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Original Contribution

Dietary Protein Intake and Risk of Type 2 Diabetes in US Men and Women

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Dietary proteins are important modulators of glucose metabolism. However, few longitudinal studies have evaluated the associations between intake of protein and protein type and risk of type 2 diabetes (T2D). We investigated the associations between total, animal, and vegetable protein and incident T2D in 72,992 women from the Nurses' Health Study (1984–2008), 92,088 women from Nurses' Health Study II (1991–2009) and 40,722 men from the Health Professionals Follow-up Study (1986–2008). During 4,146,216 person-years of follow-up, we documented 15,580 cases of T2D. In pooled multivariate models including body mass index, participants in the highest quintiles of percentage of energy derived from total protein and animal protein had 7% (95% confidence interval (CI): 1, 17) and 13% (95% CI: 6, 21) increased risks of T2D compared with those in the lowest quintiles, respectively. Percentage of energy intake from vegetable protein was associated with a moderately decreased risk of T2D (comparing extreme quintiles, hazard ratio = 0.91, 95% CI: 0.84, 0.98). Substituting 5% of energy intake from vegetable protein for animal protein was associated with a 23% (95% CI: 16, 30) reduced risk of T2D. In conclusion, higher intake of animal protein was associated with an increased risk of T2D, while higher intake of vegetable protein was associated with a modestly reduced risk.

animal protein; diabetes mellitus; dietary protein; nuts; peanuts; type 2 diabetes; vegetable protein; whole grains

Abbreviations: BMI, body mass index; CI, confidence interval; EPIC, European Prospective Investigation into Cancer and Nutrition; FFQ, food frequency questionnaire; HPFS, Health Professionals Follow-up Study; HR, hazard ratio; NHS, Nurses' Health Study; NHS II, Nurses' Health Study II; T2D, type 2 diabetes.

Diets high in protein have shown beneficial effects on glucose homeostasis in short-term trials (1, 2) and have thus been suggested as a potential strategy for type 2 diabetes (T2D) prevention. In contrast, findings from a few prospective cohort studies have shown positive associations between protein intake and risk of T2D that are driven by intake of animal protein (3, 4). However, because these studies used baseline intake only, they were not able to capture long-term intake patterns.

Findings from epidemiologic studies evaluating food sources of protein suggest divergent associations of animal and vegetable protein with T2D risk. Intake of red and processed meat has been positively associated with risk of T2D (5, 6), while intake of plant-based sources of protein, such as nuts (7, 8) and legumes (9), has been associated with decreased risk. It is unclear whether different types of protein are differentially associated with risk of T2D and whether it is the protein per se or other components of protein-rich foods that may account for observed associations. In this study, we aimed to investigate the associations between total protein intake and protein type (vegetable, animal) and risk of T2D in 3 large prospective cohort studies of US adults, using repeated measurements of protein intake taken over 18–24 years of follow-up. We also estimated the associations between substituting vegetable protein for animal protein, substituting protein for carbohydrates of differing quality, and substituting vegetable protein foods for animal protein and low-quality carbohydrate foods and T2D risk.

METHODS

Study population

Our analysis was conducted in 3 ongoing prospective cohort studies: the Nurses' Health Study (NHS), which consists of 121,700 female registered nurses who were aged 30–55 years at baseline in 1976; Nurses' Health Study II (NHS II),

Table 1. Age-Adjusted Characteristics of Participants According to Baseline (1984) Total Protein Intake Among 72,992 Women in the Nurses' Health Study, 1984–2008

Characteristic	Quintile of Protein Intake					
	1 (*n* = 14,598) (14.8% of Energy)[a]		3 (*n* = 14,598) (18.0% of Energy)		5 (*n* = 14,598) (21.6% of Energy)	
	%	Mean	%	Mean	%	Mean
Demographic and lifestyle factors						
Age, years[b]		50.1		50.1		50.7
White race/ethnicity	97.5		98.1		97.6	
Body mass index[c]		24.3		24.8		25.7
Body mass index ≥25	31.0		36.1		44.6	
Physical activity, MET-hours/week		12.8		14.2		16.1
Current smoker	30.1		22.6		21.8	
Hypertension	8.0		7.6		8.8	
High cholesterol	3.1		3.2		4.2	
Family history of diabetes[d]	26.8		28.1		31.1	
Postmenopausal hormone use	22.6		25.0		26.2	
Multivitamin use	33.6		37.0		40.8	
Alcohol, g/day		10.2		6.8		4.2
Dietary intake						
Total energy, kcal/day		1,880		1,772		1,550
Carbohydrate, % of energy intake		51.3		46.4		41.8
Total fat, % of energy intake		33.0		35.1		35.1
Saturated fat, % of energy intake		11.7		12.6		12.8
Monounsaturated fat, % of energy intake		12.0		12.9		12.8
Polyunsaturated fat, % of energy intake		6.8		6.7		6.4
Trans- fat, % of energy intake		2.0		2.0		1.7
Dietary cholesterol, mg/day		229.6		283.2		344.5
Daily dietary glycemic load[e]		111.7		99.3		86.5
Daily dietary glycemic index[f]		54.8		53.5		51.3
Total dietary fiber, g/day		15.2		16.5		17.0
Cereal fiber, g/day		4.0		4.3		4.0
Magnesium, mg/day		252.8		287.3		326.1
Heme iron, mg/day		0.76		1.1		1.5
Potassium, mg/day		2,570		2,908		3,207
Calcium, mg/day		725.2		883.4		1,040

Table continues

which consists of 116,671 female registered nurses aged 24–42 years at baseline in 1989; and the Health Professionals Follow-up Study (HPFS), which consists of 51,529 male health professionals aged 40–75 years at baseline in 1986. For each cohort, mailed questionnaires are administered biennially to collect data on lifestyle factors and health. Diet is assessed using a validated self-administered food frequency questionnaire (FFQ) every 4 years. For this analysis, we used 1984, 1991, and 1986 as the baseline years for the NHS, NHS II, and HPFS, respectively; those years respectively were then considered the year in which the FFQ was first administered in each cohort. We excluded men and women who had a diagnosis of diabetes, cardiovascular disease, or cancer at baseline, left more than 70 items on the baseline FFQ blank, reported implausible total energy intakes, or were missing baseline data on protein intake or follow-up information on date of diabetes diagnosis. After these exclusions, a total of 72,992 participants from the NHS, 92,088 participants from NHS II, and 40,722 participants from the HPFS remained. All 3 studies were approved by the institutional review boards of Brigham and Women's Hospital and the Harvard T.H. Chan School of Public Health (Boston, Massachusetts).

