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Intake of different dietary proteins and risk of type 2 diabetes in men: the Kuopio Ischaemic Heart Disease Risk Factor Study

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Abstract

The roles of different dietary proteins in the aetiology of type 2 diabetes (T2D) remain unclear. We investigated the associations of dietary proteins with the risk of incident T2D in Finnish men from the prospective Kuopio Ischaemic Heart Disease Risk Factor Study. The study included 2332 men aged 42–60 years at the baseline examinations in 1984–1989. Protein intakes were calculated from 4-d dietary records. Incident T2D was determined by self-administered questionnaires, fasting blood glucose measurements, 2-h oral glucose tolerance tests, and with national registers. The multivariable-adjusted risk of T2D on the basis of protein intakes was compared by the Cox proportional hazard ratios (HR). During the mean follow-up of 19·3 years, 432 incident T2D cases were identified. Total, animal, meat or dairy product protein intakes were not associated with risk of T2D when the potential confounders were accounted for. Plant (multivariable-adjusted extreme-quartile HR 0·65; 95 % CI 0·42, 1·00; P_{trend} 0·04) and egg (HR 0·67; 95 % CI 0·44, 1·00; P_{trend} 0·03) protein intakes were associated with a decreased risk of T2D. Adjustments for BMI, plasma glucose and serum insulin slightly attenuated associations. Replacing 1 % energy from carbohydrates with energy from protein was associated with a 5 % (95 % CI 0, 11) increased risk of T2D, but adjustment for fibre intake attenuated the association. Replacing 1 % of energy from animal protein with energy from plant protein was associated with 18 % (95 % CI 0, 32) decreased risk of T2D. This association remained after adjusting for BMI. In conclusion, favouring plant and egg proteins appeared to be beneficial in preventing T2D.

Key words: Animal protein: Dietary protein: Eggs: Plant protein: Prospective studies: Type 2 diabetes

Protein-rich diets have become a popular strategy to enhance weight management and weight loss[1]. Because obesity is one of the main risk factors of type 2 diabetes (T2D)[2], increasing protein intake may also have potential for T2D prevention[3,4]. The optimal amount and quality of protein for averting T2D is, however, controversial[3–5]. Although short-term interventions comparing higher *v.* lower protein diets have shown beneficial effects on weight loss, body composition and some metabolic markers[1,4,6,7], the results of long-term interventions have generally been modest[1,8]. Furthermore, some prospective studies have raised the concern that even moderately higher protein intake may actually increase the risk of T2D[9–14], although null associations have also been reported[15–17]. Some[18,19], but not all[20], epidemiological studies have also suggested that replacing protein with carbohydrates could decrease the risk of T2D. Contrary to short-term interventions, prospective studies have indicated that the association of high protein intake with higher T2D risk is partly mediated via the impact of higher protein intake on obesity[10–13].

Strong indications exist that different protein sources are not similar with regard to risk of T2D. Intake of red meat, especially of processed red meat, has been associated with an increased risk of T2D[21–24], whereas, for example, fermented dairy products have generally been associated with a decreased risk[24–26]. Different protein sources may also induce distinct effects on glucose and insulin metabolism or inflammation, but research findings are scarce and inconclusive[27–32].

It is not clear whether the divergent associations of protein sources with the risk of T2D are due to the differential peptide or amino acid compositions of protein sources or due to some other factors. In general, animal protein has been associated with an increased risk of T2D[10,11,13,33], whereas plant protein has had a neutral[10,11,17,33] or an inverse association[13]. However, to our knowledge, only two epidemiological studies have more comprehensively investigated the protein intake from different dietary sources with regard to T2D incidence[10,18]. van Nielen *et al.*[10] did not find evidence that protein from dairy products, fish or meat would specifically be accountable for the increased risk of T2D that was observed with higher

Abbreviations: E%, percentage of total energy intake; hsCRP, high-sensitivity C-reactive protein; HR, hazard ratio; T2D, type 2 diabetes.

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animal protein intake. Similä *et al.*[18] observed that replacing energy coming from either total, meat or milk protein with energy coming from carbohydrates was associated with a decreased risk of T2D.

