Dietary Intake of Total, Animal, and Vegetable Protein and Risk of Type 2 Diabetes in the European Prospective Investigation into Cancer and Nutrition (EPIC)-NL Study

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OBJECTIVE — Dietary recommendations are focused mainly on relative dietary fat and carbohydrate content in relation to diabetes risk. Meanwhile, high-protein diets may contribute to disturbance of glucose metabolism, but evidence from prospective studies is scarce. We examined the association among dietary total, vegetable, and animal protein intake and diabetes incidence and whether consuming 5 energy % from protein at the expense of 5 energy % from either carbohydrates or fat was associated with diabetes risk.

RESEARCH DESIGN AND METHODS — A prospective cohort study was conducted among 38,094 participants of the European Prospective Investigation into Cancer and Nutrition (EPIC)-NL study. Dietary protein intake was measured with a validated food frequency questionnaire. Incident diabetes was verified against medical records.

RESULTS — During 10 years of follow-up, 918 incident cases of diabetes were documented. Diabetes risk increased with higher total protein (hazard ratio 2.15 [95% CI 1.77–2.60] highest vs. lowest quartile) and animal protein (2.18 [1.80–2.63]) intake. Adjustment for confounders did not materially change these results. Further adjustment for adiposity measures attenuated the associations. Vegetable protein was not related to diabetes. Consuming 5 energy % from total or animal protein at the expense of 5 energy % from carbohydrates or fat increased diabetes risk.

CONCLUSIONS — Diets high in animal protein are associated with an increased diabetes risk. Our findings also suggest a similar association for total protein itself instead of only animal sources. Consumption of energy from protein at the expense of energy from either carbohydrates or fat may similarly increase diabetes risk. This finding indicates that accounting for protein content in dietary recommendations for diabetes prevention may be useful.

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Many research efforts have focused on macronutrient intake in relation to type 2 diabetes risk (1,2), but mainly on relative carbohydrate and fat content. Effects of various protein consumption are less well documented. Both Dutch and U.S. nutritional recommendations provide information on optimal dietary protein content for diabetic patients (3,4), but dietary protein content in relation to diabetes prevention received little attention (3).

In industrialized countries, dietary protein intake has increased substantially during the last few decades, exceeding 50% of the recommended dietary allowance (5). Moreover, popular weight loss diets, such as the Atkins diet, are often based on extreme low-carbohydrate, high-protein contents with favorable effects on body weight and glucose homeostasis in short-term interventions (6,7). In contrast, a cross-sectional study related long-term high-protein intake to elevated glucose concentrations and insulin resistance in healthy individuals (8).

Prospective studies addressing dietary protein and diabetes risk focused mainly on high-protein food groups, such as meat and soy. Red processed meat intake was related to increased diabetes risk, independent of fat intake (9–12), whereas intake of legumes and soy decreased diabetes risk in Asian women (13), suggesting divergent effects of animal and vegetable protein. Studies examining the relation between dietary protein and diabetes are scarce. One study reported increased diabetes risk with higher animal protein intake and no association with vegetable protein intake (11). Under isocaloric conditions, higher protein intakes will lead to lower intakes of other macronutrients, which can be investigated using substitution models in which other macronutrients are substituted for protein (14). The European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam study related consumption of 5 energy % from carbohydrate at the expense of 5 energy % from protein with decreased diabetes risk (1). However, the Nurses Health Study II did not find such an association (15). Both studies made no distinction between animal and vegetable protein. The response to dietary protein content may be dependent on an individual's degree of underlying insulin resistance (6,7), determined by adiposity.

We aimed to investigate whether higher dietary intakes of total, animal, and vegetable protein were associated with type 2 diabetes risk and whether consumption of energy from protein at the expense of the same energy percentage from fat or carbohydrate was associated with type 2 diabetes risk. Moreover,

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we examined whether an interaction with measures of adiposity was present.

