5	1.2
Whole grain, g/d	34.7	29.3	25.5	24.0	24.0	42.0	19.5	32.9
Unprocessed red meat, No. of servings/wk	2.3	3.4	3.0	4.1	3.9	2.1	4.4	2.6
Processed red meat, No. of servings/wk	1.3	1.2	2.0	1.7	1.6	0.8	2.1	1.3
Chicken, No. of servings/wk	2.0	3.8	1.9	3.4	2.5	2.5	2.4	2.5
Egg, No. of servings/wk	1.3	1.7	1.6	2.1	1.7	1.4	2.0	1.6
Fish, No. of servings/wk	1.5	2.8	1.4	2.5	1.7	2.1	1.6	2.0
High-fat dairy product, No. of servings/wk	6.8	5.2	8.7	6.4	7.8	4.9	8.9	6.0
Low-fat dairy product, No. of servings/wk	6.8	10.9	5.7	9.7	9.5	8.4	8.0	7.3

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); MET, metabolic equivalent.

[a] Healthy lifestyle was defined as never smoking or ever smoking for less than 5 pack-years, never or moderate alcohol drinking (<14 g/d in women and <28 g/d in men), BMI of 18.5 to less than 27.5, and physical activity of at least 150 min/wk at moderate level or at least 75 min/wk at vigorous level (equivalent to ≥7.5 MET h/wk). Updated information throughout follow-up was used to calculate the means for continuous variables and percentages for categorical variables. All variables are age- and sex-standardized except age and sex.

[b] Among ever smokers only.

[c] Among women only.

[d] Among postmenopausal women only.

[e] All dietary factors are energy-adjusted.

Finally, we examined the substitution association of different protein sources with mortality. The mean protein intake from various foods and their correlations are shown in eTable 5 in the Supplement, and their individual associations with mortality are summarized in eTable 6 in the Supplement. Protein intake from processed red meat was strongly associated with mortality, whereas no association was found for protein from fish or poultry. The **Figure** presents the HRs for mortality with substitution of 3% energy from plant protein for the same amount of animal protein from different food sources. The HRs for all-cause mortality were 0.66 (95% CI, 0.59-0.75) when 3% of energy from plant protein was substituted for an equivalent amount of protein from processed red meat; 0.88 (95% CI, 0.84-0.92), from unprocessed red meat; 0.94 (95% CI, 0.90-0.99), from poultry; 0.94 (95% CI, 0.89-0.99), from fish; 0.81 (95% CI, 0.75-0.88), from egg; and 0.92 (95% CI, 0.87-0.96), from dairy. The substitution associations were generally stronger for death due to CVD and other causes than those due to cancer, except substitution of egg, for which

Table 4. Risk for All-Cause and CVD Mortality According to Percentage of Energy From Animal and Plant Protein Intake Among Participants With Healthy and Unhealthy Lifestyles[a]