Because of the current limited knowledge, we investigated the associations of proteins from different dietary sources with the risk of incident T2D in middle-aged and older Finnish men. We also examined whether intakes of proteins are associated with risk factors for T2D, that is, BMI, fasting plasma glucose and serum insulin, and serum high-sensitivity C-reactive protein (hsCRP) at baseline. In the secondary analyses, we investigated the associations of the main dietary protein sources with the risk of T2D.

Methods

Study population

The Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD) was designed to investigate risk factors for CVD, atherosclerosis and related outcomes in a population-based, randomly selected sample of men from eastern Finland[34]. The baseline examinations were carried out from 1984 to 1989 (online Supplementary Fig. S1). A total of 2682 men who were 42, 48, 54, or 60 years old at baseline (83 % of those eligible) were recruited in two cohorts. The first cohort consisted of 1166 men who were 54 years old and enrolled between 1984 and 1986, and the second cohort included 1516 men who were 42, 48, 54, or 60 years old and enrolled between 1986 and 1989. Re-examination rounds were conducted 4, 11 and 20 years after the baseline (online Supplementary Fig. S1). The baseline characteristics of the entire study population have been described previously[34]. The KIHD study complies with the Declaration of Helsinki and has an approval from the Research Ethics Committee of the University of Kuopio. All subjects gave written informed consent. Subjects with T2D (n 167), impaired fasting glucose (n 127) or unknown diabetes status (n 38) at baseline, or those with missing data on dietary intakes (n 18) were excluded, which left 2332 men for the analyses of incident T2D. Data on plasma glucose, serum insulin and serum hsCRP concentrations were available for 2312 men at baseline.

Other measurements

Fasting venous blood samples were collected between 08.00 and 10.00 hours at baseline and at the follow-up examinations. Subjects were instructed to abstain from ingesting alcohol for 3 d and from smoking and eating for 12 h before providing the sample. Detailed descriptions of determining serum lipids and lipoproteins[35] and the assessment of medical history and medications[35], family history of diseases[35], smoking[35], alcohol consumption[35], serum ferritin[35] and physical activity[36] at baseline have been published. Number of years of education, annual income and marital status were obtained from self-administered questionnaires. Family history of diabetes was defined as positive if a first-degree relative of the participant had a history of diabetes. BMI was computed as the ratio of weight (kg):the square of height (m^2), both measured at the study baseline.

Assessment of dietary intakes

The consumption of foods at baseline was assessed with a guided food record of 4 d, one of which was a weekend, by using household measures. A picture book of common foods and dishes was used to help in the estimation of portion sizes. The picture book contained 126 of the most common foods and drinks consumed in Finland during the 1980s. For each food item, the participant could choose from three to five commonly used portion sizes or describe the portion size in relation to those shown in the book. To further improve accuracy, instructions were given and completed food records were checked by a nutritionist together with the participant. Nutrient intakes were estimated by using NUTRICA 2.5® software (Social Insurance Institution). The software's databank is mainly based on Finnish values of the nutrient composition of foods.

Protein intakes from different animal and plant sources were calculated (online Supplementary Table S1). Total meat included red meat, white meat and offal. Processed red meat included all red meat that had undergone industrial processing, for example, by adding salt or preservatives. Participants did not use processed white meat. Total dairy product intake was calculated as a sum of non-fermented dairy products (mainly milk, cream and ice cream) and fermented dairy products (mainly sour milk, curdled milk, yogurt and cheese) (online Supplementary Table S1).

Of the average daily protein intake, 2·2 g (0·4 % of total energy intake (E%)) was from sources that could not easily be classified as animal or plant protein (e.g. dry ready meals and chocolate) and was not included into either of the categories. For the analyses of major sources of dietary protein, we combined the most protein-rich foods of the plant protein category – that is, grain products, legumes, nuts and seeds – to assess the intake of the major plant protein sources as a whole. The carbohydrates from whole-grain products, legumes, nuts, seeds, mushrooms, fruits, berries and vegetables (excluding potatoes) were combined to assess the intake of high-quality carbohydrates. Each nutrient, except for fibre and cholesterol, was energy-adjusted by the residual method[37].