RESEARCH DESIGN AND METHODS

— EPIC-NL consists of the two Dutch contributions to the EPIC study, the Prospect-EPIC and MORGEN-EPIC cohorts. These cohorts were set up simultaneously in 1993–1997 and merged into one Dutch EPIC cohort. The design and rationale of EPIC-NL are described elsewhere (16). The Prospect-EPIC study includes 17,357 women aged 49–70 years living in Utrecht and vicinity (17). The MORGEN-EPIC cohort consists of 22,654 adults aged 21–64 years selected from random samples of the Dutch population in three Dutch towns (18). All participants provided informed consent before study inclusion. The study complies with the Declaration of Helsinki and was approved by the institutional board of the University Medical Center Utrecht (Prospect) and the Medical Ethical Committee of TNO Nutrition and Food Research (MORGEN). After exclusion of those with prevalent diabetes ($n = 615$) and of individuals with abnormal energy intake (kcal <600 or $>5,000$) ($n = 108$), missing nutritional data ($n = 213$), and missing follow-up ($n = 981$), 38,094 participants were left for analysis.

Intake of protein and other nutrients

Daily nutritional intake was obtained from a self-administered food-frequency questionnaire (FFQ) containing questions on the usual frequency of consumption of 79 main food items during the year preceding enrollment. This questionnaire allows estimation of the average daily consumption of 178 foods. The FFQ was administered once at baseline and sent to the participants by mail. Participants returned the FFQ during the physical examination screening, where difficulties in filling out the questionnaire were discussed. A registered dietitian checked the FFQ for inconsistencies, which were resolved by contacting the participant. The FFQ has been validated against 12 24-h dietary recalls (19) with Pearson correlation coefficients for protein intake of 0.67 in women and 0.71 in men (19). The glycemic index of foods, a measure of the extent to which foods raise the blood glucose level, was obtained from the Foster-Powell international table. We calculated glycemic load by multiplying the glycemic index of a food with its carbohydrate content and then multiplied this value by the frequency of consumption of this food and summed the values over all food items. Intakes of nutrients were adjusted for total energy intake by the regression residual method and by using nutrient densities (percentage of total energy intake, only for macronutrients) (14).

Diabetes

Occurrence of diabetes during follow-up was self-reported in two follow-up questionnaires with 3- to 5-year intervals. Participants were asked whether diabetes was diagnosed, in what year, and by whom and what treatment was received. In the Prospect study, incident cases of diabetes were detected via a urinary glucose strip test, sent out with the first follow-up questionnaire, for detection of glucosuria. Diagnoses of diabetes were also obtained from the Dutch Centre for Health Care Information, which holds a standardized computerized register of hospital discharge diagnoses. In this register, admission files have been entered continuously from all general and university hospitals in the Netherlands from 1990 onward. All diagnoses were coded according to the ICD-9-CM. Follow-up was complete on 1 January 2006. Potential cases identified by any of these methods were verified against participants' general practitioner or pharmacist information through mailed questionnaires. Diabetes was defined as being present when either of these confirmed the diagnosis. For 89% of participants with potential diabetes, verification information was available, and 72% were verified as having type 2 diabetes and were used for the analysis.

Other measurements

At baseline, participants filled in a mailed general questionnaire containing questions on demographics, the presence of chronic diseases, and risk factors for chronic diseases. Smoking was categorized into current, past, and never smoker and parental history of diabetes into none, one, and both parents. Physical activity was assessed using a questionnaire validated in an elderly population (20) and categorized after calculating the Cambridge Physical Activity Score. Because we could not calculate a total physical activity score for 14% of all participants, we imputed missing scores using single linear regression modeling. Participants could return the questionnaire at the physical examination screening. During the baseline physical examination screening, systolic and diastolic blood pressure measurements were performed twice in the supine position on the right arm using a Boso Oscillomat (Bosch & Son, Jungingen, Germany) (Prospect) or on the left arm using a random zero sphygmomanometer (MORGEN), from which the mean was taken. Hypertension was defined as being present when one or more of the following criteria were met: diastolic blood pressure $\geq$90 mmHg, systolic blood pressure $\geq$140 mmHg, self-reported antihypertensive medication use, or self-reported presence of hypertension. Waist circumference, height, and weight were measured, and BMI was calculated. All measurements were performed according to standard operating procedures. Weight during follow-up was derived from mailed follow-up questionnaires or physical examination (Doetinchem part). Weight change was defined as the difference between weight at baseline and follow-up. Because the follow-up period varied, we calculated annual weight change by dividing weight change by the years of follow-up.