	Category of Protein Intake							
	1	2	3	4	5	HR (95% CI)[b]	P Value for Trend	*P* Value for Interaction[c]
Animal Protein								
Intake, % of total energy	≤10	>10 to 12	>12 to 15	>15 to 18	>18	NA	N A	NA
All-cause mortality								
Healthy-lifestyle group								
No. of deaths	626	978	1712	1012	587	NA	NA	NA
HR (95% CI)[d]	1 [Reference]	0.99 (0.88-1.10)	0.97 (0.87-1.08)	1.01 (0.89-1.14)	0.94 (0.81-1.09)	0.96 (0.86-1.07)	.46	<.001
Unhealthy-lifestyle group								
No. of deaths	3110	5107	10 046	7234	5206	NA	NA	NA
HR (95% CI)[d]	1 [Reference]	1.01 (0.96-1.05)	1.01 (0.97-1.06)	1.01 (0.96-1.06)	1.03 (0.98-1.09)	1.03 (0.99-1.07)	.16	NA
CVD mortality								
Healthy-lifestyle group								
No. of deaths	172	246	369	219	134	NA	NA	NA
HR (95% CI)[d]	1 [Reference]	0.90 (0.72-1.12)	0.81 (0.65-1.01)	1.00 (0.77-1.29)	0.95 (0.70-1.29)	0.96 (0.76-1.20)	.72	.04
Unhealthy-lifestyle group								
No. of deaths	793	1273	2569	1742	1249	NA	NA	NA
HR (95% CI)[d]	1 [Reference]	0.99 (0.90-1.08)	1.07 (0.98-1.16)	1.05 (0.95-1.16)	1.10 (0.98-1.23)	1.08 (1.00-1.17)	.04	NA
Plant Protein								
Intake, % of total energy	≤3	>3 to 4	>4 to 5	>5 to 6	>6	NA	NA	NA
All-cause mortality								
Healthy-lifestyle group								
No. of deaths	467	997	1312	1210	929	NA	NA	NA
HR (95% CI)[d]	1 [Reference]	1.07 (0.95-1.21)	1.05 (0.91-1.21)	0.98 (0.83-1.16)	0.95 (0.78-1.16)	0.95 (0.83-1.08)	.44	<.001
Unhealthy-lifestyle group								
No. of deaths	5556	8497	8801	5344	2505	NA	NA	NA
HR (95% CI)[d]	1 [Reference]	0.97 (0.94-1.01)	0.95 (0.90-0.99)	0.91 (0.86-0.96)	0.89 (0.83-0.96)	0.90 (0.85-0.95)	<.001	NA
CVD mortality								
Healthy-lifestyle group								
No. of deaths	75	182	329	318	236	NA	NA	NA
HR (95% CI)[d]	1 [Reference]	1.03 (0.76-1.39)	0.98 (0.70-1.37)	0.81 (0.55-1.19)	0.69 (0.44-1.07)	0.76 (0.57-1.01)	.06	.01
Unhealthy-lifestyle group								
No. of deaths	1159	1913	2293	1487	774	NA	NA	NA
HR (95% CI)[d]	1 [Reference]	0.93 (0.86-1.01)	0.89 (0.81-0.98)	0.82 (0.73-0.92)	0.86 (0.74-1.00)	0.88 (0.79-0.98)	.02	NA

Abbreviations: BMI, (calculated as weight in kilograms divided by height in meters squared); CVD, cardiovascular disease; HR, hazard ratio; NA, not applicable.

[a] Healthy lifestyle was defined as never smoking or ever smoking for fewer than 5 pack-years, never or moderate alcohol consumption (<14 g/d in women and <28 g/d in men), BMI of 18.5 to less than 27.5, and physical activity of at least 150 min/wk at a moderate level or at least 75 min/wk at a vigorous level (equivalent to ≥7.5 MET h/wk).

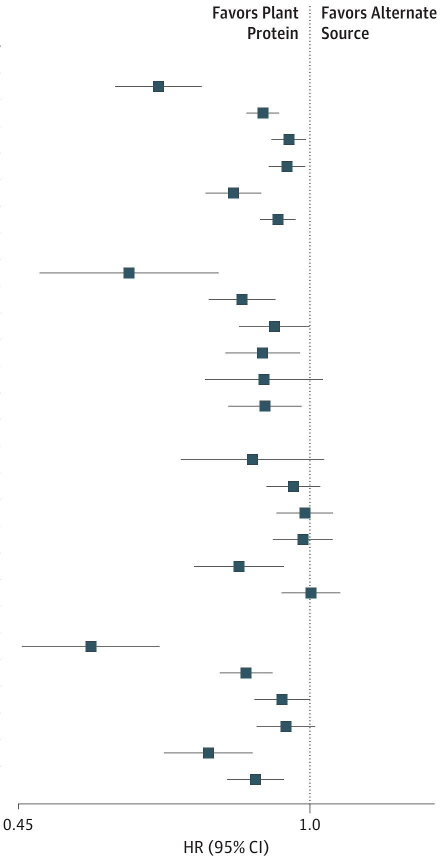
[b] Calculated per 10% increment for animal protein and per 3% increment for plant protein.

[c] Likelihood ratio test was used to calculate the *P* value for interaction by comparing the model with the product term between protein intake (continuous) and healthy lifestyle (binary).