Measurement of plasma glucose, serum insulin and high-sensitivity C-reactive protein

Plasma glucose was measured using a glucose dehydrogenase method (Merck) after precipitation of proteins with TCA using a clinical chemistry analyser (Kone Specific; KONE Instruments Oy). The serum samples for insulin determination were stored frozen at –80 °C. Serum insulin was determined with a Novo Biolabs radioimmunoassay kit (Novo Nordisk) using Multi Gamma counter with Ria Calc software. Serum hsCRP was measured with an immunometric assay (Immulite High Sensitivity CRP Assay; Diagnostic Products Corporation) using clinical chemistry analyser Kone Specific.

Diagnostic criteria for type 2 diabetes

At baseline, T2D was defined as a self-reported physician diagnosis of T2D and/or fasting plasma glucose ≥7·0 mmol/l. Impaired fasting glucose was defined by using the WHO criterion: fasting plasma glucose of 6·1–6·9 mmol/l. At the re-examination rounds 4, 11 and 20 years after the baseline, a 2-h oral glucose tolerance test was additionally performed, with criteria for T2D diagnosis as plasma glucose ≥11·1 mmol/l. During the entire study follow-up period, information about incident cases of T2D in the whole study population was also gathered from the national hospital discharge registry and the Social Insurance Institution of Finland register for reimbursement of medicine expenses used for T2D. There were no losses to follow-up.

Statistical analysis

In the main analyses, we used energy-adjusted protein intakes expressed as g/d, to allow comprehensible comparison between proteins from different food sources. The univariate relations between total, animal and plant protein intake and baseline characteristics were assessed by means and linear regression (for continuous variables) or by χ^2 tests (for categorical variables). Correlations between intakes of different proteins were estimated by Spearman's correlation coefficients. The Cox proportional hazards regression models were used to estimate hazard ratios (HR) in exposure quartiles, with the lowest category as the reference. Person-years of follow-up, which were calculated from the baseline to the date of diabetes diagnosis, death or the end of follow-up (31 December 2010), were used as the underlying time variable in these models. The validity of the proportional hazards assumptions was evaluated by using Schoenfeld residuals, and the assumptions were met. Absolute risk change was calculated by multiplying the absolute risk in the reference group by the multivariable-adjusted HR change in the comparison group.

The confounders were selected on the basis of established risk factors for T2D, previously published associations with T2D in the KIHD study, or on associations with exposures or outcomes in the present analysis. Model 1 included age (years), examination year and energy intake (kJ/d (kcal/d)). The multivariable model (model 2) included the variables in model 1 plus marital status (married/unmarried), income (euros/year), use of hypertension medication (yes or no), family history of T2D (yes or no), pack-years of smoking (packs smoked/d × years smoked), education years, leisure-time physical activity (kJ/d (kcal/d)), serum ferritin (μg/l), and intake of alcohol (g/week). Model 3 included the variables in model 2 and the dietary factors: glycaemic index, intakes of fibre (g/d), Mg (mg/d), coffee (ml/d), cholesterol (mg/d), and SFA (g/d), MUFA (g/d), PUFA (g/d) and *trans*-fatty acids (g/d). Models that include both the specific protein and fat, but not carbohydrates, can be interpreted as replacement of carbohydrates and other proteins with the protein of interest. Further adjustment for intake of fruits, berries and vegetables (excluding potatoes) (g/d) did not appreciably change the associations (change in estimates <5 %). Model 4 was further adjusted for potential effect mediators, which were measured at the study baseline: BMI (kg/m^2), fasting plasma glucose (mmol/l) and fasting serum insulin (mU/l). All quantitative variables were entered in the models as continuous variables. The cohort mean was used to replace missing values in covariates (<2·5 %)[38]. We did not observe significant multicollinearity between independent variables used in the multivariable models: variance inflation factors were <10, tolerance values were >0·10 and correlation coefficients were <0·7.

Tests of linear trend were conducted by assigning the median values for each category of exposure variable and treating those as a single continuous variable. The statistical significance of the interactions with age, BMI and physical activity level were assessed by likelihood-ratio tests with the use of a cross-product term. The median value of each of these factors was used to divide subjects into two groups in which the associations were separately assessed.

