

Major Dietary Protein Sources and Risk of Coronary Heart Disease in Women

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Background—With the exception of fish, few major dietary protein sources have been studied in relation to the development of coronary heart disease (CHD). Our objective was to examine the relation between foods that are major dietary protein sources and incident CHD.

Methods and Results—We prospectively followed 84 136 women aged 30 to 55 years in the Nurses' Health Study with no known cancer, diabetes mellitus, angina, myocardial infarction, stroke, or other cardiovascular disease. Diet was assessed by a standardized and validated questionnaire and updated every 4 years. During 26 years of follow-up, we documented 2210 incident nonfatal infarctions and 952 deaths from CHD. In multivariable analyses including age, smoking, and other risk factors, higher intakes of red meat, red meat excluding processed meat, and high-fat dairy were significantly associated with elevated risk of CHD. Higher intakes of poultry, fish, and nuts were significantly associated with lower risk. In a model controlling statistically for energy intake, 1 serving per day of nuts was associated with a 30% (95% confidence interval, 17% to 42%) lower risk of CHD compared with 1 serving per day of red meat. Similarly, compared with 1 serving per day of red meat, a lower risk was associated with 1 serving per day of low-fat dairy (13%; 95% confidence interval, 6% to 19%), poultry (19%; 95% confidence interval, 3% to 33%), and fish (24%; 95% confidence interval, 6% to 39%).

Conclusions—These data suggest that high red meat intake increases risk of CHD and that CHD risk may be reduced importantly by shifting sources of protein in the US diet. (*Circulation*. 2010;122:876-883.)

Key Words: coronary disease ■ diet ■ epidemiology ■ risk factors ■ women

With the exception of fish, relatively few foods that are major sources of dietary protein have been examined in relation to incident coronary heart disease (CHD). The relation of these foods to CHD may not be predicted by the composition of their individual nutrients because of either synergy or antagonism among the nutrients or because of the presence of other constituents in the foods. Although neither animal protein nor vegetable protein has been associated with CHD risk,¹ other nutrients in protein-rich foods, such as saturated fat,^{2,3} polyunsaturated fat,^{3,4} and vitamins E, B₆, and folate,⁵ have been associated with risk.

standing of the relation between these food groups and CHD risk over a longer period of time, as well as an examination of the individual foods that contribute to each food group and the relation of other major dietary protein sources and CHD risk, would allow individuals to reduce their risk by substituting one protein source for another. This type of dietary change may be easier to accomplish than trying to substitute one nutrient for another or one dietary pattern for another. We therefore examined the relation between major sources of dietary protein and the development of CHD in a large cohort of women with 26 years of follow-up.

Editorial see p 859
Clinical Perspective on p 883

We have reported previously on the relation between red meat, poultry, fish, and dairy products in relation to CHD risk over 14 years.² We observed that red meat was not significantly associated with CHD, but increasing poultry and fish intake was associated with a decreased risk. A clear under-

Methods

Study Population

The Nurses' Health Study began in 1976 when 121 700 female registered nurses aged 30 to 55 years and residing in 11 US states provided detailed information on their medical history and lifestyle. Every 2 years, follow-up questionnaires have been sent to update information on potential risk factors and to identify newly diagnosed

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cases of CHD and other diseases. In 1980, a 61-item food-frequency questionnaire (FFQ) was included to assess intake of specific foods. In 1984, the FFQ was expanded to include 116 food items. Similar questionnaires were used to update dietary intake in 1986, 1990, 1994, 1998, and 2002. As in our previous analyses, we excluded those who left >10 items blank on the 1980 FFQ, those with implausibly low or high scores for total food or energy intake (ie, <2094 kJ [500 kcal] or >14 650 kJ [3500 kcal] per day), and those with previously diagnosed cancer, diabetes mellitus, angina, myocardial infarction, stroke, or other cardiovascular disease (including a history of coronary artery bypass grafting).^{1-3,6} The final 1980 baseline population consisted of 84 136 women.

The study was approved by the Committee on the Use of Human Subjects in Research at Brigham and Women's Hospital. All subjects gave informed consent to participate.

Ascertainment of Diet

To calculate intakes of specific protein sources, a commonly used unit or portion size for each food was specified on the FFQ (eg, 1 slice of processed meat or 1 hamburger patty), and the nurse was asked how often on average during the previous year she had consumed that amount (for portion sizes, see the online-only Data Supplement). Nine responses were possible, ranging from "never" to ">6 times per day." The major protein sources on the FFQs include the following: chicken with and without skin, chicken sandwich, regular and lean hamburger, beef/pork hot dog, chicken/turkey hot dog, processed meat sandwich, bacon, beef/pork/lamb mixed dish, beef/pork/lamb main dish, beef liver, chicken liver, skim or low-fat milk, whole milk, yogurt, ice cream, cottage cheese, and hard cheese. The reproducibility and validity of the FFQs in measuring food intake have been described previously in detail.⁷⁻¹¹

Women were divided into fifths of cumulative average intake of various protein sources to reduce within-person variation and best represent long-term diet. These averages were calculated by calculating the mean of all FFQ intakes from all available FFQs up to the beginning of a follow-up interval. The cumulative average intake was then associated with CHD incidence from the time of the last returned questionnaire until the next follow-up cycle. If dietary data from a particular FFQ were missing, then the cumulative average of that FFQ cycle would be the last cumulative average carried forward.

