Dietary protein and risk of ischemic heart disease in women[1–3]

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ABSTRACT

Background: Ingestion of animal protein raises serum cholesterol in some experimental models but not in others, and ecologic studies have suggested a positive association between animal protein intake and risk of ischemic heart disease. Prospective data on the relation of protein intake to risk of ischemic heart disease are sparse.

Objective: The objective was to examine the relation between protein intake and risk of ischemic heart disease.

Design: The study was a prospective cohort study.

Results: We examined the association between dietary protein intake and incidence of ischemic heart disease in a cohort of 80 082 women aged 34–59 y and without a previous diagnosis of ischemic heart disease, stroke, cancer, hypercholesterolemia, or diabetes in 1980. Intakes of protein and other nutrients were assessed with validated dietary questionnaires. We documented 939 major instances of ischemic heart disease during 14 y of follow-up. After age, smoking, total energy intake, percentages of energy from specific types of fat, and other ischemic heart disease risk factors were controlled for, high protein intakes were associated with a low risk of ischemic heart disease; when extreme quintiles of total protein intake were compared, the relative risk was 0.74 (95% CI: 0.59, 0.94). Both animal and vegetable proteins contributed to the lower risk. This inverse association was similar in women with low- or high-fat diets.

Conclusions: Our data do not support the hypothesis that a high protein intake increases the risk of ischemic heart disease. In contrast, our findings suggest that replacing carbohydrates with protein may be associated with a lower risk of ischemic heart disease. Because a high dietary protein intake is often accompanied by increases in saturated fat and cholesterol intakes, application of these findings to public dietary advice should be cautious. *Am J Clin Nutr* 1999;70:221–7.

KEY WORDS Protein intake, animal protein, disease risk, ischemic heart disease, food-frequency questionnaire, women

INTRODUCTION

Experimental studies in rabbits and rats suggest that cholesterol-free, purified diets containing proteins of animal rather than vegetable origin are hypercholesterolemic and atherogenic (1). However, this cholesterol-raising effect was not observed in other species of animals (eg, pigs) or humans (2, 3). On the contrary, exchange of animal protein for carbohydrates in human diets significantly reduced LDL-cholesterol and triacylglycerol concentrations and increased HDL-cholesterol concentrations (4).

Epidemiologic data on the association between dietary protein and risk of ischemic heart disease are sparse. In ecologic studies, animal protein consumption was directly correlated (r = 0.78) and vegetable protein consumption was inversely correlated (r = −0.40) with ischemic heart disease mortality rates (5). However, these correlations are highly likely to be confounded by other aspects of diet, especially saturated fat intake and lifestyle factors. Previous prospective cohort studies focused largely on dietary fat intakes and only a few examined the association with protein intake (6–11). Most analyses of the association between protein intakes and ischemic heart disease risk are difficult to interpret because they only involved simple comparisons of means between cases and noncases. Although a significant positive association with protein intake was seen in one study (11), it was not adjusted for intake of specific types of fat.

In 1980 we used a validated, self-administered food-frequency questionnaire to assess dietary protein intake and other nutrients in the Nurses' Health Study (12). During the follow-up period, multiple assessments of dietary factors were made. In this report, we examined the intake of total protein as well as protein of animal, vegetable, and plant origin in relation to the incidence of ischemic heart disease during 14 y of follow-up.

SUBJECTS AND METHODS

Subjects

The Nurses' Health Study cohort was established in 1976 when 121 700 female registered nurses aged 30–55 y and residing in 11 large US states provided detailed information

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about their medical history and lifestyle characteristics (12). The protocol was in accordance with the ethical guidelines of Brigham and Women's Hospital.