Data analysis

Protein intake, adjusted for total energy intake by the regression residual method (14), was categorized into quartiles. Cox proportional hazard models were used to calculate crude and adjusted hazard ratios (HRs) and 95% CI for the associations between quartiles of protein intake and diabetes. We estimated P_{trend} by including median protein intakes per quartile as continuous variables in the Cox regression models. In addition, we analyzed associations between protein per 10 g of intake and diabetes risk. In the multivariate analysis, we first included sex (male or female) and age at recruitment (continuous). In the second model, we added nutritional factors: energy-adjusted intake of saturated fat, monounsaturated fat, polyunsaturated fat, cholesterol, vitamin E, magnesium, fiber, and glycemic load (continuous). In the third model, we additionally corrected for diabetes risk factors: energy-adjusted alcohol consumption (four categories), physical activity (four categories), mean systolic and diastolic blood pressure (continuous), education level (three categories), and parental history of diabetes (three categories). In the fourth model, BMI (four categories) and waist circumference (continuous) were included. To examine the influence of weight change during follow-up, we additionally corrected the analysis for annual weight change (continuous).

Table 1—*Baseline characteristics of the study population, according to quartiles of daily nutritional total protein intake*

	Quartile 1 (low)	Quartile 2	Quartile 3	Quartile 4 (high)
Participants	9,523	9,524	9,524	9,523
Male sex	2,643 (27.8)	2,685 (28.2)	2,450 (25.7)	1,962 (20.6)
Age (years)	48 ± 12	48 ± 12	50 ± 12	51 ± 11
Energy intake (kcal/day)	2,036 ± 645	2,106 ± 618	2,078 ± 602	1,998 ± 622
Animal protein intake (g/day)	35.2 ± 7.0	44.5 ± 5.2	51.3 ± 5.2	62.9 ± 8.3
Vegetable protein intake (g/day)	27.0 ± 5.5	27.6 ± 4.8	27.6 ± 4.8	26.9 ± 4.7
Saturated fat intake (g/day)	31.2 ± 6.2	32.5 ± 5.6	33.0 ± 5.6	33.4 ± 5.9
Polyunsaturated fat intake (g/day)	15.3 ± 4.3	15.3 ± 3.8	14.9 ± 3.7	14.2 ± 3.6
Monounsaturated fat intake (g/day)	29.2 ± 5.5	29.7 ± 5.0	29.6 ± 5.0	29.2 ± 5.2
Cholesterol intake (mg/day)	190.0 ± 53.0	210.4 ± 51.1	223.4 ± 53.5	245.3 ± 64.2
Carbohydrate intake (g/day)	231.5 ± 34.3	223.7 ± 29.0	219.3 ± 28.2	213.6 ± 28.8
Glycemic load intake (g/day)	120.8 ± 24.7	114.7 ± 20.1	110.8 ± 18.9	106.0 ± 18.3
Fiber intake (g/day)	22.1 ± 5.2	23.2 ± 4.6	23.8 ± 4.6	24.2 ± 4.7
Vitamin C intake (mg/day)	101.4 ± 49.7	106.6 ± 43.1	111.6 ± 42.6	118.0 ± 44.0
Vitamin E intake (mg/day)	12.9 ± 3.6	12.5 ± 3.2	12.1 ± 3.0	11.5 ± 3.0
Magnesium intake (mg/day)	304.9 ± 46.2	327.2 ± 41.7	343.9 ± 40.9	365.9 ± 43.3
Iron intake (mg/day)	10.8 ± 1.6	11.3 ± 1.5	11.7 ± 1.6	12.1 ± 1.7
Heme iron intake (mg/day)	1.6 ± 0.7	1.9 ± 0.7	2.1 ± 0.7	2.5 ± 0.9
Alcohol intake (g/day)	15.8 ± 24.4	11.0 ± 15.9	9.7 ± 13.9	8.0 ± 12.6
BMI (kg/m^2)	24.7 ± 3.7	25.3 ± 3.7	25.8 ± 3.9	26.7 ± 4.3
Waist circumference (cm)	83.1 ± 11.2	84.6 ± 11.2	85.5 ± 11.2	87.1 ± 11.7
Current smoker	3,655 (38.4)	3,014 (31.7)	2,541 (26.8)	2,420 (25.5)
Physically inactive*	3,983 (41.8)	3,609 (37.9)	3,482 (36.6)	3,413 (35.9)
Higher education	1,993 (20.9)	2,104 (22.1)	2,030 (21.3)	1,703 (17.9)
Parental history of diabetes	1,559 (16.4)	1,615 (17.0)	1,767 (18.5)	1,922 (20.2)
Systolic blood pressure (mmHg)	124.3 ± 18.6	125.3 ± 18.8	126.6 ± 18.7	127.8 ± 19.0
Diastolic blood pressure (mmHg)	77.2 ± 10.6	77.5 ± 10.5	78.0 ± 10.6	78.4 ± 10.7
Hypertension	3,108 (32.6)	3,307 (34.7)	3,491 (36.7)	3,762 (39.5)