[d] Cox proportional hazards model with age as the time scale was stratified by sex and calendar time and adjusted for total caloric intake and percentage of energy from saturated fat, polyunsaturated fat, monounsaturated fat, and *trans*-fat (all continuous), multivitamin use (yes or no), smoking status (never, past, or current [1-14, and ≥15 cigarettes/d]), pack-years of smoking (in women, ≤15, 16-25, 26-45, and ≥46; in men, <10, 11-24, 25-44, and ≥45), BMI (<23.0, 23.0-24.9, 25.0-26.9, 27.0-29.9, 30.0-34.9, and ≥35), physical activity (quintiles), alcohol consumption (in women, 0, 0.1-5.0, 5.1-15.0, and >15.0 g/d; in men, 0, 0.1-10.0, 10.1-20.0, and >20.0 g/d), history of hypertension diagnosis (yes or no), glycemic index (in quintiles), and intake of whole grains, total fiber, fruits, and vegetables (all in quintiles). Mutual adjustment was conducted for animal protein and plant protein analysis.

Figure. Risk for Mortality Associated With Replacement of 3% Energy From Various Animal Protein Sources With Plant Protein

Animal Protein Source by Cause of Death	HR (95% CI)
All cause	
Processed red meat	0.66 (0.59-0.75)
Unprocessed red meat	0.88 (0.84-0.92)
Poultry	0.94 (0.90-0.99)
Fish	0.94 (0.89-0.99)
Egg	0.81 (0.75-0.88)
Dairy	0.92 (0.87-0.96)
CVD	
Processed red meat	0.61 (0.48-0.78)
Unprocessed red meat	0.83 (0.76-0.91)
Poultry	0.91 (0.83-1.00)
Fish	0.88 (0.80- 0.97)
Egg	0.88 (0.75-1.04)
Dairy	0.89 (0.80-0.98)
Cancer	
Processed red meat	0.86 (0.71-1.04)
Unprocessed red meat	0.96 (0.89-1.03)
Poultry	0.99 (0.91-1.06)
Fish	0.98 (0.91-1.06)
Egg	0.83 (0.73-0.93)
Dairy	1.00 (0.93-1.09)
Other	
Processed red meat	0.55 (0.46-0.67)
Unprocessed red meat	0.84 (0.78-0.90)
Poultry	0.93 (0.86-1.00)
Fish	0.94 (0.87-1.01)
Egg	0.76 (0.67-0.86)
Dairy	0.86 (0.80-0.93)



Protein intake from plant sources and from all the animal food items considered were included in the multivariable model that was also adjusted for total caloric intake and percentage of energy from saturated fat, polyunsaturated fat, monounsaturated fat, and *trans*-fat (all continuous), multivitamin use (yes or no), smoking status (never, past, or current [1-14, and ≥15 cigarettes/d]), pack-years of smoking (in women, ≤15, 16-25, 26-45, and ≥46; in men, <10, 11-24, 25-44, and ≥45), body mass index (calculated as weight in kilograms divided by height in meters squared; <23.0, 23.0-24.9, 25.0-26.9, 27.0-29.9, 30.0-34.9, and ≥35), physical activity (quintiles), alcohol consumption (in women, 0, 0.1-5.0, 5.1-15.0, and >15.0 g/d; in men, 0, 0.1-10.0, 10.1-20.0, and >20.0 g/d), history of hypertension diagnosis (yes or no), glycemic index (in quintiles), and intake of whole grains, total fiber, fruits, and vegetables (all in quintiles). CVD indicates cardiovascular disease; HR, hazard ratio. Error bars indicate 95% CIs.

substitution of 3% energy plant protein was associated with 17% lower mortality due to cancer (95% CI, 7%-27%).

Discussion

After adjusting for other dietary and lifestyle factors, animal protein intake was associated with a higher risk for CVD mortality, whereas higher plant protein intake was associated with lower all-cause and cardiovascular mortality. However, in the stratified analysis, these associations were confined to participants with at least 1 lifestyle risk factor. Moreover, we observed that substitution of plant protein for animal protein from a variety of food sources, particularly processed red meat, was associated with a lower risk for mortality, suggesting that the protein source is important for long-term health.

Although short-term randomized clinical trials have shown a beneficial effect of high protein intake,[3,4,20,21] the long-term health consequences of protein intake remain controversial.[8,9,22-25] In a randomized clinical trial with a 2-year intervention, 4 calorie-restricted diets with different macronutrient compositions did not show a difference in the effects on weight loss or on improvement of lipid profiles and insulin levels.[26] When protein is substituted for other macronutrients, the dietary source of protein appears to be a critical determinant of the outcome.

