

## **Dietary Intake of Total, Animal, and Vegetable Protein and Risk of Type 2 Diabetes in the EPIC-NL Study**

**Running title:** Dietary protein intake and diabetes risk

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*Objective-* Dietary recommendations are mainly focused 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 between 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 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 follow-up, 918 incident diabetes cases were documented. Diabetes risk increased with higher total protein (HR (95%CI) highest vs. lowest quartile: 2.15 (1.77-2.60)) 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 indicates that accounting for protein content in dietary recommendations for diabetes prevention may be useful.

Many research efforts have focused on macronutrient intake in relation to type 2 diabetes risk (1,2), but mainly on relative carbohydrate (CHO) and fat content. Effects of varying protein consumption are less well documented. Both Dutch and US nutritional recommendations provide information on optimal dietary protein content for diabetes 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 decades, exceeding 50% of RDA (5). Moreover, popular weight loss diets, like Atkins, are often based on extreme low-CHO, 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 mainly focused 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), while 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 protein is substituted by other macronutrients (14). The EPIC-Potsdam study related consumption of 5 energy% from CHO 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 individuals' 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 CHO was associated with type 2 diabetes risk. Moreover, we examined whether an interaction with measures of adiposity was present.

## **Research Design and Methods**

### **Study population**

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. Its design and rationale 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 signed 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 prevalent diabetes cases (n=615), individuals with abnormal energy intake (kcal < 600 or > 5000) (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 enrolment. 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 dietician checked the FFQ for inconsistencies, which were resolved by contacting the participant. The FFQ has been validated against 12 24-hour 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 CHO content, then multiplied this value with 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 (% of total energy intake, only for macronutrients) (14).

### **Diabetes**

Occurrence of diabetes during follow-up was self-reported in two follow-up questionnaires with 3-5 year intervals. Participants were asked whether diabetes was diagnosed, in what year, by whom, and what treatment they received. In Prospect, incident diabetes cases 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 filed continuously from all general and university hospitals in the Netherlands from 1990 onwards. All diagnoses were coded according to the International Classification of Diseases, Clinical Modification, ninth revision. Follow-up was complete until 1 January 2006. Potential diabetes cases notified by any of these methods were verified against participants' general practitioner or pharmacist information through mailed questionnaires. Diabetes was defined present when either of them confirmed the diagnosis. For 89% of potential diabetes cases verification information was available and 72% were verified as having type 2 diabetes, which were used for the analysis.

### **Other measurements**

At baseline, participants filled in a mailed general questionnaire containing questions on demographics, 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 supine position on the right arm using a Boso Oscillomat (Bosch & Son, Jungingen, Germany) (Prospect) or on the left arm using a random zero Sphygmomano-meter (MORGEN), from which the mean was taken. Hypertension was defined present when one or more of the following criteria were met: diastolic blood pressure  $\geq 90$  mm Hg, systolic blood pressure  $\geq 140$  mm Hg, 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 (HR) and 95%CI for the associations between quartiles of protein intake and diabetes. We estimated P-for trend by including the median protein intake per quartile as continuous variables in the Cox regression models. Additionally, we analyzed associations between protein per 10 g of intake and diabetes risk. In the multivariate analysis, we first included sex (male / female) and age at recruitment (continuous). In the second model we added nutritional factors (energy adjusted intake of saturated fat, monounsaturated fat (MUFA), polyunsaturated fat (PUFA), cholesterol, vitamin E, magnesium, fiber, and glycemic load (continuous)). In the third model we additionally corrected for diabetes risk factors: energy adjusted alcohol consumption (4 categories), physical activity (4 categories), mean systolic and diastolic blood pressure (continuous), education level (3 categories), and parental history of diabetes (3 categories). In the fourth model BMI (4 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).

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 CHO 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 CHO, 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 ( $< 25 / \geq 25 \text{ kg/m}^2$ ) and waist circumference ( $< 84 / \geq 84 \text{ cm}$ ) 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 (version 14.0) for Windows.