Data are *n* (%) or means ± SD. *Inactive according to Cambridge physical activity index.

We used a multivariate nutrient density model by including total energy intake and energy percentages of protein and other macronutrients in the multivariate Cox regression model. Macronutrient intakes were entered into the model per 5 energy %. Total energy intake was entered into the model to keep energy intake constant, essential for creating an isocaloric model (14). By leaving out energy percentages from carbohydrate in the regression model, we created a model in which the difference in diabetes risk associated with consumption of 5 energy % from protein at the expense of 5 energy % from carbohydrate, while total energy intake is kept constant, is presented. Similarly, by leaving out energy percentages from fat, we presented the difference in diabetes risk associated with consumption of 5 energy % from protein at the expense of 5 energy % from fat, while energy intake is held constant.

Interactions of protein intake with BMI (<25 or ≥25 kg/m^2) and waist circumference (<84 or ≥84 cm) were estimated using a likelihood ratio test and by including continuous interaction terms. The proportionality assumption was checked visually using log-minus-log plots, with no deviations detected. Data were analyzed using SPSS for Windows (version 14.0).

RESULTS — Mean protein intake was 75.7 g/day; animal protein accounted for the majority. The main contributors to protein intake were meat (39%), milk (products) (29%), and cheese (18%) for animal protein and bread (43%), fruit and vegetables (14%), and potatoes (9%) for vegetable protein. Moderate correlations were present between meat intake and total ($r = 0.30$) and animal ($r = 0.36$) protein, and between intake of milk (products) and total ($r = 0.46$) and animal ($r = 0.50$) protein. Over the quartiles of total protein intake, mean age, BMI, waist circumference, and intakes of saturated fat and carbohydrates increased, whereas mean intakes of polyunsaturated fat and fiber and percentages of men, smokers, and physically inactive individuals decreased (Table 1).

During a mean follow-up of 10.1 ± 1.9 (mean ± SD) years, 918 incident cases of type 2 diabetes were documented. Diabetes risk increased significantly over the quartiles of total protein intake. Adjustment for age, sex, dietary factors, and diabetes risk factors yielded an HR in the highest versus lowest quartile (HR_{Q4}) of 1.67 (95% CI 1.29–2.16). After further adjustment for adiposity measures, this association was no longer significant (HR_{Q4} 1.18 [0.91–1.53]) (Table 2). Removing either BMI or waist circumference from model 4 yielded comparable, nonsignificant associations (excluding BMI, HR_{Q4} 1.22 [0.94–1.59]). For animal protein, we observed similar results. Vegetable protein intake was not related to diabetes. Analyzing protein per 10 g of intake showed comparable results, with significantly increased diabetes risk for higher total and animal protein intake in all models (Table 2).

Adjustment for weight change did not change these findings (model 3, HR_{Q4} 1.67 [1.28–2.16]). Moreover, additional correction for meat and poultry intake in

Table 2—*Univariable and adjusted HRs (95% CI) for the association between intake of protein, in quartiles and per 10 g, and incident type 2 diabetes*