To our knowledge, only 2 cohort studies[8,9] have examined animal and plant protein intake in relation to mortality. In the Iowa Women's Health Study,[9] although neither animal nor plant protein was associated with all-cause mortality, an inverse association was found between plant protein and CVD mortality, and substituting plant protein for animal protein was associated with a substantially lower CVD mortality. In a recent report from the National Health and Nutrition Examination Survey III,[8] higher protein intake was related to an increased risk for all-cause mortality among participants younger than 65 years. However, when animal protein intake was controlled for, this association was eliminated, suggesting that animal protein was responsible for the effect of higher protein intake, if any, on increased mortality. Although a direct comparison of these studies is difficult, given the variation in the study methods,[27] these data together with our current findings support the importance of protein sources for the long-term health outcome and suggest that plants constitute a preferred protein source compared with animal foods.

Indeed, unlike animal protein, plant protein has not been associated with increased insulinlike growth factor 1 levels[28,29] and has been linked to lower blood pressure,[30-32] reduced low-density lipoprotein levels,[32-34] and improved insulin sensitivity.[35] Substitution of plant protein for animal protein has been related to a lower incidence of CVD[36-39] and type 2 diabetes.[40-42] Moreover, although a high intake of red meat, particularly processed red meat, has been associated with increased mortality in a recent meta-analysis of 13 cohort studies,[43] high consumption of nuts, a major contributor to plant protein, has been associated lower CVD and all-cause mortality.[44] These results underscore the importance of protein sources for risk assessment and suggest that other components in protein-rich foods (eg, sodium,[45] nitrates, and nitrites[46] in processed red meat), in addition to protein per se, may have a critical health effect.

Interestingly, in this study, we found that the association of animal and plant protein with mortality varied by lifestyle factors, and any statistically significant protein-mortality associations were restricted to participants with at least 1 of the unhealthy behaviors, including smoking, heavy alcohol intake, overweight or obesity, and physical inactivity. Several reasons may explain these findings. First, given the remaining variation of health behaviors across protein intake categories in the unhealthy-lifestyle group, residual confounding from lifestyle factors may contribute to the observed protein-mortality associations. However, our results are robust to adjustment for a wide spectrum of potential confounders and the propensity score. Second, our results suggest that the adverse effects of high animal protein intake and beneficial effects of plant protein may be enhanced by other unhealthy lifestyle choices and become evident among the subgroup of individuals with these behaviors who may already have had some underlying inflammatory or metabolic disorders. Finally, as shown in Table 3, participants with a similar intake and with and without a healthy lifestyle demonstrated distinct profiles of protein sources. Those with unhealthy lifestyles consumed more processed and unprocessed red meat, whereas the healthy-lifestyle group consumed more fish and chicken as animal protein sources, suggesting that different protein sources, at least in part, contributed to the observed variation in the protein-mortality associations according to lifestyle factors. This hypothesis is supported by our substitution analysis results. Although substituting plant foods for various animal foods was associated with a lower mortality, red meat, especially processed red meat, showed a much stronger association than fish and poultry, which themselves were not associated with mortality (eTable 6 in the Supplement). In fact, protein from certain fish, such as cod, has been suggested to improve the lipid profile, glycemic control, and insulin sensitivity.[35,47,48]

The strengths of the present study included the large sample size, repeated dietary assessments, and high follow-up rate of the 2 well-established cohorts for up to 32 years. Moreover, we collected detailed data on a wide spectrum of lifestyle factors that allowed for rigorous confounding adjustment and subgroup analysis. In addition, to facilitate public health recommendations, we calculated protein intake according to food sources and assessed the substitution effect for protein of various origins.

A limitation of the study is the moderately higher protein consumption (median, 19% of calories) in our study population compared with the general US population (15%-16%),[49,50] thus limiting our ability to assess the effect of the very low end of intake. Furthermore, as an observational study, residual confounding could not be excluded. However, our results are robust to the multivariable adjustment and propensity score analysis, and any confounding effect may have been minimized in our stratified analysis according to lifestyle profile.

Conclusions

Although higher intake of animal protein was associated with higher cardiovascular mortality and higher intake of plant protein was associated with lower mortality, these associations were confined to participants with at least 1 lifestyle risk factor. Substitution of plant protein for animal protein, especially from processed red meat, may confer a substantial health benefit. Therefore, public health recommendations should focus on improvement of protein sources.

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