## **Results**

Mean protein intake was 75.7 g/day; animal protein contributed 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 CHO increased, while mean intakes of PUFA and fiber and percentages of males, smokers, and physically inactive persons decreased. (Table 1)

During a mean follow-up of 10.1 (SD 1.9) years, 918 incident type 2 diabetes cases were documented. Diabetes risk increased significantly over the quartiles of total protein intake. Adjustment for age, sex, dietary factors and diabetes risk factors yielded a HR (95%CI) in the highest vs. lowest quartile ( $\text{HR}_{\text{Q4}}$ ) of 1.67 (1.29-2.16). After further adjustment for adiposity measures this association was no longer significant ( $\text{HR}_{\text{Q4}}$  1.18 (0.91-1.53)). (Table 2) Removing either BMI or waist circumference from model 4 yielded comparable, non-significant associations (excluding BMI,  $\text{HR}_{\text{Q4}}$  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)

Adjusting for weight change did not change these findings (Model 3,  $\text{HR}_{\text{Q4}}$  1.67 (1.28-2.16)). Moreover, additional correction for meat and poultry intake in model 3 did not substantially change associations for both total and animal protein ( $\text{HR}_{\text{Q4}}$  1.50 (1.14-1.98)), nor did adjustment for dairy intake ( $\text{HR}_{\text{Q4}}$  1.62 (1.24-2.11)). Excluding participants who followed a diet did not change the results (model 3, total protein  $\text{HR}_{\text{Q4}}$  1.51 (1.11-2.06)), neither did exclusion of baseline cardiovascular disease, hypertension and hyperlipidemia cases ( $\text{HR}_{\text{Q4}}$  1.68 (1.17-2.43)).

Consumption of 5 energy% from protein at the expense of 5 energy% from fat increased diabetes risk, with a HR of 1.31 (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 CHO, we observed a 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-for 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<sub>Q4</sub> 2.15 (1.24-3.15), and 2.36 (1.30-4.29) for low BMI and waist circumference group respectively), while there was no relation in obese participants. Similar results were found for animal protein. Additionally, similar results were obtained when analyzing these interactions continuously (P-for interaction < 0.05). Correction for annual weight change did not change the associations (Low BMI group, total protein, HR<sub>Q4</sub> 2.16 (1.25-3.75)).

## **Discussion**

In this study, high total and animal protein intake, but not vegetable protein, was associated with increased diabetes risk. This relation was not explained by specific protein sources like meat or by weight change during follow-up, but attenuated after adjustment for baseline adiposity measures. Consuming 5 energy% from protein at the expense of 5 energy% from CHO or fat increased diabetes risk by approximately 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, presence of diabetes goes often undetected, and may be preclinical up to 9-12 years (21). Undetected diabetes cases may have been misclassified as non-diabetic individuals, resulting in attenuated associations. Strengths of our study include its prospective design, large sample size and long follow-up. Use of validated diabetes cases minimized presence of false-positive diabetes cases, 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 present (9-11), indicating 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). They 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 to 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 was 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 is further supported by the finding that consuming energy from protein at the expense of energy from either fat or CHO increased diabetes risk. We found no difference in risk when energy from protein was consumed at the expense CHO or fat, suggesting that the increase of protein itself, and not the decrease in fat or CHO, caused this effect. Only one previous study, which focused on consuming CHO 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 present (model 3, HR<sub>Q4</sub> 1.46 (0.96-2.25)). Similarly, adjusting total protein for several sources of animal protein intake, like meat, did not explain the entire association. This 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 for animal protein, although this did not reach statistical significance. Varying 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 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 might not sufficiently compensate in subjects with impaired insulin secretion. (6,7)

A persons' 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 to our hypothesis, we only found an association in lean individuals. In the EPIC-Potsdam study, a similar, but non-significant interaction with adiposity was observed (1). Several potential mechanisms may underlie our findings. First, iron metabolism might 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 an increased diabetes risk (25). Since increased animal protein intake may contribute to increased body iron load, the association between high (animal) protein intake and diabetes in the non-obese 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 non-obese 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 this is unlikely. Finally, because of the direction of the interactions, borderline significance and relatively few non-obese diabetes cases, we cannot exclude these interactions are due to chance.

In conclusion, 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 CHO, increased diabetes risk with approximately 30%. More research into the effect of total protein intake in different populations with different intakes of protein sources is needed to establish 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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**Table 1** Baseline characteristics of the study population, according to quartiles of daily nutritional total protein intake\*