We stopped updating a participant's cumulative average intake when she developed an intermediate outcome on the causal pathway between diet and CHD, such as diabetes mellitus, hypertension, angina, or hypercholesterolemia. We also stopped updating diet at the diagnosis of cancer or stroke or after coronary artery bypass. This was done because changes in diet after these diagnoses or events may bias the diet-disease association (ie, these diagnoses can be confounders).^{2,3} In sensitivity analyses, however, we examined cumulative average intake that was continually updated even after intermediate outcomes.

Ascertainment of End Point

The primary end point for this study was nonfatal myocardial infarction or fatal CHD occurring after the return of the 1980 FFQ but before June 1, 2006. After report of an infarction, permission to obtain medical records was requested, and these were reviewed by study physicians with no knowledge of the subjects' self-reported risk factor status. Myocardial infarction was confirmed by World Health Organization criteria: symptoms plus either diagnostic ECG changes or elevated cardiac enzymes.¹² Infarctions that required hospital admission and for which confirmatory information was obtained by interview or letter, but for which no medical records were available, were designated as probable (527 of 2210 cases of nonfatal myocardial infarction [24%]). We included all confirmed and probable cases in our report because results were similar after probable cases were excluded.

Deaths were identified from state vital records and the National Death Index or were reported by next of kin or the postal system. Follow-up for deaths has been shown to be >98% complete.¹³ Fatal CHD was defined as a fatal myocardial infarction only if confirmed by medical records, next-of-kin or contact person, or autopsy report

or if CHD was listed as cause of death on the death certificate and there was evidence of previous coronary disease. We considered sudden death within 1 hour of the onset of symptoms in women with no other plausible cause of death other than coronary disease as fatal CHD; these cases constituted 146 of 952 or 15% of fatal CHD cases. Fatal CHD was designated as presumed if the underlying cause on the death certificate was CHD but no records were available. These cases constituted 193 of 952 or 20% of fatal CHD cases. Analyses limited to confirmed cases yielded results similar to those of analyses with both confirmed and presumed cases.

Data Analysis

We evaluated the individual associations between red meat, poultry, canned tuna, dark- and light-fleshed fish, nuts, and beans in relation to the risk of CHD. We also examined protein source "groups"—total meat (including chicken with and without skin, chicken sandwich, hamburger, beef and chicken/turkey hot dog, processed meat and processed meat sandwich, bacon, beef/pork/lamb as a mixed and main dish, beef and chicken liver), red meat (hamburger, beef hot dog, processed meat and processed meat sandwich, bacon, beef/pork/lamb as a mixed and main dish), poultry (chicken with and without skin, chicken sandwich, and chicken/turkey hot dog), fish (canned tuna, dark- and light-fleshed fish, and breaded fish), high-fat dairy (whole milk, ice cream, hard cheese, full-fat cheese, cream, sour cream, cream cheese, butter), low-fat dairy (skim/low-fat milk, 1% and 2% milk, yogurt, cottage and ricotta cheeses, low-fat cheese, sherbert)—and their relation to CHD. We also analyzed the ratio of red meat to poultry and red meat to poultry plus fish in relation to incidence of CHD. We estimated the association between animal and vegetable protein and incidence of CHD. We investigated the effect of "substituting" a serving of one major protein source for another by including both as continuous variables in the same multivariable model. The difference in their coefficients plus their covariance was used to estimate the relative risk (RR) and 95% confidence interval (CI) for the substitution.¹⁴

Person-years of follow-up for each participant were calculated from the return of the 1980 FFQ to the date of the first CHD event, death, or June 1, 2006, whichever came first. The RR was computed with the use of a proportional hazards regression model, with the rate in a specific fifth divided by that in the lowest fifth, with adjustment by age in months and calendar time (2-year follow-up period). Median values of intake for each fifth were used to test for a linear trend across fifths.

In multivariable proportional hazards models, in addition to age and calendar time, we simultaneously controlled for intakes of total energy (kcal), cereal fiber (g/d), alcohol (g/d), and trans unsaturated fatty acids (g/d), as well as other potential nondietary confounding variables. These variables were updated biennially and include body mass index (<22, 22 to 24, 24 to 25, 25 to 27, 27 to 29, 29 to 30, 30 to 32, 32 to 35, 35 to 40, >40), physical exercise (<3, 3 to 9, 9 to 18, 18 to 27, ≥ 27 metabolic equivalents per week), cigarette smoking (never, past, current 1 to 14 cigarettes per day, current 14 to 25 cigarettes per day, current ≥ 25 cigarettes per day), menopausal status (premenopausal, postmenopausal with no history of hormone replacement, postmenopausal with a history of hormone replacement, postmenopausal with current hormone replacement), parental history of early myocardial infarction (defined as infarction before age 65 years for a participant's mother or before age 55 years for a participant's father), years of multivitamin use, vitamin E supplement use (yes/no), and aspirin use at least once per week (yes/no). The last value was carried forward for one 2-year cycle to replace missing values. If the last value was missing, then a missing value indicator was created. In the models with animal and vegetable protein, we built 2 models. The first included the main exposures plus the aforementioned covariates, and the second included main exposures plus the aforementioned covariates and saturated fat, monounsaturated fat, and polyunsaturated fatty acids. Neither the diagnoses of diabetes mellitus, angina, hypertension, or hypercholesterolemia nor use of the medications that are prescribed to treat these diseases were included in any of the multivariable models because these may all be considered intermediate outcomes on the