Assessment of dietary protein

Diet was assessed using a 131-item FFQ, administered at baseline and every 4 years. Each FFQ asked participants how

Table 1. Continued

Characteristic	Quintile of Protein Intake					
	1 (*n* = 14,598) (14.8% of Energy)[a]		3 (*n* = 14,598) (18.0% of Energy)		5 (*n* = 14,598) (21.6% of Energy)	
	%	Mean	%	Mean	%	Mean
Vitamin C, mg/day		301.2		326.0		389.8
Vitamin E, mg/day		71.1		83.2		104.3
Whole grains, servings/day		1.0		1.2		1.2
Fruit, servings/day		1.3		1.4		1.4
Vegetables, servings/day		2.4		2.8		3.2
Red meat, servings/day		0.62		0.88		0.93
Processed meat, servings/day		0.35		0.32		0.23
Fish, servings/day		0.10		0.15		0.28
Chicken, servings/day		0.20		0.29		0.46
Eggs, servings/day		0.30		0.36		0.38
Dairy foods, servings/day		1.7		2.0		2.1
Legumes, servings/day		0.35		0.40		0.42
Nuts, servings/day		0.31		0.33		0.25
Peanuts, servings/day		0.12		0.12		0.09
Peanut butter, servings/day		0.20		0.21		0.16
Potatoes, servings/day		0.43		0.41		0.29
Coffee, servings/day		1.9		1.8		1.6
Sugar-sweetened beverages, servings/day		0.69		0.23		0.10

Abbreviations: GI, glycemic index; MET, metabolic equivalent of task.
[a] Median percentage of energy intake in the quintile.
[b] Not adjusted for age.
[c] Weight (kg)/height (m)^2.
[d] First-degree relatives.
[e] Average glycemic load was calculated by multiplying the amount of carbohydrates in the diet by the average glycemic index. For 1 serving of a food, a glycemic load of ≥20 is considered high, 11–19 is considered medium, and ≤10 is considered low. Among these 72,992 women from the Nurses' Health Study, the mean glycemic load was 102.5 (range, 0–213).
[f] Average dietary glycemic index (GI) was calculated by summing the products of 1) the carbohydrate content of each food item per serving, 2) the average daily number of servings of that food, and 3) the food's GI value (derived from available databases and publications) and dividing by total daily carbohydrate content. Foods with a GI value of ≤55 are considered to have a low GI, foods with a value of 56–69 are considered to have a medium GI, and foods with a value of ≥70 are considered to have a high GI. Among these 72,992 women from the Nurses' Health Study, the mean GI was 52.8 (range, 0.01–70.9).

often, on average, they consumed standard portions of foods and beverages, using 9 possible responses ranging from "never or less than once per month" to "6 or more times per day." Nutrient and energy intakes were calculated by multiplying the frequency of consumption of each unit of food and beverage by nutrient and energy contents and summing across all items. Values were obtained using the US Department of Agriculture food composition database (10). Intakes of total, animal, and vegetable protein were calculated for each participant and expressed as a percentage of total energy by multiplying the grams of protein consumed per day by the number of kilocalories in 1 gram of protein (4 kcal/g) and then dividing by total caloric intake (11). The reproducibility and validity of these FFQs have been described in detail elsewhere (12–16). In a subsample of NHS participants, the coefficients for correlations between the FFQ and multiple dietary records were 0.50 for protein, 0.57 for fat, and 0.64 for carbohydrate (14, 16). Similar values were reported in a subsample of HPFS participants (13).

Assessment of T2D

Participants who reported a diagnosis of T2D on the biennial questionnaire were mailed a supplementary questionnaire about symptoms and treatment. In accordance with the National Diabetes Data Group criteria (17), a case of T2D was confirmed if at least 1 of the following was reported on the supplementary questionnaire: 1) 1 or more classic symptoms of T2D (excessive thirst, polyuria, weight loss, hunger) and fasting plasma glucose concentration of ≥11.1 mmol/L; 2) ≥2 elevated plasma glucose measurements taken on different occasions (fasting concentrations of ≥7.8 mmol/L, random plasma

Table 2. Age-Adjusted Characteristics of Participants According to Baseline (1991) Total Protein Intake Among 92,088 Women in Nurses' Health Study II, 1991–2009

Characteristic	Quintile of Protein Intake					
	1 (*n* = 18,417) (15.3% of Energy)[a]		3 (*n* = 18,466) (18.8% of Energy)		5 (*n* = 18,417) (22.6% of Energy)	
	%	Mean	%	Mean	%	Mean
Demographic and lifestyle factors						
Age, years[b]		35.9		36.0		36.4
White race/ethnicity	95.6		96.8		96.1	
Body mass index[c]		23.8		24.5		25.5
Body mass index ≥25	31.0		36.1		44.6	
Physical activity, MET-hours/week		20.7		20.3		22.4
Current smoker	14.6		11.8		11.7	
Hypertension	5.8		5.9		7.3	
High cholesterol	13.7		13.8		16.4	
Family history of diabetes[d]	32.4		34.3		36.5	
Postmenopausal hormone use	4.3		4.4		5.0	
Oral contraceptive use	12.0		10.4		10.1	
Multivitamin use	42.7		43.9		44.5	
Alcohol, g/day		4.0		3.1		2.3
Dietary intake						
Total energy, kcal/day		1,901		1,815		1,626
Carbohydrate, % of energy intake		55.8		49.4		44.6
Total fat, % of energy intake		30.4		32.1		31.5
Saturated fat, % of energy intake		10.7		11.4		11.2
Monounsaturated fat, % of energy intake		11.7		12.2		11.7
Polyunsaturated fat, % of energy intake		5.6		5.7		5.6
Trans- fat, % of energy intake		1.8		1.7		1.4
Dietary cholesterol, mg/day		187.1		241.7		297.6
Daily dietary glycemic load[e]		139.1		120.3		106.4
Daily dietary glycemic index[f]		55.3		53.9		52.4
Total dietary fiber, g/day		17.7		18.3		18.6
Cereal fiber, g/day		5.6		5.7		5.4
Magnesium, mg/day		285.2		314.9		345.2
Heme iron, mg/day		0.75		1.1		1.5