Food-frequency questionnaires

Every 2 y, follow-up questionnaires have been sent to update information on potential risk factors and to identify newly diagnosed cases of ischemic heart disease and other diseases. In 1980, a 61-item food-frequency questionnaire was included to assess intake of specific fats and other nutrients. In 1984, the food-frequency questionnaire was expanded to include 116 items. Similar questionnaires were used in 1986 and 1990 to update diet. The primary change in the revised questionnaire was to create individual questions from groups of nutritionally similar foods that had been collapsed into single items on the more compressed original questionnaire. The reproducibility and validity of the food-frequency questionnaires were described in detail elsewhere (13, 14). Pearson correlation coefficients between energy-adjusted protein intakes assessed by the 61- and 116-item food-frequency questionnaires compared with the four 1-wk diet records were 0.44 and 0.52, respectively, in validation studies performed in samples of the main study. The correlation for energy-adjusted protein intakes assessed by the 2 questionnaires administered 4 y apart was 0.53. In this cohort, the correlations for protein intake expressed as a percentage of energy were 0.47 between 1980 and 1984, 0.54 between 1984 and 1986, and 0.47 between 1986 and 1990.

To calculate intakes of protein and other nutrients, a commonly used unit or portion size for each food (eg, one egg or one slice of bread) was specified and the participants were asked how often on average during the previous year they had consumed that amount. Nine responses were possible, ranging from "never" to "six or more times per day." The intake of nutrients was computed by multiplying the frequency of consumption of each unit of food by the nutrient content of the specified portions. Composition values for protein and other nutrients were obtained from US Department of Agriculture sources (15).

After up to 4 mailings, 98 462 women returned the 1980 diet questionnaire. We excluded those women who left ≥10 items blank, those with implausibly high or low scores for total food intake or energy intake [ie, <2094 kJ (500 kcal) or >14 650 kJ (3500 kcal)/d], and those with previously diagnosed cancer, angina, myocardial infarction, stroke, or other cardiovascular diseases. Women reporting diabetes or high serum cholesterol concentrations were excluded because these disorders are associated with the risk of ischemic heart disease and also could have caused the women to change their diets. The final 1980 baseline population consisted of 80 082 women. On average, >90% of the women responded to the biennial questionnaires (12) and ≈80% responded to each of the dietary questionnaires during the follow-up periods.

Ascertainment of endpoint

The primary endpoint for this study was nonfatal myocardial infarction or fatal ischemic heart disease occurring after return of the 1980 questionnaire but before 1 June 1994. We sought to review medical records for all such reports. Records were reviewed by the study physicians with no knowledge of the self-reported risk factor status of the women. Myocardial infarction was confirmed by using World Health Organization criteria (16): symptoms plus either diagnostic electrocardiographic changes or elevated cardiac enzymes. Women with infarctions who required hospital admission and for whom confirmatory information was obtained by interview or letter, but for whom no medical records were available, were designated as probable cases (17%). We included all confirmed and probable cases in our analyses because results were the same after probable cases were excluded.

Deaths were identified from state vital records and the National Death Index or reported by next of kin and the postal system. Follow-up for the deaths was >98% complete (17). Fatal ischemic heart disease was defined as fatal myocardial infarction if it was confirmed by hospital records or autopsy, if it was listed as the cause of death on the death certificate, if it was the underlying and most plausible cause of death, or if evidence of previous ischemic heart disease was available.

Data analysis

Person-years were calculated for each participant from the date of return of the 1980 questionnaire to the date of first ischemic heart disease event, death, or 1 June 1994. Women were divided into quintiles according to the percentage of energy from protein in their diet. Incidence rates were calculated by dividing the number of events by person-years of follow-up in each quintile. The relative risk (RR) was computed as the rate in a specific category of protein intake divided by that in the lowest quintile, with adjustment for 5-y age categories. In multivariate nutrient density models (18), we simultaneously included intakes of total energy, percentages of energy from specific types of fat (saturated, monounsaturated, polyunsaturated, and *trans* fats), and other potential confounding variables so that the effects of protein intake were compared with those of carbohydrates. We evaluated the nature of the relation between protein intake and risk of ischemic heart disease by comparing a model with a linear term only and an alternative model including a linear and a quadratic term; the latter model had a better fit (likelihood ratio test: $\chi^2 = 6.7$, df = 1, $P < 0.05$). Thus, in our main analyses, we treated protein intake as a categorical variable (quintiles) to capture potential nonlinear effects. In addition, we evaluated potential interactions between protein intake and fat or fiber intake by stratifying the analyses according to low-fat, high-fat, or fiber intake (by using median values as cutoff points). We tested the significance of the interaction using the likelihood ratio test by comparing a model with both the main effects of protein and the stratifying variable and the interaction terms with a reduced model with only the main effects. Pooled logistic regression with 2-y follow-up increments was used for all multivariate analyses (19).