Table 1. Age-Standardized Characteristics of Study Participants, 1980–2006*

	Red Meat Consumption, Fifths of Cumulative Average Intake					Poultry Consumption, Fifths of Cumulative Average Intake					Fish Consumption, Fifths of Cumulative Average Intake				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Median intake, servings per day	0.49	0.83	1.14	1.50	2.11	0.07	0.14	0.24	0.40	0.56	0.07	0.11	0.14	0.25	0.43
Age, y	58	58	58	58	57	58	57	58	58	58	57	58	58	58	58
Body mass index, kg/m ²	25	26	26	26	26	25	26	26	26	27	26	25	26	26	27
Current smoker, %	13	15	17	17	19	21	19	17	13	12	19	17	17	14	13
Family history of early MI, %	13	13	13	13	13	12	13	13	13	13	13	12	13	12	14
History of angina, %	7	6	7	8	10	8	9	6	7	7	9	5	9	5	8
History of high blood pressure, %	30	30	32	36	41	34	38	30	32	31	41	23	38	23	35
History of high cholesterol, %	31	33	36	39	40	35	37	35	35	35	37	34	36	34	36
History of diabetes mellitus, %	8	8	10	11	14	10	11	9	10	10	12	7	11	7	12
Aspirin use at least once per week, %	22	21	22	22	24	23	24	21	21	20	25	17	24	17	23
Current hormone use, %	25	26	26	25	23	23	22	26	27	27	23	29	23	30	25
Vitamin E use, %	26	25	24	23	21	22	22	24	25	26	20	26	22	28	25
Multivitamin use, y	7.8	7.7	7.4	7.0	6.6	6.6	6.6	7.4	7.8	8.2	6.3	7.7	7.0	8.4	7.8
Calories, kcal/d	1326	1492	1623	1761	2015	1480	1565	1656	1698	1815	1535	1612	1620	1739	1723
Cereal fiber, g/d	3.3	3.5	3.5	3.5	3.3	3.0	3.0	3.5	3.7	3.9	2.7	3.8	3.1	4.3	3.7
Trans fat, g/d	2.4	3.0	3.6	4.1	5.2	3.6	3.8	3.7	3.5	3.5	3.9	3.6	3.8	3.5	3.4
Alcohol, g/d	5.8	6.1	6.3	6.5	6.6	5.6	6.5	6.3	6.2	6.2	5.9	5.4	6.6	6.4	6.4
Activity, MET/wk	21	18	17	16	15	15	15	17	19	20	14	15	16	19	21

MI indicates myocardial infarction; MET, metabolic equivalent.

*Median values are given for continuous variables; percentages of population are given for dichotomous variables.

causal pathway between diet and CHD. However, in sensitivity analyses, these intermediates outcomes were included.

Results

During 2 050 071 person-years of follow-up from 1980 through 2006, we documented 2210 nonfatal infarctions and 952 deaths from CHD. Our follow-up rate (defined as person-years contributed by study participants until their last returned FFQ or death divided by person-years contributed by study participants until death) was 97%. Between 1980 and 2002, red meat consumption decreased from a median intake of 1.06 to 0.49 servings per day, whereas poultry consumption increased from 0.14 to 0.28 servings per day, and fish consumption remained unchanged. Characteristics of the study participants during the 26 years of follow-up, averaged according to proportion of person-time in each fifth of intake, are shown in Table 1. High red meat consumption was associated with higher rates of smoking, angina, hypertension, hypercholesterolemia, and diabetes mellitus, lower physical activity, increased intake of trans fat, and decreased intake of vitamin E and multivitamins. The major groups of protein sources were not highly correlated with each other: The Spearman correlation coefficient of red meat with poultry was -0.09 ; that of red meat with fish was -0.17 ; and that of poultry with fish was 0.39 .

In age-adjusted analyses, higher intakes of red meat, red meat excluding processed meat, red meat to poultry ratio, red meat to poultry plus fish ratio, and high-fat dairy were significantly associated with increased risk of CHD. Higher intakes of poultry, fish, and low-fat dairy sources were significantly associated with decreased risk (Table 2). When we controlled for age, follow-up period, and smoking status,

the significant associations seen in the age-adjusted analyses remained. In multivariable analyses (Table 2) that adjusted for dietary and nondietary cardiovascular disease risk factors, the associations were reduced in magnitude but remained significant for all of the protein groups except low-fat dairy. Fruit and vegetable intake was only weakly to moderately correlated with intake of all of the major protein sources, and further adjustment for it had no effect on the risk associations. Egg intake was not associated with risk in either age-adjusted or multivariable models.