Table continues

glucose concentrations of ≥11.1 mmol/L, and/or concentrations of ≥11.1 mmol/L after ≥2 hours shown by an oral glucose tolerance test) in the absence of symptoms; or 3) treatment with hypoglycemic medication (insulin or oral hypoglycemic agent). For cases identified after 1998, we applied the American Diabetes Association criteria (18), in which the threshold for diagnosis of diabetes changed from a fasting plasma glucose concentration of 7.8 mmol/L to a concentration of 7.0 mmol/L. The validity of the supplementary questionnaire has been documented through medical record review (19, 20).

Covariates

The biennial follow-up questionnaires collected updated information on lifestyle factors and medical history, including age, body weight, smoking status, physical activity, medication use, and history of chronic diseases. Family history of diabetes in first-degree relatives was assessed in 1982 and 1988 in the NHS; in 1989, 1997, 2001, and 2005 in NHS II; and in 1987 in the HPFS. Information on dietary factors was obtained from the FFQs.

Statistical analysis

We calculated person-time for each participant from the date of return of the baseline questionnaire to the date of diagnosis of T2D, death, loss to follow-up, or the end of the follow-up period, whichever occurred first. Cox proportional hazards regression was used to model the association between protein intake and risk of T2D. Protein intake was expressed as a

Table 2. Continued

Characteristic	Quintile of Protein Intake					
	1 (n = 18,417) (15.3% of Energy)[a]		3 (n = 18,466) (18.8% of Energy)		5 (n = 18,417) (22.6% of Energy)	
	%	Mean	%	Mean	%	Mean
Potassium, mg/day		2,649		2,944		3,189
Calcium, mg/day		857.7		1,028		1,145
Vitamin C, mg/day		264.5		246.5		268.3
Vitamin E, mg/day		47.1		41.2		48.6
Whole grains, servings/day		1.3		1.4		1.3
Fruit, servings/day		1.2		1.2		1.2
Vegetables, servings/day		3.0		3.3		3.6
Red meat, servings/day		0.57		0.77		0.75
Processed meat, servings/day		0.25		0.24		0.17
Fish, servings/day		0.18		0.27		0.42
Chicken, servings/day		0.39		0.66		1.1
Eggs, servings/day		0.17		0.19		0.17
Dairy foods, servings/day		2.2		2.5		2.4
Legumes, servings/day		0.35		0.38		0.40
Nuts, servings/day		0.29		0.26		0.18
Peanuts, servings/day		0.05		0.04		0.02
Peanut butter, servings/day		0.20		0.20		0.14
Potatoes, servings/day		0.39		0.39		0.31
Coffee, servings/day		1.5		1.6		1.5
Sugar-sweetened beverages, servings/day		1.1		0.36		0.13

Abbreviations: GI, glycemic index; MET, metabolic equivalent.

[a] Median percentage of energy intake in the quintile.

[b] Not adjusted for age.

[c] Weight (kg)/height (m)^2.

[d] First-degree relatives.

[e] Average glycemic load was calculated by multiplying the amount of carbohydrates in the diet by the average glycemic index. For 1 serving of a food, a glycemic load of ≥20 is considered high, 11–19 is considered medium, and ≤10 is considered low. Among these 92,088 women from Nurses' Health Study II, the mean glycemic load was 119.0 (range, 47.8–222.4).

[f] Average dietary glycemic index (GI) was calculated by summing the products of 1) the carbohydrate content of each food item per serving, 2) the average daily number of servings of that food, and 3) the food's GI value (derived from available databases and publications) and dividing by total daily carbohydrate content. Foods with a GI value of ≤55 are considered to have a low GI, foods with a value of 56–69 are considered to have a medium GI, and foods with a value of ≥70 are considered to have a high GI. Among these 92,088 women from Nurses' Health Study II, the mean GI was 52.9 (range, 37.0–64.8).

percentage of total energy using the nutrient density method (16) and categorized into quintiles. Regression models included age in years as the time scale, stratified by calendar time in 2-year intervals. Multivariate models adjusted for race/ethnicity (white or nonwhite), family history of diabetes (yes/no), and various lifestyle factors, including smoking status (never smoker, past smoker, or current smoker of 1–14, 15–24, or ≥25 cigarettes/day), alcohol intake (0, 0.1–4.9, 5.0–14.9, or ≥15 g/day in women; 0, 0.1–4.9, 5.0–29.9, or ≥30 g/day in men), physical activity (3.0, 3.0–8.9, 9.0–17.9, 18.0–26.9, or ≥27.0 metabolic equivalent-hours/week), total energy intake (kcal/day; quintiles), and, for women, menopausal status, postmenopausal hormone use (NHS and NHS II; premenopausal, postmenopausal with no history of hormone replacement, or postmenopausal with current hormone replacement), and oral contraceptive use (NHS II only; never user, past user, or current user). Results were also adjusted for percentages of energy derived from *trans*- fat, saturated fat, monounsaturated fat, and polyunsaturated fat; dietary fiber; dietary cholesterol; and glycemic index, and included mutual adjustment for percentages of energy derived from animal protein and vegetable protein (quintiles). Since body weight may partly mediate the association between protein intake and risk of T2D (21), we subsequently adjusted for body mass index (BMI), defined as weight (kg)/height (m)^2 (<23, 23–24.9, 25–29.9, 30–34.9 or ≥35). We also conducted sensitivity analyses by adding red and processed meat, heme iron, and branched chain and aromatic

Table 3. Age-Adjusted Characteristics of Participants According to Baseline (1986) Total Protein Intake Among 40,722 Men in the Health Professionals Follow-up Study, 1986–2008