TABLE 1
Pearson correlation coefficients between intakes of protein and fats (% of energy), carbohydrates (% of energy), and fiber at baseline

	Total protein intake	Animal protein intake	Vegetable protein intake
Total fat	0.08	0.16	−0.34
Saturated fat	0.12	0.24	−0.47
Monounsaturated fat	0.10	0.19	−0.34
Polyunsaturated fat	−0.12	−0.19	0.27
trans Fat	−0.21	−0.17	−0.11
Carbohydrates	−0.40	−0.48	0.36
Fiber[1]	0.07	−0.11	0.62

[1] Fiber intake was energy-adjusted by using the regression method described in reference 17.

TABLE 2
Distribution of potential age-adjusted ischemic heart disease risk factors among median quintiles of total protein intake (% of total energy) in 1980

	Quintiles of total protein intake				
	1 (14.7%)	2 (17.1%)	3 (18.8%)	4 (20.6%)	5 (24.0%)
Subject characteristics					
Current smokers (%)	34.2	28.8	27.4	26.8	25.0
History of hypertension (%)	13.3	13.3	13.4	14.3	15.8
Parental myocardial infarction before age 65 y (%)	19.4	19.5	19.4	20.0	21.4
Postmenopausal women taking hormones (%)	14.8	17.1	16.7	16.7	16.5
Regular exercise (%)	38.4	43.0	45.6	48.3	50.8
Multivitamin use (%)	29.5	32.0	33.7	34.8	38.6
Vitamin E supplement use (%)	9.8	11.1	12.7	13.6	15.8
Current aspirin use (%)	47.8	46.7	45.5	46.7	45.8
Age (y)	45.3 ± 7.1[1]	45.6 ± 7.1	45.8 ± 7.1	46.0 ± 7.2	46.2 ± 7.1
Alcohol (g/d)	10.1 ± 15.1	6.9 ± 10.6	6.1 ± 9.1	5.3 ± 8.0	4.1 ± 6.6
Body mass index (kg/m^2)	23.8 ± 4.3	23.9 ± 4.2	24.0 ± 4.2	24.4 ± 4.3	25.3 ± 4.5
Nutrient intakes					
Carbohydrates (% of energy)	45.3 ± 9.2	40.5 ± 7.6	37.8 ± 7.8	35.9 ± 8.1	34.3 ± 8.9
Total fat (% of energy)	35.9 ± 7.3	39.2 ± 6.8	40.4 ± 7.2	40.8 ± 7.8	38.7 ± 8.9
Saturated fat (% of energy)	14.1 ± 3.4	15.6 ± 3.2	16.2 ± 3.3	16.6 ± 3.5	15.9 ± 4.5
Monounsaturated fat (% of energy)	14.3 ± 3.2	16.0 ± 3.2	16.8 ± 3.5	17.0 ± 3.9	16.0 ± 4.4
Polyunsaturated fat (% of energy)	5.5 ± 1.9	5.4 ± 1.6	5.3 ± 1.5	5.2 ± 1.4	4.9 ± 1.4
trans Fat (g/d)	2.3 ± 0.8	2.4 ± 0.7	2.3 ± 0.7	2.2 ± 0.66	1.9 ± 0.6
Cholesterol (mg·MJ^{-1}·d^{-1})	164 ± 56	194 ± 58	211 ± 64	230 ± 72	269 ± 97
Folate (μg/d)	320 ± 244	345 ± 257	359 ± 264	372 ± 279	422 ± 318
Fiber (g/d)	13.4 ± 5.0	13.5 ± 4.5	13.4 ± 4.5	13.5 ± 4.6	14.2 ± 5.1
Food intakes (servings·MJ^{-1}·d^{-1})					
Red meat[2]	0.62 ± 0.28	0.73 ± 0.30	0.78 ± 0.32	0.80 ± 0.34	0.72 ± 0.38
White meat[3]	0.16 ± 0.08	0.19 ± 0.10	0.23 ± 0.12	0.30 ± 0.15	0.57 ± 0.31
Dark bread	0.40 ± 0.55	0.46 ± 0.57	0.49 ± 0.57	0.51 ± 0.55	0.53 ± 0.57
White bread	0.69 ± 0.72	0.60 ± 0.67	0.49 ± 0.57	0.40 ± 0.54	0.31 ± 0.48
Rice or pasta	0.14 ± 0.16	0.15 ± 0.15	0.15 ± 0.14	0.15 ± 0.15	0.13 ± 0.14
Potatoes	0.22 ± 0.20	0.22 ± 0.19	0.20 ± 0.18	0.19 ± 0.17	0.14 ± 0.16
Sweets and desserts[4]	0.86 ± 0.84	0.63 ± 0.58	0.50 ± 0.47	0.41 ± 0.40	0.29 ± 0.34
Fruit and vegetables[5]	2.42 ± 1.38	2.54 ± 1.22	2.63 ± 1.22	2.75 ± 1.23	3.19 ± 1.43