In age-adjusted analyses, animal protein was associated with increased risk, and vegetable protein was associated with decreased risk (animal protein RR across fifths: 1.00, 0.94, 0.96, 0.96, 1.14 [P for trend=0.01]; vegetable protein RR across fifths: 1.00, 0.84, 0.77, 0.69, 0.62 [P for trend <0.0001]). In the multivariable analyses, vegetable protein but not animal protein remained significantly associated with decreased risk (vegetable protein RR across fifths: 1.00, 0.88, 0.85, 0.80, 0.72 [P for trend <0.001]). However, when we included polyunsaturated fat, monounsaturated fat, and saturated fat in the multivariable models, we found no relation between animal or vegetable protein and CHD risk.

When we continued updating a participant's diet even after she had been diagnosed with angina, diabetes mellitus, hypertension, hypercholesterolemia, cancer, or stroke or had a coronary artery bypass, we saw associations with red meat that were similar to those obtained when we stopped updating diet at these diagnoses (RR for highest fifth=1.31; 95% CI, 1.15 to 1.50; P for trend <0.0001); however, poultry, fish, and high-fat dairy were no longer

Table 2. RRs and 95% CIs for CHD According to Fifths of Intake of Major Sources of Dietary Protein

	Fifths					P for Trend	RR for 1 Serving per Day
	1	2	3	4	5		
Total meat							
Median servings per day	0.79	1.15	1.46	1.82	2.48		
Age-adjusted	1.00	1.04 (0.93, 1.16)	1.09 (0.97, 1.22)	1.19 (1.07, 1.34)	1.59 (1.43, 1.77)	<0.0001	1.26 (1.21, 1.32)
Multivariable*	1.00	1.01 (0.90, 1.13)	1.00 (0.88, 1.13)	1.01 (0.89, 1.15)	1.22 (1.06, 1.40)	<0.01	1.13 (1.07, 1.20)
Red meat							
Median servings per day	0.49	0.83	1.14	1.50	2.11		
Age-adjusted	1.00	1.15 (1.02, 1.29)	1.18 (1.05, 1.32)	1.33 (1.19, 1.49)	1.75 (1.57, 1.95)	<0.0001	1.32 (1.26, 1.38)
Multivariable*	1.00	1.09 (0.97, 1.23)	1.06 (0.93, 1.20)	1.10 (0.97, 1.26)	1.29 (1.12, 1.49)	<0.001	1.16 (1.09, 1.23)
Red meat, excluding processed meat							
Median servings per day	0.28	0.45	0.64	0.84	1.17		
Age-adjusted	1.00	0.95 (0.84, 1.06)	1.11 (0.99, 1.24)	1.07 (0.95, 1.20)	1.50 (1.35, 1.66)	<0.0001	1.46 (1.36, 1.58)
Multivariable*	1.00	0.91 (0.81, 1.03)	0.99 (0.88, 1.12)	0.93 (0.81, 1.06)	1.13 (0.99, 1.30)	0.02	1.19 (1.07, 1.32)
Poultry							
Median servings per day	0.07	0.14	0.24	0.40	0.56		
Age-adjusted	1.00	1.10 (0.98, 1.23)	0.84 (0.74, 0.96)	0.83 (0.73, 0.93)	0.80 (0.70, 0.92)	<0.0001	0.73 (0.61, 0.88)
Multivariable*	1.00	1.07 (0.96, 1.20)	0.91 (0.80, 1.04)	0.94 (0.83, 1.06)	0.92 (0.80, 1.06)	0.02	0.90 (0.75, 1.08)
Fish							
Median servings per day	0.07	0.11	0.14	0.25	0.43		
Age-adjusted	1.00	0.64 (0.55, 0.74)	0.86 (0.78, 0.94)	0.59 (0.53, 0.66)	0.67 (0.60, 0.74)	<0.0001	0.54 (0.43, 0.68)
Multivariable*	1.00	0.76 (0.65, 0.87)	0.94 (0.85, 1.03)	0.76 (0.68, 0.86)	0.81 (0.72, 0.90)	<0.001	0.81 (0.66, 1.00)
Ratio of red meat to poultry							
Median servings per day	1.41	2.83	4.53	7.32	13.86		
Age-adjusted	1.00	1.12 (0.99, 1.26)	1.35 (1.21, 1.52)	1.41 (1.25, 1.58)	1.78 (1.59, 1.99)	<0.0001	
Multivariable*	1.00	1.00 (0.89, 1.14)	1.14 (1.01, 1.28)	1.11 (0.98, 1.25)	1.26 (1.11, 1.43)	<0.0001	
Ratio of red meat to poultry and fish							
Median servings per day	0.77	1.59	2.48	3.83	7.14		
Age-adjusted	1.00	1.17 (1.04, 1.32)	1.32 (1.17, 1.49)	1.54 (1.37, 1.74)	1.82 (1.62, 2.05)	<0.0001	
Multivariable*	1.00	1.07 (0.94, 1.21)	1.12 (0.98, 1.27)	1.22 (1.07, 1.39)	1.28 (1.12, 1.46)	0.0001	
High-fat dairy							
Median servings per day	0.33	0.69	1.07	1.57	3.00		
Age-adjusted	1.00	0.79 (0.71, 0.89)	0.86 (0.77, 0.96)	0.89 (0.80, 0.99)	1.15 (1.04, 1.27)	<0.0001	1.07 (1.04, 1.10)
Multivariable*	1.00	0.86 (0.77, 0.96)	0.91 (0.81, 1.02)	0.93 (0.83, 1.04)	1.09 (0.97, 1.22)	<0.01	1.03 (1.00, 1.06)
Low-fat dairy							
Median servings per day	0.07	0.32	0.69	1.14	2.32		
Age-adjusted	1.00	0.68 (0.61, 0.76)	0.63 (0.57, 0.70)	0.63 (0.56, 0.70)	0.67 (0.60, 0.74)	<0.0001	0.91 (0.88, 0.95)
Multivariable*	1.00	0.79 (0.71, 0.89)	0.81 (0.73, 0.91)	0.83 (0.75, 0.93)	0.90 (0.80, 1.01)	0.66	1.01 (0.96, 1.04)
Eggs							
Median servings per day	0.07	0.15	0.33	0.43	0.79		
Age-adjusted	1.00	0.92 (0.82, 1.03)	0.74 (0.64, 0.85)	1.17 (1.05, 1.30)	0.98 (0.87, 1.11)	0.12	1.14 (1.02, 1.27)
Multivariable*	1.00	0.96 (0.85, 1.07)	0.83 (0.72, 0.96)	1.11 (1.00, 1.23)	0.96 (0.85, 1.09)	0.74	1.04 (0.93, 1.16)
Nuts							
Median servings per day	0.00	0.04	0.07	0.12	0.40		
Age-adjusted	1.00	0.61 (0.54, 0.68)	0.85 (0.77, 0.94)	0.63 (0.57, 0.70)	0.54 (0.48, 0.60)	<0.0001	0.63 (0.52, 0.75)
Multivariable*	1.00	0.73 (0.65, 0.82)	0.91 (0.82, 1.00)	0.76 (0.67, 0.84)	0.68 (0.60, 0.76)	<0.0001	0.78 (0.66, 0.93)
Beans							
Median servings per day	0.00	0.02	0.05	0.07	0.14		
Age-adjusted	1.00	0.56 (0.48, 0.66)	0.73 (0.65, 0.82)	0.83 (0.76, 0.91)	0.75 (0.67, 0.83)	<0.0001	0.48 (0.31, 0.73)
Multivariable*	1.00	0.68 (0.58, 0.81)	0.89 (0.78, 1.00)	0.92 (0.84, 1.01)	0.89 (0.80, 0.99)	0.17	0.76 (0.50, 1.14)