In the substitution models, we assessed the isoenergetic replacement of 1 E% coming from total or high-quality carbohydrates with an equal amount of energy from different proteins. All the macronutrients except the one that was replaced were simultaneously added into the Cox proportional hazards regression models. We also assessed the replacement of protein from animal sources with protein from plant sources. All proteins were simultaneously added into the models, and the difference of regression coefficients of two proteins of interest, their variance and covariance were used for calculating HR and 95 % CI for substitution models. The adjustments in the substitution models were the same as those in model 3 in the main analyses (listed above), except that glycaemic index and fibre intake were not included in the models where carbohydrates were replaced with protein to allow comparison of different types of carbohydrates.

The mean values of BMI, plasma glucose, serum insulin and serum hsCRP in quartiles of different proteins were analysed using ANCOVA. The same adjustments were used as in model 3 in the main analyses (listed above), but the models for glucose, insulin and hsCRP were further adjusted for BMI, to observe the associations independently of BMI.

In the secondary analyses, we investigated the associations of the major protein sources with the risk of T2D. Exposure quartiles were based on intakes calculated as g/d. The same covariates were used as in the protein models, but the intake of fruits, berries and vegetables (excluding potatoes) (g/d) was used as an additional covariate in models 3, 4 and 5. The possible effect mediators – that is, serum ferritin, glycaemic index, and intakes of fibre, Mg, cholesterol, and SFA, MUFA, PUFA and *trans*-fatty acids – were only added to models 4 and 5. These factors have been suggested to explain the associations of protein sources with the risk of T2D[2,23,39,40]. The possible mediators were not used in the substitution models either, where we assessed the replacement of 50 g portions of protein sources with each other. All protein sources were simultaneously added into the model, and HR and CI were calculated as in the protein substitution models (see above). All *P* values were two-tailed (α 0·05). Data were analysed using SPSS 21.0 for Windows (IBM Corp.).

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Results

Baseline characteristics

The average protein intake was 92·9 g/d (15·7 E%), of which 69·8 % was from animal sources (online Supplementary Table S2). Main contributors for animal protein intake were dairy products (44·4 % of the animal protein), meat (37·7 %) and fish (12·5 %), whereas grain products provided the majority of the plant protein (79·5 %), followed by potatoes (9·3 %) and other vegetables, fruits and berries (7·9 %). Animal and plant protein intakes were negatively correlated (online Supplementary Table S2). Meat and dairy product protein intakes were equally correlated with animal protein intake, but negatively correlated with each other.

Table 1 and the online Supplementary Table S3 show the baseline characteristics according to intakes of total, animal and plant protein. Men with a higher total protein intake were more likely to be married and have higher education and income than men with a lower intake. Higher total protein intake was associated with favourable dietary factors, such as higher fibre intake and lower intake of alcohol, but with higher BMI. Associations with animal protein were more mixed: higher animal protein intake was associated with higher BMI, higher proportion of current smokers and lower fibre intake, but with higher intake of PUFA and lower intake of *trans*-fatty acids. Those with a higher plant protein intake had, in general, a healthier lifestyle: they were physically more active, had lower BMI and a healthier diet, were less likely to smoke and used less alcohol than those with lower intake.

Associations of dietary proteins with risk factors of type 2 diabetes

At baseline, higher total, animal and fish protein intakes were associated with higher BMI after multivariable adjustments, but intake of other types of protein did not associate with BMI (online Supplementary Table S4). Protein from plant sources, especially from grain products, was associated with lower fasting plasma glucose concentrations. Proteins from red meat and non-fermented dairy products were associated with higher fasting serum insulin concentrations and proteins from fish, cheese and grain products were associated with lower insulin concentrations. Other proteins did not associate with glucose metabolism markers, and no associations were observed with serum hsCRP.