[1] $\bar{x}$ ± SD.
[2] A composite score of beef, pork, or lamb as a main dish; beef as a sandwich or mixed dish; hamburger; hot dog; processed meat; and bacon.
[3] A composite score of fish and chicken.
[4] A composite score of chocolate, candy without chocolate, homemade pie, and ready-made pie, cake, and cookies.
[5] A composite score of 6 fruit and 11 vegetable foods.

To reduce within-subject variation and best represent long-term diet, we used repeated measures of diet in the analyses (20). In particular, the incidence of ischemic heart disease was related to the cumulative average of protein intake from all available questionnaires up to the start of each 2-y follow-up interval in a pooled logistic model. For example, ischemic heart disease incidence during the 1980–1984 time period was related to the protein intake from the 1980 questionnaire and ischemic heart disease incidence during the 1984–1986 time period was related to the average intake from the available 1980 and 1984 questionnaires. Because changes in diet after development of intermediate endpoints such as angina, hypercholesterolemia, and diabetes may confound diet-disease associations (21), we stopped updating the diet at the beginning of the time interval during which individuals developed those intermediate endpoints. Most covariates were updated biennially, including age (5-y category), time period (7 periods), body mass index (5 categories), cigarette smoking (never, past, and current smoking of 1–14, 15–24, and ≥25 cigarettes/d), menopausal status (premenopausal, postmenopausal without hormone replacement, postmenopausal with past hormone replacement, and postmenopausal with current hormone replacement), parental history of myocardial infarction before 65 y of age, multivitamin use, vitamin E supplement use, and alcohol consumption (0, 0–4, 5–14, and ≥15 g/d). Aspirin use (nonuser, 1–6/wk, ≥7/wk, and dose unknown) was assessed in 1980, 1982, 1984, and 1988. Regular vigorous exercise (≥1 time/wk) was assessed in 1980.

RESULTS

During 1 057 269 person-years of follow-up from 1980 to 1994, we documented 658 nonfatal myocardial infarctions and 281 deaths from ischemic heart disease. The mean intakes in 1980, expressed as a percentage of total energy, were 19.1% for total protein, 15.5% for animal protein, and 3.6% for vegetable protein. On the basis of the assessment of the average diet in 1984, the largest contributors (as a percentage of absolute intake) to the overall intake of animal protein were beef as a main dish (20%), chicken (15%), fish (13%), skim milk (10%), and cheese (10%). The largest contributors to the overall intake of vegetable protein were dark bread (8%), white bread (7%), and cold cereal

(5%). Intakes of animal and vegetable proteins were inversely correlated ($r = -0.31$). Protein intake was slightly correlated with intakes of total fat and specific types of fat and modestly correlated with intake of carbohydrates (**Table 1**). Vegetable protein was positively correlated with fiber intake, whereas animal protein intake was negatively correlated with fiber intake.

Median protein intakes ranged from 14.7% of energy in the lowest quintile to 24.0% of energy in the top quintile (**Table 2**). Participants with higher protein intake were less likely to smoke and drink alcohol and more likely to exercise regularly as well as to take multivitamin and vitamin E supplements than were those with lower protein intakes. Those with higher protein intakes also tended to consume more total fat, saturated fat, cholesterol, folate, and less carbohydrate. In terms of foods, they tended to consume more meats, dark bread, and fruit and vegetables, and less white bread, potatoes, and sweets and desserts.