Characteristic	Quintile of Protein Intake					
	1 ($n = 7,921$) (14.7% of Energy)[a]		3 ($n = 7,921$) (18.0% of Energy)		5 ($n = 7,921$) (21.9% of Energy)	
	%	Mean	%	Mean	%	Mean
Demographic and lifestyle factors						
Age, years[b]		52.9		52.9		53.1
White race/ethnicity	94.7		95.5		94.4	
Body mass index[c]		25.0		25.4		25.9
Body mass index ≥25	47.0		52.1		57.7	
Physical activity, MET-hours/week		20.8		21.0		21.5
Current smoker	12.3		9.1		8.3	
Hypertension	18.1		19.3		21.4	
High cholesterol	8.8		10.1		12.0	
Family history of diabetes[d]	17.1		18.6		19.6	
Multivitamin use	39.9		40.8		44.8	
Alcohol, g/day		17.4		10.7		6.8
Dietary intake						
Total energy, kcal/day		2,076		1,986		1,764
Carbohydrate, % of energy intake		50.9		46.9		42.7
Total fat, % of energy intake		30.7		32.5		32.4
Saturated fat, % of energy intake		10.5		11.2		11.1
Monounsaturated fat, % of energy intake		11.8		12.4		12.2
Polyunsaturated fat, % of energy intake		5.9		6.0		6.0
Trans- fat, % of energy intake		1.4		1.3		1.1
Dietary cholesterol, mg/day		236.2		300.2		374.2
Daily dietary glycemic load[e]		136.8		124.6		111.0
Daily dietary glycemic index[f]		54.0		53.3		52.0
Total dietary fiber, g/day		19.9		21.1		21.4
Cereal fiber, g/day		5.6		6.0		5.8
Magnesium, mg/day		324.2		351.5		380.6
Heme iron, mg/day		0.93		1.3		1.7
Potassium, mg/day		3,084		3,426		3,708
Calcium, mg/day		767.3		906.3		1,002

Table continues

amino acids to multivariate models, since these factors may partially mediate associations. For dietary measures, we used the cumulative average of intakes recorded from baseline to the censoring event (22). We replaced missing values with the cumulative average from preceding FFQ cycles. Because a diagnosis of cardiovascular disease or cancer may lead to changes in diet that may confound the relationship between diet and T2D, we stopped updating dietary variables when participants reported a diagnosis of one of these conditions (22, 23). To test the robustness of our findings, we repeated the analysis while continuing to update diet after a report of an intermediate chronic disease and also by using baseline diet and using the most recent measure of diet instead of repeated measures. We conducted tests for linear trend by assigning the median value to each quintile and treating this as a continuous variable in the regression model. Potential effect modification by age, BMI, physical activity, diet quality as assessed by the Alternate Healthy Eating Index (24), and family history of diabetes was evaluated using cross-product terms based on median protein intake and respective binary stratification variables.

We also estimated the association between substituting vegetable protein for an equal exchange of animal protein and T2D risk and simulated the isocaloric substitution of dietary protein for total carbohydrate, carbohydrate from food sources with low or medium glycemic index values (including intact and milled whole grains), and carbohydrate from food sources with high glycemic index values (including refined grains, potatoes, and added sugar). To fit these models, we simultaneously included total energy, percentage of energy derived from protein, and the substitution macronutrient of interest as

Table 3. Continued

Characteristic	Quintile of Protein Intake					
	1 (*n* = 7,921) (14.7% of Energy)[a]		3 (*n* = 7,921) (18.0% of Energy)		5 (*n* = 7,921) (21.9% of Energy)	
	%	Mean	%	Mean	%	Mean
Vitamin C, mg/day		389.9		426.0		493.1
Vitamin E, mg/day		82.5		91.2		116.9
Whole grains, servings/day		1.5		1.7		1.5
Fruit, servings/day		2.4		2.4		2.1
Vegetables, servings/day		2.7		3.1		3.3
Red meat, servings/day		0.62		0.82		0.78
Processed meat, servings/day		0.41		0.38		0.26
Fish, servings/day		0.24		0.38		0.66
Chicken, servings/day		0.33		0.54		0.87
Eggs, servings/day		0.29		0.34		0.34
Dairy foods, servings/day		1.7		2.0		1.9
Legumes, servings/day		0.37		0.43		0.45
Nuts, servings/day		0.51		0.49		0.34
Peanuts, servings/day		0.17		0.17		0.12
Peanut butter, servings/day		0.27		0.23		0.16
Potatoes, servings/day		0.43		0.43		0.35
Coffee, servings/day		2.0		2.0		1.8
Sugar-sweetened beverages, servings/day		0.68		0.30		0.14

Abbreviations: GI, glycemic index; MET, metabolic equivalent.

[a] Median percentage of energy intake in the quintile.

[b] Not adjusted for age.

[c] Weight (kg)/height (m)^2.

[d] First-degree relatives.

[e] Average glycemic load was calculated by multiplying the amount of carbohydrates in the diet by the average glycemic index. For 1 serving of a food, a glycemic load of ≥20 is considered high, 11–19 is considered medium, and ≤10 is considered low. Among these 40,722 men from the Health Professionals Follow-up Study, the mean glycemic load was 128.3 (range, 6–263).

[f] Average dietary glycemic index (GI) was calculated by summing the products of 1) the carbohydrate content of each food item per serving, 2) the average daily number of servings of that food, and 3) the food's GI value (derived from available databases and publications) and dividing by total daily carbohydrate content. Foods with a GI value of ≤55 are considered to have a low GI, foods with a value of 56–69 are considered to have a medium GI, and foods with a value of ≥70 are considered to have a high GI. Among these 40,722 men from the Health Professionals Follow-up Study, the mean GI was 53.1 (range, 15.4–72.4).

continuous variables along with the covariates listed above, except for total fiber and glycemic index. Coefficients were multiplied by 5 to estimate the association of substituting 5% of energy intake. We also investigated the association between substituting 1 serving of foods rich in vegetable protein (composite variable comprised of legumes, peanuts, peanut butter, other nuts, and whole grains) for 1 serving of major food sources of animal protein, refined grains, and potatoes and T2D risk by simultaneously modeling all terms as continuous variables (servings/day) in the same multivariate model, adjusted for the nondietary covariates listed above and intakes of total energy, alcohol, sugar-sweetened beverages, fruit, and vegetables (quintiles). For all substitution models, hazard ratios and 95% confidence intervals were estimated using the difference between coefficients for 2 foods or macronutrients of interest and their variance and covariance (6). All analyses were conducted separately in each cohort, and results were then combined using fixed-effects meta-analysis. All statistical tests were 2-sided and performed using SAS, version 9.2 for UNIX (SAS Institute, Inc., Cary, North Carolina).