*Multivariable model includes age (months), time period (13 periods), total energy (fifths of kilocalories), cereal fiber (fifths of grams per day), alcohol (fifths of grams per day), trans fat (fifths of grams per day), body mass index (10 categories), cigarette smoking (never, past, current 1–14, current 14–25, current ≥ 25 cigarettes per day), menopausal status (premenopausal, postmenopausal with no history of hormone replacement, postmenopausal with history of hormone replacement, postmenopausal with current hormone replacement), parental history of early myocardial infarction (before age 65 years for mother or age 55 years for father), multivitamin use (fifths of years), vitamin E supplement use (yes/no), aspirin use at least once per week (yes/no), physical exercise (< 3 , 3–9, 9–18, 18–27, ≥ 27 metabolic equivalents per week).

Table 3. RRs and 95% CIs for CHD According to Fifths of Intake of Individual Types of Red Meat, Poultry, and Fish

	Fifths					P for Trend	RR for 1 Serving per Day
	1	2	3	4	5		
Chicken without skin							
Median servings per day	0.00	0.07	0.14	0.24	0.43		
Multivariable*	1.00	0.99 (0.88, 1.11)	1.11 (1.00, 1.23)	0.81 (0.71, 0.93)	1.02 (0.90, 1.14)	0.64	0.97 (0.79, 1.19)
Chicken with skin							
Median servings per day	0.00	0.04	0.07	0.14	0.31		
Multivariable*	1.00	0.89 (0.78, 1.03)	0.93 (0.84, 1.03)	1.02 (0.92, 1.13)	0.91 (0.82, 1.02)	0.31	0.94 (0.72, 1.23)
Bacon							
Median servings per day	0.00	0.04	0.07	0.14	0.33		
Multivariable*	1.00	0.80 (0.70, 0.91)	1.05 (0.95, 1.17)	1.04 (0.94, 1.16)	1.03 (0.91, 1.15)	0.17	1.41 (1.12, 1.76)
Beef hot dog							
Median servings per day	0.00	0.04	0.07	0.11	0.14		
Multivariable*	1.00	0.86 (0.76, 0.97)	1.14 (1.03, 1.26)	0.88 (0.77, 1.01)	1.11 (0.99, 1.23)	0.07	1.35 (0.94, 1.93)
Beef, mixed dish							
Median servings per day	0.07	0.11	0.14	0.24	0.43		
Multivariable*	1.00	0.80 (0.70, 0.91)	1.13 (1.02, 1.25)	0.80 (0.71, 0.90)	1.09 (0.98, 1.22)	0.22	1.23 (1.01, 1.49)
Beef, main dish							
Median servings per day	0.07	0.14	0.29	0.43	0.70		
Multivariable*	1.00	1.18 (1.05, 1.33)	0.90 (0.79, 1.03)	1.14 (1.00, 1.30)	1.10 (0.96, 1.27)	0.70	1.08 (0.92, 1.27)
Hamburger							
Median servings per day	0.07	0.11	0.14	0.21	0.43		
Multivariable*	1.00	0.83 (0.73, 0.94)	1.14 (1.03, 1.27)	0.86 (0.75, 0.99)	1.09 (0.98, 1.23)	0.11	1.42 (1.10, 1.84)
Processed meat							
Median servings per day	0.00	0.04	0.07	0.14	0.43		
Multivariable*	1.00	0.89 (0.79, 1.01)	0.98 (0.88, 1.08)	0.98 (0.88, 1.10)	1.05 (0.93, 1.17)	0.11	1.20 (1.03, 1.40)
Canned tuna							
Median servings per day	0.02	0.07	0.11	0.14	0.33		
Multivariable*	1.00	1.07 (0.91, 1.25)	0.89 (0.74, 1.06)	1.13 (0.96, 1.33)	0.97 (0.82, 1.15)	0.64	1.05 (0.72, 1.54)
Dark fish							
Median servings per day	0.00	0.07					
Multivariable*	1.00	0.92 (0.82, 1.02)				0.13	0.96 (0.37, 2.52)
Light fish							
Median servings per day	0.00	0.04	0.07	0.14	0.29		
Multivariable*	1.00	0.95 (0.78, 1.16)	1.05 (0.90, 1.23)	1.15 (0.98, 1.34)	0.91 (0.75, 1.11)	0.74	0.88 (0.52, 1.49)