After adjustment for age, a higher intake of total protein was associated with a lower risk of ischemic heart disease (**Table 3**). The RR was 0.75 (95% CI: 0.61, 0.92) when extreme quintiles of total protein intake were compared. This inverse association became slightly stronger after adjustment for smoking and dietary cholesterol and fiber intakes, which are other known risk factors; the RR for the highest compared with the lowest quintiles was 0.72 (95% CI: 0.57, 0.91). Additional adjustment for saturated fat, monounsaturated fat, polyunsaturated fat, and *trans* fat did not alter the RRs, although the CIs became wider. We conducted alternative analyses using protein intake assessed at baseline only and obtained slightly attenuated result; the RR for the highest compared with the lowest quintiles was 0.80 (95% CI: 0.64, 1.02).

Weaker and nonsignificant inverse associations between protein intake and risk of total ischemic heart disease were observed for both animal and vegetable protein intakes when examined separately (Table 3). After adjustment for age, other known risk factors, intake of specific types of fats, and vegetable protein intake, the RR was 0.84 (95% CI: 0.65, 1.07) when extreme quintiles of animal protein intake were compared. The comparable estimate for vegetable protein intake was 0.89 (95% CI: 0.68, 1.18).

To examine potential interactions between dietary protein and fat intakes, we conducted analyses stratified by low and high fat intakes using median values as cutoff points (**Table 4**). The apparent inverse association for the top quintile of protein intake did not differ appreciably among women with low and with high total or saturated fat intakes. There was some suggestion that the inverse association may have been stronger among women with higher intakes of polyunsaturated fats, *trans* fats, and fiber. However, interactions were far from being significant. In addition, the inverse association was persistent across categories of smoking, alcohol drinking, multivitamin use, and regular exercise.

DISCUSSION

In this prospective study, we found that a high protein intake was not associated with an increased risk of ischemic heart disease, but rather it had a modest association with decreased risk. This inverse association could not be explained by other dietary and lifestyle

TABLE 3
Relative risks (RR) of ischemic heart disease and 95% CIs according to quintiles of protein intake[1]

	Quintiles of total protein intake				
	1	2	3	4	5[2]
Total protein intake					
Median (% of energy)	14.7	17.1	18.8	20.6	24.0
Number of cases	211	184	182	193	169
Person-years	211 420	218 846	215 949	209 797	201 258
Age-adjusted RR	1.0	0.82	0.81	0.86	0.75 (0.61, 0.92)
Multivariate RR[3]	1.0	0.86	0.84	0.91	0.72 (0.57, 0.91)
Additional adjustment for specific fats[4]	1.0	0.86	0.84	0.92	0.74 (0.59, 0.95)
Animal protein intake					
Median (% of energy)	11.6	13.3	15.2	17.2	20.6
Number of cases	195	197	174	201	172
Age-adjusted RR	1.0	0.97	0.85	0.99	0.84 (0.68, 1.03)
Multivariate RR	1.0	1.01	0.92	0.87	0.86 (0.68, 1.09)
Additional adjustment for specific fats	1.0	1.01	0.91	0.85	0.85 (0.67, 1.09)
Further adjustment for vegetable protein	1.0	1.00	0.90	0.84	0.84 (0.65, 1.07)
Vegetable protein intake					
Median (% of energy)	2.4	3.0	3.5	4.1	5.0
Number of cases	219	197	187	173	163
Age-adjusted RR	1.0	0.87	0.81	0.77	0.74 (0.60, 0.90)
Multivariate RR	1.0	0.95	0.95	0.85	0.84 (0.66, 1.08)
Additional adjustment for specific fats	1.0	0.98	1.00	0.92	0.94 (0.71, 1.23)
Further adjustment for animal protein	1.0	0.96	0.97	0.89	0.89 (0.68, 1.18)

[1] Values were computed as the cumulative updated average (*see* Methods).
[2] 95% CIs in parentheses.
[3] Models include the following: age (5-y category), time period (7 periods), body mass index (5 categories), cigarette smoking (never, past, and current smoking of 1–14, 15–24, and ≥25 cigarettes/d), menopausal status (premenopausal, postmenopausal without hormone replacement, postmenopausal with past hormone replacement, and postmenopausal with current hormone replacement), parental history of myocardial infarction before 65 y of age, multiple vitamin use, vitamin E supplement use, alcohol consumption (4 categories), history of hypertension, aspirin use (nonuser, 1–6/wk, ≥7/wk, and dose unknown), vigorous exercise ≥1 time/wk; total energy intake, dietary cholesterol intake (quintiles), and dietary fiber intake (quintiles).
[4] Includes saturated fat, monounsaturated fat, polyunsaturated fat, and *trans* fat (all in quintiles).