Characteristics	Q1 (low)	Q2	Q3	Q4 (high)
Participants, <i>n</i>	9523	9524	9524	9523
Male gender, <i>n</i> (%)	2643 (27.8)	2685 (28.2)	2450 (25.7)	1962 (20.6)
Age, years	48 (12)	48 (12)	50 (12)	51 (11)
Energy intake, <i>kcal/d</i>	2036 (645)	2106 (618)	2078 (602)	1998 (622)
Animal protein intake, <i>g/d</i>	35.2 (7.0)	44.5 (5.2)	51.3 (5.2)	62.9 (8.3)
Vegetable protein intake, <i>g/d</i>	27.0 (5.5)	27.6 (4.8)	27.6 (4.8)	26.9 (4.7)
Saturated fat intake, <i>g/d</i>	31.2 (6.2)	32.5 (5.6)	33.0 (5.6)	33.4 (5.9)
PUFA intake, <i>g/d</i>	15.3 (4.3)	15.3 (3.8)	14.9 (3.7)	14.2 (3.6)
MUFA intake, <i>g/d</i>	29.2 (5.5)	29.7 (5.0)	29.6 (5.0)	29.2 (5.2)
Cholesterol intake, <i>mg/d</i>	190.0 (53.0)	210.4 (51.1)	223.4 (53.5)	245.3 (64.2)
Carbohydrate intake, <i>g/d</i>	231.5 (34.3)	223.7 (29.0)	219.3 (28.2)	213.6 (28.8)
Glycemic load intake, <i>g/d</i>	120.8 (24.7)	114.7 (20.1)	110.8 (18.9)	106.0 (18.3)
Fiber intake, <i>g/d</i>	22.1 (5.2)	23.2 (4.6)	23.8 (4.6)	24.2 (4.7)
Vitamin C intake, <i>mg/d</i>	101.4 (49.7)	106.6 (43.1)	111.6 (42.6)	118.0 (44.0)
Vitamin E intake, <i>mg/d</i>	12.9 (3.6)	12.5 (3.2)	12.1 (3.0)	11.5 (3.0)
Magnesium intake, <i>mg/d</i>	304.9 (46.2)	327.2 (41.7)	343.9 (40.9)	365.9 (43.3)
Iron intake, <i>mg/d</i>	10.8 (1.6)	11.3 (1.5)	11.7 (1.6)	12.1 (1.7)
Heme iron intake, <i>mg/d</i>	1.6 (0.7)	1.9 (0.7)	2.1 (0.7)	2.5 (0.9)
Alcohol intake, <i>g/d</i>	15.8 (24.4)	11.0 (15.9)	9.7 (13.9)	8.0 (12.6)
BMI, <i>kg/m<sup>2</sup></i>	24.7 (3.7)	25.3 (3.7)	25.8 (3.9)	26.7 (4.3)
Waist circumference, <i>cm</i>	83.1 (11.2)	84.6 (11.2)	85.5 (11.2)	87.1 (11.7)
Current smoker, <i>n</i> (%)	3655 (38.4)	3014 (31.7)	2541 (26.8)	2420 (25.5)
Physically inactive <sup>†</sup> , <i>n</i> (%)	3983 (41.8)	3609 (37.9)	3482 (36.6)	3413 (35.9)
Higher education, <i>n</i> (%)	1993 (20.9)	2104 (22.1)	2030 (21.3)	1703 (17.9)
Parental history diabetes, <i>n</i> (%)	1559 (16.4)	1615 (17.0)	1767 (18.5)	1922 (20.2)
Systolic BP, <i>mm Hg</i>	124.3 (18.6)	125.3 (18.8)	126.6 (18.7)	127.8 (19.0)
Diastolic BP, <i>mm Hg</i>	77.2 (10.6)	77.5 (10.5)	78.0 (10.6)	78.4 (10.7)
Hypertension, <i>n</i> (%)	3108 (32.6)	3307 (34.7)	3491 (36.7)	3762 (39.5)

BP, blood pressure; CPAI, Cambridge physical activity index; d, day; MUFA, monounsaturated fat; PUFA, polyunsaturated fat; Q, quartile.

\* Values are expressed as mean (SD), unless stated otherwise.

† Inactive according to CPAI index.