Associations of dietary proteins with risk of type 2 diabetes

During the mean follow-up time of 19·3 years, 432 incident cases of T2D were identified. Total protein intake or proteins from total red meat, unprocessed red meat or fish were not associated with the risk of T2D (Table 2). Animal protein intake, protein from total meat and protein from processed meat were associated with an increased risk of T2D in the model that was adjusted for age, examination year and energy intake (Table 2, model 1), but these associations were not statistically significant after further adjustments for potential non-dietary and dietary

Table 1. Baseline characteristics according to total, animal and plant protein intake among 2332 men from the Kuopio Ischaemic Heart Disease Risk Factor Study (Mean values and standard deviations; percentages)

	Total protein intake				Animal protein intake				Plant protein intake			
	Quartile 1 (<83·7 g/d)		Quartile 4 (>101·1 g/d)		Quartile 1 (<55·0 g/d)		Quartile 4 (>74·0 g/d)		Quartile 1 (<22·1 g/d)		Quartile 4 (>29·2 g/d)	
Characteristics	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Subjects (*n*)	583		583		583		583		583		583	
Age (years)	53·6	4·7	52·5*	5·6	53·7	4·7	52·4*	5·8	52·3	5·2	53·3*	5·2
Married (%)	84		89*		85		88*		84		89*	
Current smokers (%)	34		31		28		35*		48		18*	
Family history of type 2 diabetes (%)	28		26		27		26		25		27	
CHD (%)	25		22		26		23		24		21	
Use of hypertension medication (%)	18		20		19		18		15		22*	
BMI (kg/m^2)	26·2	3·3	27·2*	3·5	26·0	3·0	27·5*	3·6	26·8	3·5	26·0*	3·1
Education (years)	8·2	3·1	9·1*	3·7	8·4	3·2	8·9*	3·6	8·3	3·2	8·8*	3·6
Income (1000 €)	11·6	7·8	14·7*	9·7	12·1	7·6	14·3*	9·8	12·8	9·7	13·5	8·7
Leisure-time physical activity (kJ/d)	561	736	619	799	573	686	577	770	506	707	690*	845
Leisure-time physical activity (kcal/d)	134	176	148	191	137	164	138	184	121	169	165*	202
Alcohol intake (g/week)	99	213	67*	85	75	197	80	99	138	215	31*	54
Serum LDL-cholesterol (mmol/l)	4·06	1·02	3·98	1·00	4·02	1·02	4·00	1·01	4·09	1·05	3·96*	0·98
Serum HDL-cholesterol (mmol/l)	1·32	0·32	1·32	0·31	1·31	0·31	1·33	0·30	1·33	0·32	1·28*	0·28
Serum TAG (mmol/l)	1·24	0·75	1·29	0·77	1·24	0·74	1·27	0·76	1·22	0·73	1·31	0·84
Serum ferritin (μg/l)	148	156	178*	146	135	141	179*	144	183	152	130*	134
Energy (kJ/d)	10 694	2812	10 694	2682	10 665	2841	10 652	2711	10 661	2728	10 711	2569
Energy (kcal/d)	2556	672	2556	641	2549	679	2546	648	2548	652	2560	614

*$P_{\text{for trend}}$ across quartiles <0·05; $P_{\text{for trend}}$ was assessed with linear regression (continuous variables) or with χ^2 test (categorical variables).

Table 2. Type 2 diabetes incidence according to protein intake among 2332 men from the Kuopio Ischaemic Heart Disease Risk Factor Study (Hazard ratios (HR) and 95 % confidence intervals derived from the Cox proportional hazards regression models)