	Quartile 1 (low)	Quartile 2	Quartile 3	Quartile 4 (high)	P_{trend}	Per 10 g
Total protein						
Cases/at risk (*n*)	153/9,523	185/9,524	249/9,524	331/9,523		
Quartile median total protein (g/day)	64	72	79	88		
Univariable	1	1.20 (0.97–1.49)	1.63 (1.33–1.99)*	2.15 (1.77–2.60)*	<0.001	1.36 (1.28–1.44)*
Model 1†: age and sex	1	1.17 (0.94–1.45)	1.50 (1.23–1.84)*	1.85 (1.53–1.25)*	<0.001	1.28 (1.22–1.36)*
Model 2‡: model 1 + dietary intake	1	1.24 (0.99–1.54)	1.65 (1.32–2.07)*	2.16 (1.69–2.76)*	<0.001	1.45 (1.34–1.56)*
Model 3§: model 2 + diabetes risk factors	1	1.16 (0.92–1.45)	1.45 (1.15–1.83)*	1.67 (1.29–2.16)*	<0.001	1.33 (1.22–1.45)*
Model 4‖: model 3 + waist and BMI	1	1.03 (0.82–1.29)	1.20 (0.95–1.51)	1.18 (0.91–1.53)	0.15	1.16 (1.06–1.26)¶
Animal protein						
Cases/at risk (*n*)	155/9,523	182/9,524	243/9,524	338/9,523		
Quartile median animal protein (g/day)	35	44	52	62		
Univariable	1	1.16 (0.94–1.44)	1.56 (1.28–1.91)*	2.18 (1.80–2.63)*	<0.001	1.32 (1.25–1.39)*
Model 1†: age and sex	1	1.08 (0.87–1.33)	1.35 (1.10–1.65)¶	1.73 (1.43–2.10)*	<0.001	1.24 (1.17–1.30)*
Model 2‡: model 1 + dietary intake	1	1.17 (0.94–1.46)	1.54 (1.23–1.92)*	2.09 (1.64–2.67)*	<0.001	1.40 (1.30–1.51)*
Model 3§: model 2 + diabetes risk factors	1	1.09 (0.87–1.36)	1.31 (1.05–1.65)¶	1.58 (1.23–2.04)*	<0.001	1.28 (1.18–1.39)*
Model 4‖: model 3 + waist and BMI	1	0.99 (0.79–1.23)	1.11 (0.89–1.40)	1.14 (0.88–1.47)	0.22	1.13 (1.04–1.22)¶
Vegetable protein						
Cases/at risk (*n*)	245/9,524	228/9,524	235/9,523	210/9,523		
Quartile median vegetable protein (g/day)	22	26	29	33		
Univariable	1	0.92 (0.76–1.10)	0.95 (0.79–1.13)	0.84 (0.70–1.01)	0.10	0.87 (0.76–0.99)¶
Model 1†: age and sex	1	0.94 (0.79–1.13)	1.02 (0.85–1.22)	1.02 (0.85–1.23)	0.64	1.01 (0.88–1.15)
Model 2‡: model 1 + dietary intake	1	0.89 (0.73–1.08)	0.96 (0.77–1.19)	0.91 (0.70–1.19)	0.63	0.85 (0.69–1.06)
Model 3§: model 2 + diabetes risk factors	1	0.95 (0.78–1.16)	1.03 (0.83–1.27)	1.05 (0.80–1.37)	0.63	0.97 (0.78–1.20)
Model 4‖: model 3 + waist and BMI	1	0.99 (0.82–1.21)	1.11 (0.89–1.38)	1.15 (0.88–1.50)	0.23	1.04 (0.83–1.29)

Data are HRs (95% CI). *Significant at $P < 0.001$ level. †Model 1: Corrected for sex (male or female) and age at recruitment (continuous). ‡Model 2: model 1 + energy-adjusted intake of saturated fat, monounsaturated fat, polyunsaturated fat, cholesterol, vitamin E, magnesium, fiber, and glycemic load (continuous). §Model 3: model 2 + energy-adjusted alcohol consumption (<11, 11–25, 26–50, or >50 g/day), physical activity (not active, moderately inactive, moderately active, or active), mean systolic and diastolic blood pressure (continuous), education level (high, middle, or low), and parental history of diabetes (no, one parent, or both parents). ‖Model 4: model 3 + BMI (<20, 20–25 [reference group], 25–30, or >30 kg/m^2), and waist circumference (continuous). ¶Significant at $P < 0.05$ level.

model 3 did not substantially change associations for either total or animal protein (1.50 [1.14–1.98]) nor did adjustment for dairy intake (1.62 [1.24–2.11]). Excluding participants who followed a diet did not change the results (model 3, total protein 1.51 [1.11–2.06]) nor did exclusion of participants with baseline cardiovascular disease, hypertension, or hyperlipidemia (1.68 [1.17–2.43]).