*Multivariable model includes age (months), time period (13 periods), total energy (fifths of kilocalories), cereal fiber (fifths of grams per day), alcohol (fifths of grams per day), trans fat (fifths of grams per day), body mass index (10 categories), cigarette smoking (never, past, current 1–14, current 14–25, current ≥ 25 cigarettes per day), menopausal status (premenopausal, postmenopausal with no history of hormone replacement, postmenopausal with history of hormone replacement, postmenopausal with current hormone replacement), parental history of early myocardial infarction (before age 65 years for mother or age 55 years for father), multivitamin use (fifths of years), vitamin E supplement use (yes/no), aspirin use at least once per week (yes/no), physical exercise (<3, 3–9, 9–18, 18–27, ≥ 27 metabolic equivalents per week).

associated with CHD risk. When we stopped updating diet at the diagnosis of an intermediate outcome and also controlled for their presence in the multivariable model, red meat, poultry, fish, and high-fat dairy all remained significantly related to risk of CHD.

In the analyses of individual protein sources, higher intake of red and processed meats were associated with increased risk, whereas higher intakes of fish, nuts, and beans were associated with decreased risk (Table 3). When compared with 1 serving per day of red meat, 1 serving per day of nuts, fish, poultry, or dairy was associated with a decreased risk of

CHD (Figure). One serving per day of nuts was associated with a 30% (95% CI, 17% to 42%) lower risk of CHD compared with 1 serving per day of red meat. Similarly, when compared with 1 serving per day of red meat, 1 serving per day of low-fat dairy was associated with a 13% (95% CI, 6% to 19%) lower risk, 1 serving per day of poultry was associated with a 19% (95% CI, 3% to 33%) lower risk, and 1 serving per day of fish was associated with a 24% (95% CI, 6% to 39%) lower risk.

When compared with 1 serving per day of fish, 1 serving per day of dairy products was associated with an increased

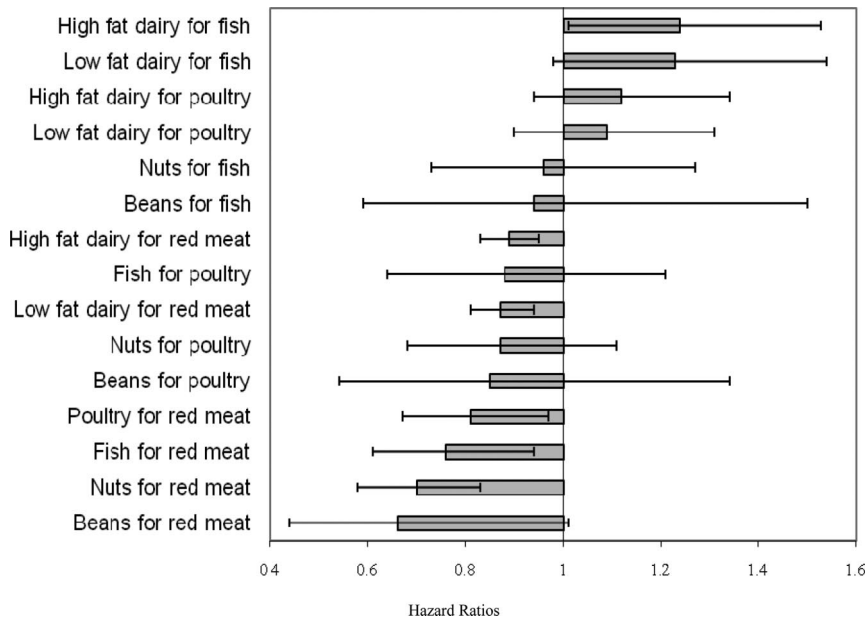


Figure. RRs and 95% CIs for CHD associated with replacement of a major dietary protein source with another.

risk, whereas 1 serving per day of nuts or beans was not significantly associated with risk.