RESULTS

During 4,146,216 person-years of follow-up among 205,802 participants (72,992 from NHS, 92,088 from NHS II, and 40,722 from HPFS), we documented 15,580 cases of T2D (7,214 in NHS, 5,032 in NHS II, and 3,334 in HPFS). Mean percentages of energy intake from total, animal, and vegetable protein were 18.1%, 15.1%, and 5%, respectively, in the NHS; 18.9%, 13.7%, and 7.3% in the NHS2; and 18.2%, 13.0%, and 5.1% in the HPFS. Intake of protein was fairly stable across FFQ cycles in the 3 cohorts, and animal

Table 4. Hazard Ratios for the Association Between Protein Intake and Risk of Type 2 Diabetes in the Nurses' Health Study (1984–2008), Nurses' Health Study II (1991–2009), and the Health Professionals Follow-up Study (1986–2008)

Cohort and Quintile of Protein Intake	Median Intake, % of Energy	No. of Cases	No. of Person-Years	Age-Adjusted HR	95% CI	Multivariate-Adjusted HR[a]	95% CI	Multivariate- and BMI[b]-Adjusted HR[c]	95% CI
Total Protein									
NHS									
1	14.8	1,220	309,146	1.00		1.00		1.00	
2	16.7	1,248	309,248	1.02	0.94, 1.10	1.04	0.96, 1.13	0.99	0.91, 1.07
3	18.0	1,408	309,052	1.15	1.06, 1.24	1.16	1.06, 1.25	1.04	0.96, 1.13
4	19.4	1,565	308,986	1.27	1.18, 1.37	1.27	1.17, 1.39	1.08	0.99, 1.17
5	21.6	1,773	308,742	1.43	1.33, 1.54	1.38	1.26, 1.51	1.05	0.95, 1.15
P for trend				<0.001		<0.001		0.14	
NHS II									
1	15.3	857	354,913	1.00		1.00		1.00	
2	17.4	839	355,439	0.98	0.89, 1.08	0.99	0.89, 1.09	0.92	0.83, 1.02
3	18.8	927	355,299	1.08	0.99, 1.19	1.07	0.97, 1.19	0.91	0.82, 1.01
4	20.2	1,062	354,884	1.23	1.12, 1.34	1.19	1.07, 1.32	0.96	0.86, 1.07
5	22.6	1,347	353,537	1.53	1.41, 1.67	1.45	1.30, 1.62	1.03	0.92, 1.15
P for trend				<0.001		<0.001		0.24	
HPFS									
1	14.7	596	165,260	1.00		1.00		1.00	
2	16.7	567	165,866	0.95	0.84, 1.06	0.90	0.80, 1.01	0.87	0.77, 0.98
3	18.0	614	165,809	1.02	0.91, 1.14	0.95	0.84, 1.07	0.90	0.80, 1.02
4	19.5	679	165,606	1.12	1.00, 1.25	1.02	0.90, 1.15	0.93	0.83, 1.06
5	21.9	878	164,429	1.45	1.30, 1.61	1.35	1.19, 1.53	1.18	1.04, 1.34
P for trend				<0.001		<0.001		0.001	
Pooled									
1						1.00		1.00	
2						0.99	0.94, 1.05	0.94	0.89, 0.99
3						1.08	1.02, 1.14	0.97	0.92, 1.03
4						1.19	1.20, 1.26	1.01	0.95, 1.07
5						1.39	1.31, 1.48	1.07	1.01, 1.17
P for trend						<0.001		0.001	

Table continues

protein contributed to the majority of total protein intake (see Web Figure 1, available at http://aje.oxfordjournals.org/). Major food sources of animal and vegetable protein are listed in Web Table 1. For all cohorts, participants who had a higher percentage of energy derived from protein as compared with a lower percentage were less likely to smoke, tended to have higher BMIs, and were more likely to be overweight, to be physically active, and to have a family history of diabetes (Tables 1–3). They also had a lower total energy intake, a lower percentage of energy from carbohydrate, lower intakes of sugar-sweetened beverages, processed meat, and nuts, and a lower glycemic index. Similar trends were noted for baseline characteristics according to quintile of percentage of energy from animal protein (Web Table 2). In contrast, persons with higher intakes of percentage of energy from vegetable protein tended to have a lower BMI and were less likely to be overweight. They also had higher glycemic index values and higher intakes of whole grains, legumes, peanuts, fruits, and vegetables (Web Table 3).

A higher intake of percentage of energy from total protein was associated with a higher risk of T2D in age- and multivariate-adjusted models across all 3 cohorts (all *P*'s for trend < 0.0001) (Table 4). After further adjustment for BMI, associations were attenuated and no longer statistically significant in the NHS (*P* for trend = 0.14) and NHS II (*P* for trend = 0.24) but remained statistically significant in the HPFS (*P* for trend = 0.001). In the pooled analysis of the 3 cohorts, persons in the highest quintile of intake compared with the lowest quintile had a 7% increased risk of T2D after adjustment for BMI (comparing extreme quintiles, hazard ratio (HR) = 1.07, 95% confidence interval (CI): 1.01, 1.17; *P* for trend = 0.001). Estimates were also attenuated but remained statistically significant after adjustment for red and processed meat and heme iron (Web Table 4). Adjusting