Consumption of 5 energy % from protein at the expense of 5 energy % from fat increased diabetes risk, with an HR of 1.31 (95% CI 1.06–1.61) for each 5 energy % from protein exchanged for 5 energy % from fat in the final model. For consuming 5 energy % from protein at the expense of 5 energy % from carbohydrate, we observed an HR of 1.28 (1.01–1.61) in the final model. Similar results were observed for animal protein. We observed no associations with consuming 5 energy % from vegetable protein (Table 3).

We observed borderline significant interactions with BMI and waist circumference ($P_{interaction} = 0.08$ for both) in the relation between total protein and diabetes. For lean individuals, diabetes risk increased with increasing total protein intake (HR_{Q4} 2.15 [95% CI 1.24–3.15] and 2.36 [1.30–4.29] for low BMI and waist circumference groups, respectively), whereas there was no relation in obese participants. Similar results were found for animal protein. In addition, similar results were obtained when these interactions were analyzed continuously ($P_{interaction} < 0.05$). Correction for annual weight change did not change the associations (low BMI group, total protein, HR_{Q4} 2.16 [1.25–3.75]).

CONCLUSIONS — In this study, high total and animal protein intake, but

Table 3—*Multivariable HRs (95% CI) for the association between the consumption of 5% energy from protein at the expense of 5% energy from fat or carbohydrates while keeping total energy intake constant and incident type 2 diabetes*

	Model 3*	Model 4†
Total protein		
Substitution protein for fat	1.72 (1.41–2.12)‡	1.31 (1.06–1.61)§
Substitution protein for carbohydrates	1.91 (1.52–2.40)‡	1.28 (1.01–1.61)§
Animal protein		
Substitution protein for fat	1.51 (1.26–1.82)‡	1.19 (0.99–1.44)
Substitution protein for carbohydrates	1.72 (1.39–2.12)‡	1.20 (0.97–1.49)
Vegetable protein		
Substitution protein for fat	1.13 (0.67–1.92)	1.32 (0.82–2.13)
Substitution protein for carbohydrates	0.97 (0.57–1.65)	1.17 (0.73–1.89)

Data are HRs (95% CI). *Model 3: corrected for sex (male or female), age at recruitment (continuous), energy-adjusted intake of cholesterol, vitamin E, magnesium, fiber, and glycemic load (continuous), total energy intake (continuous), energy densities of fat, carbohydrates, and alcohol (per 5 energy %), physical activity (not active, moderately inactive, moderately active, or active), mean systolic and diastolic blood pressure (continuous), education level (high, middle, or low), and parental history of diabetes (no, one parent, or both parents). †Model 4: model 3 + BMI (<20, 20–25 [reference group], 25–30, or >30 kg/m^2) and waist circumference (continuous). ‡Significant at $P < 0.001$ level. §Significant at $P < 0.05$ level.

not vegetable protein intake, was associated with increased diabetes risk. This relation was not explained by specific protein sources such as meat or by weight change during follow-up but was attenuated after adjustment for baseline adiposity measures. Consuming 5 energy % from protein at the expense of 5 energy % from carbohydrate or fat increased diabetes risk by ~30%.

Some aspects of the study need to be addressed. First, although we corrected for all possible available confounders, we cannot exclude unknown or unmeasured confounding. Second, the presence of diabetes goes often undetected and may be preclinical up to 9–12 years (21). Individuals with undetected diabetes may have been misclassified as nondiabetic individuals, resulting in attenuated associations. Strengths of our study include its prospective design, large sample size, and long follow-up. Use of validated cases of diabetes minimized the presence of false-positive cases of diabetes, reducing dilution of associations.

Thus far, it is unclear whether a potential harmful effect of protein on diabetes is caused by high protein sources, such as meat, or by protein per se. Several studies related higher red, mainly processed, meat intake with increased diabetes risk (2,9–12). When corrections for fat intake were made, associations remained (9–11), indicating that the association is not caused by fat intake. However, as most studies did not further address which nutrients were responsible for the increased diabetes risk with high meat intake, one cannot conclude whether it is the protein or other nutrients in meat, such as iron, that promoted diabetes risk. Only one prospective study in women further investigated which nutrients in meat (several types of fat and protein, heme, and total iron) could promote diabetes (11). These researchers observed no relationship with vegetable protein, consistent with our study. Animal protein intake significantly increased diabetes risk. After correction for BMI, this association attenuated but remained significant, in contrast with our findings. Differences in study population and range of protein intake might explain this difference. Unfortunately, the study did not address total protein intake.