Discussion

In this prospective cohort study with 26 years of follow-up, we observed that a higher consumption of red meat was associated with an increased risk of CHD. The positive association was independent of established dietary and non-dietary cardiovascular risk factors as well as fruit and vegetable intake. When compared with red meat, intakes of dairy, poultry, fish, and especially nuts were associated with substantially lower risk of coronary disease.

To date, only a few prospective and case-control studies have examined major dietary protein sources and risk of CHD. Snowdon et al¹⁵ followed 25 153 California Seventh-Day Adventists between 1960 and 1980 and observed that their daily meat consumption was associated with a 70% greater risk of fatal CHD in men and a 37% greater risk in women. Gramenzi et al¹⁶ conducted a case-control study among women with 287 cases and 649 controls. Women who consumed ham and salami >2 times per week had a significant 60% increased odds of myocardial infarction. Sinha et al¹⁷ followed 322 263 men and 223 390 women in the National Institutes of Health–American Association of Retired Persons study for 10 years, documenting 71 252 deaths, including 5356 deaths from cardiovascular disease among women. They reported an increased risk of cardiovascular mortality among women consuming high intakes of red and processed meats (red meat: RR=1.50 for highest versus lowest fifth; processed meat: RR=1.38 for highest versus lowest fifth).

In an earlier analysis of the Nurses' Health Study with 14 years of follow-up, increasing the ratio of red meat to poultry plus fish was associated with a highly significant positive association.² A separate analysis with 16 years of follow-up showed that higher fish consumption was associated with a lower risk of CHD.⁴ Compared with these studies, our

analysis here has a longer follow-up period and greater precision in dietary measurement because of a greater number of cases and repeated dietary questionnaires.

When we continued updating diet throughout follow-up, we no longer observed significant associations between poultry, fish, or high-fat dairy and CHD risk. This suggests that other diseases and events do in fact lead nurses to change their diets and thus confound the diet-disease association. Red meat, however, continued to be significantly related to CHD risk even when we continually updated diet and when we controlled for the intermediate outcomes. These results suggest a strong association between red meat and CHD, independent of measured confounders and known intermediate outcomes.

The positive association between total meat and CHD risk is likely driven by the high amount of red meat that makes up total meat intake (Spearman coefficient of correlation=0.94). The strong association between red meat and CHD is not entirely explained by processed meat intake because red meat remained associated with CHD risk even after processed meat was excluded.

Dietary iron, and particularly the heme iron found in red meat, has been positively associated with myocardial infarction and fatal CHD in most^{18–22} but not all studies.^{23,24} Through its effect on systolic blood pressure, the high sodium content of processed meats may be associated with CHD risk.^{25,26} Compounds created by cooking red meat, including heterocyclic amines and advanced glycation end-products, may also be linked with CHD.^{27–34} Compared with polyunsaturated fat, high intakes of saturated fat and cholesterol have been linked with increased CHD risk.^{2,3} A recent meta-analysis reported that saturated fat consumption was not significantly associated with CHD risk³⁵; however, in this meta-analysis saturated fat was compared with other calorie sources, primarily refined carbohydrates, and high intake of refined carbohydrates has been associated with a high risk of CHD.³⁶ In another recent meta-analysis with findings similar

to ours, total red meat and processed red meat were both positively associated with risk of CHD³⁷; however, in this meta-analysis, red meat was compared with other sources of calories, and the published data did not allow for an explicit comparison with protein sources. When major sources of protein, such as nuts and fish, are used to replace red meat, saturated fat, heme iron, and sodium decrease, whereas intake of polyunsaturated fat increases. The benefit on CHD risk of such a substitution is thus likely to be due to multiple simultaneous changes in nutrient intake.

Our analysis has several important strengths and limitations. The 26-year follow-up with updated dietary data, high follow-up rate, and large number of study participants provided excellent power to detect differences in RRs across fifths (for example, power for test for trend across 5 quantiles, with 3162 cases and 80 974 controls and a 2-sided test with $\alpha=0.05$, was 99.67%³⁸). The ability to measure and control for known cardiovascular risk factors in multivariable models permitted less biased estimates of the independent association of protein-rich foods with CHD risk. Measurement of dietary data is imperfect, and measurement error would likely lead to an underestimation of the true effect of the exposures with outcome.⁶ The repeated dietary data serve to reduce random error to some extent. Nevertheless, we cannot exclude the possibility of residual and unmeasured confounding due to the observational nature of this study.

In conclusion, in this large study of US women, we found that greater consumption of red meat was associated with a higher risk of CHD. Compared with the same number of servings of red meat, consumption of fish, poultry, dairy products, and especially nuts was associated with lower risk. These findings suggest that the risk of CHD may be reduced importantly by shifting the sources of protein in the US diet.