TABLE 4
Multivariate relative risks (RR) of ischemic heart disease and 95% CIs for each quintile (median percentages of energy) of protein intake according to dietary and nondietary characteristics[1]

	Quintiles of total protein intake				
	1 (14.7%)	2 (17.1%)	3 (18.8%)	4 (20.6%)	5[2] (24.0%)
Dietary variables[3]					
Total fat					
Low (33% of energy)	1.0	0.90	0.72	0.97	0.76 (0.55, 1.06)
High (42% of energy)	1.0	0.83	0.92	0.90	0.72 (0.52, 1.01)
Saturated fat					
Low (12% of energy)	1.0	0.92	0.87	0.97	0.77 (0.56, 1.04)
High (17% of energy)	1.0	0.83	0.85	0.95	0.78 (0.53, 1.15)
Polyunsaturated fat					
Low (5% of energy)	1.0	0.75	0.86	1.00	0.88 (0.62, 1.24)
High (7% of energy)	1.0	0.92	0.83	0.87	0.67 (0.48, 0.93)
Monounsaturated fat					
Low (13% of energy)	1.0	0.87	0.86	0.92	0.74 (0.55, 0.99)
High (17% of energy)	1.0	0.94	0.90	1.05	0.88 (0.57, 1.37)
trans Fat					
Low (2% of energy)	1.0	0.75	0.80	1.00	0.87 (0.57, 1.37)
High (3% of energy)	1.0	0.95	0.88	0.88	0.67 (0.48, 0.94)
Fiber					
Low (10 g/d)	1.0	0.84	0.92	1.03	0.80 (0.57, 1.38)
High (16 g/d)	1.0	0.91	0.76	0.83	0.69 (0.49, 0.98)
Nondietary variables					
Current smoking					
No	1.0	0.71	0.73	0.85	0.67 (0.49, 0.93)
Yes	1.0	1.04	0.99	1.03	0.87 (0.61, 1.24)
Current alcohol drinking					
No	1.0	0.87	0.86	0.94	0.65 (0.43, 0.99)
Yes	1.0	0.91	0.86	0.86	0.77 (0.54, 1.10)
Multivitamin use					
No	1.0	0.85	0.75	0.91	0.71 (0.53, 0.93)
Yes	1.0	0.88	1.10	0.90	0.85 (0.53, 1.35)
Regular exercise (≥1 time/wk)					
No	1.0	0.81	0.92	0.93	0.72 (0.53, 0.98)
Yes	1.0	0.94	0.67	0.88	0.78 (0.52, 1.16)

[1] RRs adjusted for the same covariates as in Table 3 (including specific types of fat), except for the stratifying variable used to divide the subjects into the "low" or "high" groups.
[2] 95% CIs in parentheses.
[3] Values in parentheses are medians and were used as cutoff points.

variables for which we had information. Both animal and vegetable protein intakes contributed to the inverse association.

Protein intake in our cohort was relatively high compared with the general population (22). In ecologic studies (5), those countries with low rates of ischemic heart disease tend to have low protein intakes. However, this result should be interpreted with caution because these countries also have low intakes of saturated fat and cholesterol and high intakes of fiber. In some animal studies, high protein intakes raise serum cholesterol concentrations (1). However, metabolic studies have suggested either neutral (3) or beneficial effects (4) of animal protein on serum cholesterol concentrations in humans. Our findings strongly reject the hypothesis that a high protein intake, including animal protein, is associated with an increased risk of ischemic heart disease. On the contrary, our results suggest that a diet with a relatively high protein and low carbohydrate content may reduce the risk. These results are consistent with results of controlled feeding studies that suggest improvements in plasma lipid profiles when carbohydrates are replaced with proteins. In a crossover study (23), subjects with moderate hypercholesterolemia were randomly assigned to either a high-protein (23% of energy), low-carbohydrate (53% of energy) diet or a low-protein (11% of energy), high-carbohydrate (65% of energy) diet for 4–5 wk. Intakes of dietary fat, cholesterol, and fiber were kept constant. The main sources of protein were turkey, cottage cheese, beef, fish, and ham. Replacement of carbohydrate with protein significantly reduced LDL-cholesterol (by 6.4%) and triacylglycerol (by 23%) concentrations and increased HDL-cholesterol concentrations (by 12%). Favorable effects on plasma lipids of replacing carbohydrates with protein were also observed among subjects with familial hypercholesterolemia (24) and normolipidemia (25).