Table 4. Continued

Cohort and Quintile of Protein Intake	Median Intake, % of Energy	No. of Cases	No. of Person-Years	Age-Adjusted HR	95% CI	Multivariate-Adjusted HR[a]	95% CI	Multivariate- and BMI[b]-Adjusted HR[c]	95% CI
Animal Protein									
NHS									
1	9.7	1,158	309,267	1.00		1.00		1.00	
2	11.6	1,233	309,319	1.07	0.98, 1.15	1.06	0.97, 1.15	0.99	0.91, 1.08
3	12.9	1,383	309,141	1.19	1.10, 1.29	1.16	1.06, 1.26	1.03	0.95, 1.12
4	14.4	1,565	309,024	1.35	1.25, 1.46	1.27	1.17, 1.38	1.06	0.97, 1.16
5	16.8	1,875	308,423	1.61	1.50, 1.74	1.43	1.31, 1.57	1.08	0.99, 1.19
P for trend				<0.001		<0.001		0.04	
NHS II									
1	9.9	722	355,405	1.00		1.00		1.00	
2	12.0	876	355,377	1.23	1.12, 1.36	1.15	1.04, 1.28	1.03	0.92, 1.14
3	13.5	931	355,474	1.31	1.19, 1.44	1.17	1.05, 1.31	0.97	0.87, 1.09
4	15.1	1,106	354,678	1.55	1.41, 1.70	1.35	1.20, 1.51	1.03	0.92, 1.16
5	17.6	1,397	353,138	1.94	1.77, 2.12	1.62	1.44, 1.83	1.11	0.98, 1.25
P for trend				<0.001		<0.001		0.05	
HPFS									
1	9.4	515	165,717	1.00		1.00		1.00	
2	11.4	567	165,969	1.11	0.98, 1.25	1.00	0.88, 1.13	0.96	0.85, 1.09
3	12.8	610	165,831	1.19	1.05, 1.33	1.02	0.90, 1.16	0.96	0.85, 1.10
4	14.4	731	165,387	1.41	1.26, 1.58	1.18	1.03, 1.34	1.06	0.93, 1.21
5	17.0	911	164,067	1.77	1.59, 1.97	1.46	1.27, 1.68	1.27	1.11, 1.46
P for trend				<0.001		<0.001		<0.001	
Pooled									
1						1.00		1.00	
2						1.07	1.01, 1.13	0.99	0.94, 1.05
3						1.13	1.07, 1.20	1.00	0.94, 1.06
4						1.27	1.20, 1.35	1.05	0.99, 1.12
5						1.49	1.40, 1.59	1.13	1.06, 1.21
P for trend						<0.001		<0.001	

Table continues

for branched chain and aromatic amino acids also attenuated associations (Web Table 4).

Associations with percentage of energy derived from animal protein were stronger than those observed for total protein and persisted after further adjustment for BMI across all cohorts (all P's for trend < 0.05). Comparing extreme quintiles from the pooled analysis, the hazard ratio was 1.13 (95% CI: 1.06, 1.21; P for trend < 0.0001). Percentage of energy from vegetable protein was associated with decreased risk of T2D in age-adjusted models (P for trend < 0.0001), but associations were attenuated after further adjustment for lifestyle and dietary factors. In the pooled analyses, the association persisted (comparing extreme quintiles from the fully adjusted model including BMI, HR = 0.91, 95% CI: 0.84, 0.98; P for trend = 0.01).

Substituting 5% of energy intake from total and animal protein for an equal exchange of total carbohydrate was not associated with risk of T2D, while substitution with vegetable protein was associated with reduced risk (HR = 0.78, 95% CI: 0.71, 0.86; P < 0.001) (Table 5). Similar estimates were observed after making substitutions for carbohydrate from refined grains, potatoes, and added sugar. In contrast, substituting total and animal protein for carbohydrate from whole grains was associated with 20% (95% CI: 14, 28) and 18% (95% CI: 11, 25) increased risks of T2D, respectively (P < 0.001), while substitution with vegetable protein was not associated with risk of T2D. Substituting vegetable protein for animal protein was associated with a 23% reduced risk of T2D (HR = 0.77, 95% CI: 0.70, 0.84; P < 0.001). Substituting 1 serving per day of vegetable protein foods for an equal exchange of animal protein foods, refined grains, or potatoes was associated with reduced T2D risks ranging from 6% for refined grains to 21% for processed meat (Figure 1). The substitution for dairy foods was not statistically significant. In our

Table 4. Continued

Cohort and Quintile of Protein Intake	Median Intake, % of Energy	No. of Cases	No. of Person-Years	Age-Adjusted HR	95% CI	Multivariate-Adjusted HR[a]	95% CI	Multivariate- and BMI[b]-Adjusted HR[c]	95% CI
Plant Protein									
NHS									
1	3.9	1,698	308,253	1.00		1.00		1.00	
2	4.6	1,551	308,928	0.91	0.85, 0.97	0.96	0.89, 1.04	0.95	0.88, 1.02
3	5.0	1,428	309,125	0.83	0.77, 0.89	0.94	0.86, 1.02	0.91	0.84, 0.99
4	5.4	1,335	309,343	0.77	0.72, 0.83	0.93	0.85, 1.02	0.89	0.81, 0.97
5	6.1	1,202	309,525	0.69	0.64, 0.74	0.96	0.86, 1.06	0.91	0.82, 1.02
P for trend				<0.001		0.34		0.05	
NHS II									
1	4.0	1,109	348,121	1.00		1.00		1.00	
2	4.7	1,061	356,756	0.82	0.76, 0.89	0.92	0.84, 1.00	0.95	0.87, 1.04
3	5.1	941	358,050	0.73	0.68, 0.80	0.89	0.80, 0.99	0.92	0.92, 1.02
4	5.6	959	356,832	0.70	0.65, 0.76	0.93	0.83, 1.04	0.95	0.85, 1.06
5	6.6	962	354,313	0.53	0.48, 0.58	0.85	0.75, 0.98	0.90	0.79, 1.04
P for trend				<0.001		0.03		0.12	
HPFS									
1	3.9	833	163,820	1.00		1.00		1.00	
2	4.6	710	165,172	0.85	0.77, 0.94	0.91	0.82, 1.02	0.93	0.93, 1.04
3	5.1	644	165,617	0.76	0.69, 0.84	0.87	0.77. 0.98	0.87	0.77, 0.99
4	5.6	637	166,037	0.74	0.67, 0.82	0.93	0.81, 1.07	0.96	0.84, 1.10
5	6.6	510	166,324	0.59	0.53, 0.66	0.88	0.75, 1.04	0.91	0.77, 1.07
P for trend				<0.001		0.20		0.34	
Pooled									
1						1.00		1.00	
2						0.94	0.89, 0.99	0.94	0.90, 0.99
3						0.91	0.86, 0.96	0.91	0.85, 0.96
4						0.93	0.88, 0.99	0.92	0.86, 0.98
5						0.91	0.85, 0.98	0.91	0.84, 0.98
P for trend						0.01		0.01	