We observed that both high total and animal protein were associated with higher diabetes risk. Fat intake did not change much over the quartiles of protein intake, and the association was not altered after correction for fat intake. Moreover, after correction for meat or dairy intake, the association between total and animal protein and diabetes remained, suggesting a detrimental role for protein per se in diabetes risk. This association is further supported by the finding that consuming energy from protein at the expense of energy from either fat or carbohydrate increased diabetes risk. We found no difference in risk when energy from protein was consumed at the expense of carbohydrate or fat, suggesting that the increase in protein itself and not the decrease in fat or carbohydrate caused this effect. Only one previous study, which focused on consuming carbohydrate at the expense of protein, reported similar findings (1). Yet, in that study, exchanging energy from protein for fat was not accounted for, and no differentiation was made for total protein content and protein source.

Because the majority of protein intake in our study is from animal sources, one might think the association with total protein is merely driven by the association with animal protein. However, when we corrected the association between total protein and diabetes for animal protein, the association attenuated but remained (model 3, HR_{Q4} 1.46 [0.96–2.25]). Similarly, adjusting total protein for several sources of animal protein intake, such as meat, did not explain the entire association. This finding indicates that part of the association between total protein and diabetes indeed seems to be explained by animal protein intake, but that a role for total protein cannot be excluded. For vegetable protein, we found an association in the same direction as that for animal protein, although this result did not reach statistical significance. Different effects of amino acids in animal and vegetable proteins on glucose metabolism may underlie the difference found between animal and vegetable protein (22,23). Further studies addressing the effect of total protein intake in populations with differing intakes of protein sources are needed to establish the effects of total protein intake and specific protein sources on diabetes risk.

Several mechanisms may explain the relationship between protein intake and diabetes. Insulin resistance may arise, as amino acids can inhibit glucose transport and phosphorylation, leading to impaired glucose synthesis. Furthermore, amino acids intervene with glucose metabolism via stimulation of insulin and glucagon secretion and by serving as substrates for gluconeogenesis. Although stimulation of insulin secretion is expected to prevent hyperglycemia due to increased gluconeogenesis, this process might not sufficiently compensate in subjects with impaired insulin secretion (6,7).

An individual's degree of insulin sensitivity is determined by the degree of adiposity. We therefore investigated whether adiposity modified the relation between protein intake and diabetes. In contrast with our hypothesis, we only found an association in lean individuals. In the EPIC-Potsdam study, a similar but nonsignificant interaction with adiposity

was observed (1). Several potential mechanisms may underlie our findings. First, iron metabolism may be involved. A recent study showed that soluble transferrin receptor was inversely associated with insulin sensitivity only in normal glucose tolerant and lean individuals, suggesting a mechanism through iron metabolism (24). Iron overload is associated with increased diabetes risk (25). Because increased animal protein intake may contribute to increased body iron load, the association between high (animal) protein intake and diabetes in the nonobese people might be (partly) explained markers of body iron load. However, markers of body iron load (serum ferritin, iron, total iron binding capacity, and transferrin saturation) could not explain this association in a random sample of our cohort (data not shown). Second, it is unlikely that weight gain during follow-up explains the increased diabetes risk in nonobese individuals, as correction for annual weight change did not change these findings. Third, the associations between protein and diabetes were largely attenuated after correction for adiposity measures, raising the possibility for adiposity to be an intermediate in this relationship. However, when we adjusted the association between protein intake and diabetes for BMI (continuous), the positive association in the lean group remained present, indicating that this possibility is unlikely. Finally, because of the direction of the interactions, borderline significance, and relatively few nonobese diabetic participants, we cannot exclude the possibility that these interactions are due to chance.

In summary, diets high in animal protein are associated with an increased risk of incident diabetes. Our findings also suggest a similar association for protein itself instead of only animal sources. The consumption of energy from protein, at the expense of the same percentage of energy from either fat or carbohydrate, increased diabetes risk by ~30%. More research into the effect of total protein intake in different populations with intakes of protein from different sources is needed to establish the effects of total protein intake and differing sources on diabetes risk. Yet, these results underline the importance of taking into account the protein content of diet in dietary recommendations to prevent diabetes.

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