It is known that low-fat, high-carbohydrate diets reduce LDL concentrations when saturated or *trans* fats are replaced with carbohydrates, but these diets also reduce HDL concentrations and raise fasting triacylglycerol concentrations (26). However, the observed apparent benefit of replacing carbohydrate with protein cannot be attributed simply to displacement of carbohydrate in the diet because LDL-cholesterol concentrations also decreased

in metabolic studies (4). A high-protein diet may decrease triacylglycerol secretion by hepatocytes (27). Rats fed a protein-deficient diet (8% of energy) had slight hypercholesterolemia and increased activity of liver hydroxymethylglutaryl-CoA reductase compared with controls (16% casein diet) (28). These rats also showed increased susceptibility of lipoprotein to peroxidation. In addition, dietary arginine is a precursor of nitric oxide, which is an endothelium-derived relaxing factor (29) and can induce vascular smooth muscle relaxation and inhibit platelet adhesion and aggregation. Furthermore, high consumption of dietary protein has been inversely associated with blood pressure in several observational studies and animal experiments (30).

Although some animal studies suggested a hypercholesterolemic effect of animal protein relative to that of vegetable protein (31), we observed no differential effect of animal compared with vegetable protein intakes on the risk of ischemic heart disease. Most animal studies used rabbits; experiments that used other species such as pigs yielded discrepant results (32). Recently, a meta-analysis of 38 controlled feeding studies in humans suggested that replacement of animal protein with soy protein significantly decreased total and LDL-cholesterol concentrations (33). Because soy protein, the usual experimental source of vegetable protein, is not commonly consumed in the United States, we were not able to examine the effect of this specific protein. However, tofu or soybean consumption (reported in 1984) was nonsignificantly associated with a lower risk of ischemic heart disease between 1984 and 1994. The multivariate RR for those who ate tofu or soybean ≥1 time/mo compared with those who never ate it was 0.79 (95% CI: 0.54, 1.17), but in this cohort only 8% of participants reported this modest level of consumption.

Many null findings for diet-ischemic heart disease relations have been attributed to wide intraindividual variation in diet (18). In the present study we used repeated measures of protein intake instead of a single measurement in the analyses to reduce measurement error resulting from intraindividual variation. In addition, by updating dietary intake during follow-up, we accounted for changes in eating behavior and food composition over time.

Although we assessed and adjusted for a multitude of potential confounding variables, we cannot rule out the possibility of residual confounding, especially because the observed inverse association in our study was modest and only significant among women in the highest quintile of protein intake. It is possible that the inverse association was due to some unmeasured variable, such as socioeconomic status. However, because the population we studied is relatively homogeneous in terms of education and occupation, confounding by socioeconomic status was likely to be small relative to that in the general population. In addition, the study was restricted to women without cardiovascular disease; therefore, the findings might not apply to women with ischemic heart disease.

In conclusion, our results do not support the hypothesis that a high protein intake is associated with increased risk of ischemic heart disease. Our findings suggest that given the same intake of total energy and dietary fat, an increase in the percentage of energy from protein relative to carbohydrates is associated with a decreased risk. This result is consistent with evidence from metabolic studies that replacement of dietary carbohydrate with protein has a favorable effect on plasma lipoprotein and lipid concentrations. Because an increase in protein intake from animal products such as meats, dairy products, and eggs is often accompanied by increases in intakes of saturated fat and cholesterol and possible adverse effects on renal disease and osteoporosis (22), dietary advice to improve public health on the basis of these findings should be made with caution.

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