Abbreviations: CI, confidence interval; BMI, body mass index; HPFS, Health Professionals Follow-up Study; HR, hazard ratio; NHS, Nurses' Health Study; NHS II, Nurses' Health Study II.

[a] Adjusted for family history of diabetes, smoking, alcohol intake, physical activity, race/ethnicity, total energy intake, postmenopausal hormone use (NHS, NHS II), oral contraceptive use (NHS II), percentages of energy from *trans*- fat, saturated fat, monounsaturated fat, and polyunsaturated fat, dietary cholesterol, dietary fiber, and glycemic index. Results were mutually adjusted for percentage of energy derived from animal protein and vegetable protein.

[b] Weight (kg)/height (m)^2.

[c] Adjusted for family history of diabetes, smoking, alcohol intake, physical activity, race/ethnicity, total energy intake, postmenopausal hormone use (NHS, NHS II), oral contraceptive use (NHS II), percentages of energy from *trans*- fat, saturated fat, monounsaturated fat, and polyunsaturated fat, dietary cholesterol, dietary fiber, and glycemic index. Results were mutually adjusted for percentage of energy derived from animal protein and vegetable protein + BMI.

cohorts, whole grains and peanuts and peanut butter were the most commonly consumed major food sources of vegetable protein. Substituting these individual vegetable protein foods for animal protein foods, refined grains, and potatoes yielded similar reductions in T2D as those reported above (Web Figure 2).

In stratified analysis, associations between total and animal protein and T2D were greater among participants with BMI <30 compared with those with BMI ≥30 (*P* for interaction < 0.001) (Web Table 5). No significant effect modification by age, physical activity, Alternate Healthy Eating Index score, or family history of T2D was observed. When we used baseline diet and most recent diet as our exposure to examine associations between protein intake and risk of T2D, our results were similar, and results remained largely unchanged when we continued to update diet after the occurrence of coronary heart disease, stroke, or cancer (not shown).

Table 5. Hazard Ratios for the Association Between Protein Intake and Risk of Type 2 Diabetes (Pooled Estimates) After Substitution of 5% of Energy From Protein for Equal Exchanges of Total and Different-Quality Carbohydrates and Substitution of Vegetable Protein for Animal Protein, Nurses' Health Study (1984–2008), Nurses' Health Study II (1991–2009), and Health Professionals Follow-up Study (1986–2008)

Substitution	Hazard Ratio[a]	95% Confidence Interval	*P* Value
Substitution for total carbohydrate			
Total protein	0.99	0.95, 1.02	0.37
Animal protein	0.99	0.96, 1.03	0.76
Vegetable protein	0.78	0.71, 0.86	<0.001
Substitution for carbohydrate from intact and milled whole grains			
Total protein	1.20	1.14, 1.28	<0.001
Animal protein	1.18	1.11, 1.25	<0.001
Vegetable protein	1.02	0.90, 1.16	0.76
Substitution for carbohydrate from refined grains, potatoes, and added sugar			
Total protein	1.00	0.97, 1.04	0.90
Animal protein	1.01	0.98, 1.05	0.49
Vegetable protein	0.81	0.73, 0.89	<0.001
Substitution of vegetable protein for animal protein	0.77	0.70, 0.84	<0.001

[a] Adjusted for age, family history of diabetes, smoking, alcohol intake, physical activity, race/ethnicity, postmenopausal hormone use (Nurses' Health Study, Nurses' Health Study II), oral contraceptive use (Nurses' Health Study II), total energy intake, percentage of energy from fat, dietary cholesterol, and body mass index.

DISCUSSION

In these 3 large prospective cohort studies of US adults, we found that total protein intake was positively associated with risk of T2D, largely due to intake of animal protein. In contrast, intake of vegetable protein was moderately inversely associated with risk of T2D. Substitution of 5% of energy intake from vegetable protein for an equal exchange of animal protein and carbohydrate from refined grains, potatoes, and added sugar was associated with decreased risk of T2D. These findings suggest a benefit of replacing animal protein and low-quality carbohydrates with vegetable protein in regard to T2D risk, which was corroborated in our food substitution models. To our knowledge, this is the first study that has examined long-term intake of protein in relation to T2D risk using repeated measurements taken over many years of