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CLINICAL PERSPECTIVE

With the exception of fish, few major dietary protein sources have been studied in relation to the development of coronary heart disease (CHD). Our objective was to examine the relation between foods that are major dietary protein sources and incident CHD. We prospectively followed 84 136 women aged 30 to 55 years in the Nurses' Health Study with no known cancer, diabetes mellitus, angina, myocardial infarction, stroke, or other cardiovascular disease. During 26 years of follow-up, we documented 2210 incident nonfatal infarctions and 952 deaths from CHD. In multivariate analyses including age, smoking, and other known cardiovascular risk factors, a higher intake of red meat was significantly associated with an elevated risk of CHD. Higher intakes of poultry, fish, and nuts were significantly associated with lower risk. In a model controlling statistically for total energy intake, 1 serving per day of nuts was associated with a 30% (95% confidence interval, 17% to 42%) lower risk of CHD compared with 1 serving per day of red meat. Similarly, compared with 1 serving per day of red meat, 1 serving per day of low-fat dairy was associated with a 13% (95% confidence interval, 6% to 19%) lower risk, 1 serving per day of poultry was associated with a 19% (95% confidence interval, 3% to 33%) lower risk, and 1 serving per day of fish was associated with a 24% (95% confidence interval, 6% to 39%) lower risk. These data suggest that high red meat intake increases risk of CHD and that risk of CHD may be reduced importantly by shifting the sources of protein in the US diet.

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Major Dietary Protein Sources and Risk of Coronary Heart Disease in Women
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SUPPLEMENTAL MATERIAL

**Appendix Table: Serving Sizes of Major Protein Sources on Food Frequency Questionnaires
in the Nurses' Health Study, 1980-2002**

	1980	1984	1986	1990	1994	1998	2002
Milk (incl. skim, 1%, 2%, whole)	8oz	8oz	8oz	8oz	8oz	8oz	8oz
Cream		1 tbs	1 tbs	1 tbs	1 tbs	1 tbs	1 tbs
Sour cream		1 tbs	1 tbs	1 tbs	1 tbs	1 tbs	1 tbs
Yogurt (incl. flavored and plain)	1 cup	1 cup	1 cup	1 cup	1 cup	1 cup	1 cup
Ice cream	½ cup	½ cup	½ cup	½ cup	½ cup	½ cup	½ cup
Cottage or ricotta cheese	½ cup	½ cup	½ cup	½ cup	½ cup	½ cup	½ cup
Hard cheese	1 slice or svg	1 slice or 1oz svg	1 slice or 1oz svg	1 slice or 1oz svg	1 slice or 1oz svg	1 slice or 1oz svg	1 slice or 1oz svg
Cream cheese		1 oz	1 oz	1 oz	1 oz	1 oz	1 oz
Butter	1 pat	1 pat	1 pat	1 pat	1 pat	1 pat	1 pat
Chicken w/o skin	6-8oz	4-6oz	4-6oz	4-6oz	4-6oz	3oz	3oz
Chicken w/skin	6-8oz	4-6oz	4-6oz	4-6oz	4-6oz	3oz	3oz

Hamburger (incl. regular and lean)	1 patty	1 patty	1 patty	1 patty	1 patty	1 patty	1 patty
Hot dog	1 hot dog	1 hot dog	1 hot dog	1 hot dog	1 hot dog	1 hot dog	1 hot dog
Chicken or turkey dog					1 chicken or turkey dog	1 chicken or turkey dog	1 chicken or turkey dog
Processed meats * Sandwich * Other (sausage, kielbasa, et)	1 piece or slice	1 piece or slice	1 piece or slice	1 piece or slice	1 piece or slice	1 sandwich 2oz	1 sandwich 2oz
Bacon	2 slices	2 slices	2 slices	2 slices	2 slices	2 slices	2 slices
Beef, pork, lamb sandwich or mixed dish (stew, casserole, lasagna, etc)	Not quantified	Not quantified	Not quantified	Not quantified	Not quantified	Not quantified	Not quantified
Beef, pork, lamb as main dish (steak, roast, ham,	6-8oz	4-6oz	4-6oz	4-6oz	4-6oz	4-6oz	4-6oz

etc.)							
Pork as a main dish (eg, ham or chops)					4-6oz	4-6oz	4-6oz
Beef as a main dish (eg, steak or roast)					4-6oz	4-6oz	4-6oz
Liver		3-4oz					
* Beef			4oz	4oz	4oz	4oz	4oz
* Chicken			1oz	1oz	1oz	1oz	1oz
Eggs	1 egg	1 egg	1 egg	1 egg	1 egg	1 egg	1 egg
Fish	6-8oz						
* Canned tuna		3-4oz	3-4oz	3-4oz	3-4oz	2-3oz	2-3oz
* Dark meat fish (mackerel, salmon, sardines, bluefish, swordfish)		3-5oz	3-5oz	3-5oz	3-5oz	3-5oz	3-5oz
* Other fish (eg, Cod, Haddock, Halibut)		3-5oz	3-5oz	3-5oz	3-5oz	3-5oz	3-5oz

* Breaded fish cakes, pieces, or fish sticks					1 svg	1 svg	1 svg
Nuts (incl. peanuts, walnuts, other nuts)	1oz	Small packet or 1oz	Small packet or 1oz	Small packet or 1oz	Small packet or 1oz	Small packet or 1oz	Small packet or 1oz
Beans or lentils, baked or dry	½ cup	½ cup	½ cup	½ cup	½ cup	½ cup	½ cup