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

	Q1 (low)	Q2	Q3	Q4 (high)	P-trend	Per 10 g
<b>Total protein</b>						
Cases / at risk, <i>n</i>	153 / 9523	185 / 9524	249 / 9524	331 / 9523		
Quartile median total protein, <i>g/d</i>	64	72	79	88		
Univariable	1	1.20 (0.97-1.49)	1.63 (1.33-1.99) <sup>¶</sup>	2.15 (1.77-2.60) <sup>¶</sup>	< 0.001	1.36 (1.28-1.44) <sup>¶</sup>
Model 1 <sup>*</sup> : age, sex	1	1.17 (0.94-1.45)	1.50 (1.23-1.84) <sup>¶</sup>	1.85 (1.53-2.25) <sup>¶</sup>	< 0.001	1.28 (1.22-1.36) <sup>¶</sup>
Model 2 <sup>†</sup> : Model 1 + dietary intake	1	1.24 (0.99-1.54)	1.65 (1.32-2.07) <sup>¶</sup>	2.16 (1.69-2.76) <sup>¶</sup>	< 0.001	1.45 (1.34-1.56) <sup>¶</sup>
Model 3 <sup>‡</sup> : Model 2 + diabetes risk factors	1	1.16 (0.92-1.45)	1.45 (1.15-1.83) <sup>¶</sup>	1.67 (1.29-2.16) <sup>¶</sup>	< 0.001	1.33 (1.22-1.45) <sup>¶</sup>
Model 4 <sup>§</sup> : 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) <sup>¶</sup>
<b>Animal protein</b>						
Cases / at risk, <i>n</i>	155 / 9523	182 / 9524	243 / 9524	338 / 9523		
Quartile median animal protein, <i>g/d</i>	35	44	52	62		
Univariable	1	1.16 (0.94-1.44)	1.56 (1.28-1.91) <sup>¶</sup>	2.18 (1.80-2.63) <sup>¶</sup>	< 0.001	1.32 (1.25-1.39) <sup>¶</sup>
Model 1 <sup>*</sup> : age, sex	1	1.08 (0.87-1.33)	1.35 (1.10-1.65) <sup>¶</sup>	1.73 (1.43-2.10) <sup>¶</sup>	< 0.001	1.24 (1.17-1.30) <sup>¶</sup>
Model 2 <sup>†</sup> : Model 1 + dietary intake	1	1.17 (0.94-1.46)	1.54 (1.23-1.92) <sup>¶</sup>	2.09 (1.64-2.67) <sup>¶</sup>	< 0.001	1.40 (1.30-1.51) <sup>¶</sup>
Model 3 <sup>‡</sup> : Model 2 + diabetes risk factors	1	1.09 (0.87-1.36)	1.31 (1.05-1.65) <sup>¶</sup>	1.58 (1.23-2.04) <sup>¶</sup>	< 0.001	1.28 (1.18-1.39) <sup>¶</sup>
Model 4 <sup>§</sup> : 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) <sup>¶</sup>
<b>Vegetable protein</b>						
Cases / at risk, <i>n</i>	245 / 9524	228 / 9524	235 / 9523	210 / 9523		
Quartile median vegetable protein, <i>g/d</i>	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) <sup>¶</sup>
Model 1 <sup>*</sup> : age, 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 <sup>†</sup> : 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 <sup>‡</sup> : 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 <sup>§</sup> : 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)

d, day; Q, quartile

\* Model 1: Corrected for sex (male / female), age at recruitment (continuous).

† Model 2: Model 1 + energy adjusted intake of saturated fat, monounsaturated fat, polyunsaturated fat, cholesterol, vitamin E, magnesium, fibre, and glycaemic load (continuous).

‡ Model 3: Model 2 + energy adjusted alcohol consumption (<11 / 11-25 / 26-50 / >50 g/day), physical activity (not active / moderately inactive / moderately active / active), mean systolic and diastolic blood pressure (continuous), education level (high / middle / low), parental history of diabetes (no / one parent / both parents).

§ Model 4: Model 3 + BMI (<20 / 20-25 (reference group) / 25-30 / >30 kg/m<sup>2</sup>), waist circumference (continuous).

¶ Significant at P < 0.05 level. ¶¶ Significant at P < 0.001 level.

**Table 3** Multivariable HR (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 <sup>*</sup>	Model 4 <sup>†</sup>
<b>TOTAL PROTEIN</b>		
Substitution protein for fat	1.72 (1.41-2.12) <sup>§</sup>	1.31 (1.06-1.61) <sup>‡</sup>
Substitution protein for carbohydrates	1.91 (1.52-2.40) <sup>§</sup>	1.28 (1.01-1.61) <sup>‡</sup>
<b>ANIMAL PROTEIN</b>		
Substitution protein for fat	1.51 (1.26-1.82) <sup>§</sup>	1.19 (0.99-1.44)
Substitution protein for carbohydrates	1.72 (1.39-2.12) <sup>§</sup>	1.20 (0.97-1.49)
<b>VEGETABLE PROTEIN</b>		
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)

<sup>\*</sup> Model 3: Corrected for sex (male / 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 / active), mean systolic and diastolic blood pressure (continuous), education level (high / middle / low), parental history of diabetes (no / one parent / both parents).

<sup>†</sup> Model 4: Model 3 + BMI (<20 / 20-25 (reference group) / 25-30 >30 kg/m<sup>2</sup>), waist circumference (continuous).

<sup>‡</sup> Significant at P < 0.05 level. <sup>§</sup> Significant at P < 0.001 level.