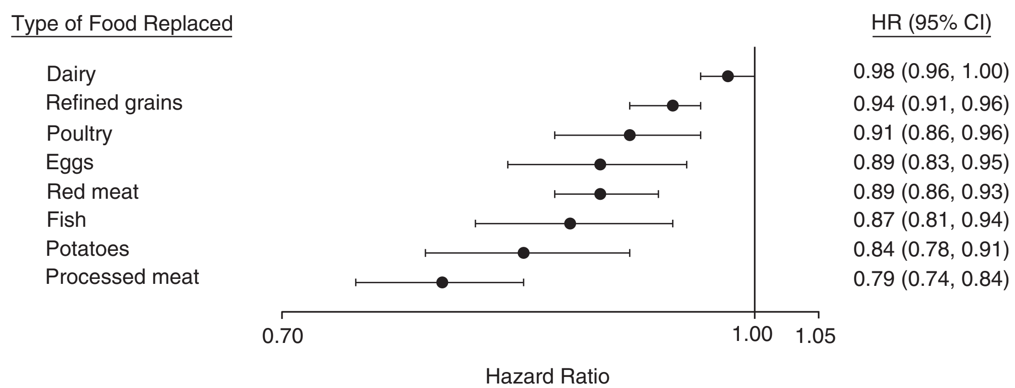

Figure 1. Pooled hazard ratios (HRs) and 95% confidence intervals (CIs) for type 2 diabetes associated with replacement of 1 serving of individual animal protein foods (dairy foods, poultry, eggs, red meat, and processed meat), refined grains, and potatoes with 1 serving of vegetable protein foods (composite variable comprised of whole grains, legumes, peanuts, peanut butter, and other nuts) in the Nurses' Health Study (1984–2008), Nurses' Health Study II (1991–2009), and the Health Professionals Follow-up Study (1986–2008). The models adjusted for age, family history of diabetes, smoking, alcohol intake, physical activity, race/ethnicity, total energy intake, postmenopausal hormone use (Nurses' Health Study, Nurses' Health Study II), oral contraceptive use (Nurses' Health Study II), intakes of sugar-sweetened beverages, fruit, and vegetables, and body mass index. Results were mutually adjusted for other food sources of animal protein, refined grains, and potatoes.

follow-up and that has examined the role of substitution of protein and protein type by carbohydrate type in T2D risk. This has important public health implications, since protein and carbohydrate are often exchanged for one another in the diet, and both type of protein and type of carbohydrate have been associated with T2D risk.

Our results support those of previous studies that have found positive associations between total and animal protein and risk of T2D. In the European Prospective Investigation into Cancer and Nutrition (EPIC)-InterAct Case-Cohort Study, there were 17% and 22% increased risks of T2D when comparing extreme quintiles of total protein intake and animal protein intake, respectively (3). Similar associations were reported in the Dutch cohort of EPIC-InterAct, although estimates were not statistically significant after adjustment for BMI and waist circumference (4). However, both of these studies used baseline protein intake only, which may underestimate associations, and were not able to assess longitudinal intake. Other epidemiologic studies (25–28) have also found positive associations between total or animal protein and risk of T2D, which is consistent with findings from mid- (29) and long-term (30) trials.

Similar to previous observational studies (3, 4, 25), in our analysis, estimates were attenuated after adjustment for BMI, and this was more evident in the NHS and NHS II, suggesting that body weight may partly mediate the association between total and animal protein and risk of T2D, particularly among women (21). However, BMI could be both a confounder and an intermediate factor, and it is difficult to know which is driving the attenuation. In contrast to our findings, van Nielen et al. (3) reported greater attenuation of estimates in men compared with women after adjusting for BMI and waist circumference. They also found that associations were stronger among women who were obese (3). In our analysis, associations between total and animal protein and T2D were stronger among participants who had a BMI less than 30. Similarly, Sluijs et al. (4) reported weaker associations with increasing BMI. It is unclear why associations were stronger in nonobese participants than in obese participants, but it is possible that the positive associations between protein and T2D risk are more pronounced among persons who are presumably more insulin-sensitive (1). Further studies are warranted to evaluate the role of BMI in this association.

Unlike previous studies that did not observe associations between vegetable protein intake and risk of T2D, we found a modest inverse association that remained statistically significant in the pooled analysis. This discrepancy may be due to differences in sources of vegetable protein across study populations. In our cohorts, the main sources of vegetable protein intake included whole grains, nuts, peanut butter, and beans, whereas in EPIC-InterAct the main sources of vegetable protein intake were bread, pasta, rice, and potatoes (3), which may contribute to a high dietary glycemic load. Diets high in glycemic load have been shown to increase risk of T2D (31).

Potential biological mechanisms supporting divergent associations of animal and vegetable protein with risk of T2D are unknown but may relate to different protein-rich food sources, co-occurrence of other nutrients in protein-rich foods, and variations in the amino acid composition of these foods. In our cohorts, intake of red and processed meat has been positively associated with weight gain (32) and with risk of T2D (6), coronary heart disease (33), stroke (34), and mortality (35). Various nutrients in red and processed meat, including heme iron, advanced glycation end products, and nitrites, are thought to mediate the association between meat intake and risk of T2D (6). In our analysis, adjusting for red and processed meat and heme iron attenuated the estimates, although they remained statistically significant, suggesting that they are partial mediators. Inconsistent findings have been reported for consumption of fish (36, 37), while intake of low-fat and fermented dairy products may be beneficial (38). In contrast, plant-based sources of protein, such as nuts (8), legumes (9), and whole grains (39), have been associated with a decreased risk of T2D. These foods have healthful nutritional profiles characterized by low glycemic index values and a high content of fiber and micronutrients. Nuts are also rich in monounsaturated and polyunsaturated fatty acids.

In a metabolomics study, Wang et al. (40) found strong positive associations between branched chain and aromatic amino acids and incident T2D. These amino acids have also been found to be associated with increased T2D risk (41) and represent the majority of amino acids entering circulation after consumption of red meat (42). In our analysis, adjustment for these amino acids attenuated associations between protein intake and risk of T2D, suggesting that they may be partial mediators.

Our study had important strengths and limitations. The large sample size, long duration of follow-up, and high response rate provided us with the statistical power to detect meaningful differences in estimates. We also used repeated measurements of diet, which better represents long-term dietary intake. Because diet was assessed using FFQs, some measurement error in assessment of protein intake was inevitable. However, given the prospective study design, any measurement error in protein intake was independent of outcome assessment and thus was more likely to attenuate associations. Although we adjusted for a number of potential confounders, the possibility of residual confounding cannot be dismissed and thus precludes inference of causation. Our study population primarily consisted of white health-care professionals, which may have helped reduce confounding by socioeconomic status but also limits the generalizability of these associations to other populations.

In conclusion, we found that greater intakes of total and animal protein were associated with a higher risk of T2D, while intake of vegetable protein had a modest inverse association. Substituting vegetable protein for animal protein and low-quality carbohydrates was associated with reduced risk of T2D. These data suggest that adopting a diet rich in plant-based proteins should be considered for T2D prevention. Confirmatory results from dietary intervention studies are warranted and will provide further support for dietary recommendations to increase intake of vegetable protein in place of animal protein.

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