	Intake quartile								
	Quartile 1 (*n* 583)	Quartile 2 (*n* 583)		Quartile 3 (*n* 583)		Quartile 4 (*n* 583)			Per 5 g increase
	HR	HR	95 % CI	HR	95 % CI	HR	95 % CI	P_{trend}	HR	95 % CI
Total protein										
Median intake (g/d)	78·3	87·5		96·1		108·4				
Number of events, incidence rate/1000 PY	101, 9·07	109, 9·68		113, 9·93		109, 9·77				
Model 1*	1	1·05	0·80, 1·38	1·07	0·81, 1·40	1·05	0·80, 1·37	0·76	1·01	0·98, 1·05
Model 2†	1	1·07	0·81, 1·40	1·07	0·81, 1·40	1·00	0·76, 1·31	0·93	1·01	0·97, 1·04
Model 3‡	1	1·03	0·77, 1·38	1·03	0·75, 1·40	0·97	0·66, 1·40	0·82	1·02	0·97, 1·07
Model 4§	1	1·04	0·78, 1·39	1·03	0·76, 1·41	0·91	0·62, 1·33	0·59	1·01	0·96, 1·06
Animal protein										
Median intake (g/d)	48·8	59·5		68·3		81·6				
Number of events, incidence rate/1000 PY	90, 7·98	102, 8·92		126, 11·30		114, 10·32				
Model 1*	1	1·11	0·84, 1·48	1·43	1·09, 1·88	1·31	0·99, 1·73	0·03	1·04	1·01, 1·07
Model 2†	1	1·07	0·80, 1·43	1·33	1·01, 1·74	1·20	0·91, 1·59	0·11	1·03	0·99, 1·06
Model 3‡	1	1·05	0·78, 1·42	1·30	0·94, 1·79	1·20	0·82, 1·76	0·27	1·03	0·98, 1·08
Model 4§	1	1·04	0·77, 1·41	1·27	0·92, 1·76	1·04	0·71, 1·54	0·74	1·02	0·98, 1·07
Protein from total meat‖										
Median intake (g/d)	12·3	19·8		26·7		37·4				
Number of events, incidence rate/1000 PY	104, 9·50	99, 8·66		94, 8·24		135, 12·14				
Model 1*	1	0·88	0·67, 1·16	0·84	0·64, 1·11	1·27	0·98, 1·65	0·05	1·03	0·99, 1·07
Model 2†	1	0·84	0·64, 1·11	0·81	0·61, 1·08	1·13	0·86, 1·47	0·24	1·01	0·96, 1·05
Model 3‡	1	0·86	0·65, 1·15	0·87	0·64, 1·18	1·22	0·88, 1·70	0·13	1·01	0·96, 1·07
Model 4§	1	0·87	0·65, 1·16	0·87	0·64, 1·18	1·24	0·90, 1·73	0·11	1·01	0·96, 1·07
Protein from red meat										
Median intake (g/d)	10·5	17·8		24·2		34·2				
Number of events, incidence rate/1000 PY	97, 8·90	119, 10·40		99, 8·79		117, 10·34				
Model 1*	1	1·14	0·87, 1·49	0·97	0·73, 1·28	1·12	0·85, 1·48	0·62	1·02	0·97, 1·06
Model 2†	1	1·13	0·87, 1·49	0·89	0·67, 1·19	1·06	0·80, 1·40	0·94	1·00	0·95, 1·04
Model 3‡	1	1·11	0·84, 1·47	0·87	0·64, 1·18	1·01	0·72, 1·40	0·74	0·99	0·94, 1·04
Model 4§	1	1·15	0·87, 1·52	0·93	0·68, 1·27	1·08	0·78, 1·51	0·90	0·99	0·94, 1·04
Protein from processed red meat										
Median intake (g/d)	1·7	5·8		9·9		16·9				
Number of events, incidence rate/1000 PY	101, 8·93	92, 8·27		116, 10·34		123, 10·93				
Model 1*	1	0·90	0·68, 1·20	1·16	0·88, 1·51	1·25	0·95, 1·63	0·03	1·07	1·00, 1·14
Model 2†	1	0·88	0·66, 1·17	1·05	0·80, 1·38	1·10	0·84, 1·45	0·26	1·02	0·96, 1·09
Model 3‡	1	0·90	0·67, 1·20	1·05	0·79, 1·40	1·14	0·82, 1·57	0·28	1·03	0·94, 1·12
Model 4§	1	0·90	0·67, 1·20	1·05	0·79, 1·39	1·07	0·77, 1·48	0·48	1·02	0·93, 1·11
Protein from unprocessed red meat										
Median intake (g/d)	3·9	9·2		14·2		23·0				
Number of events, incidence rate/1000 PY	101, 9·24	112, 9·82		113, 10·15		106, 9·26				
Model 1*	1	1·04	0·79, 1·36	1·08	0·83, 1·41	0·96	0·73, 1·26	0·74	0·98	0·93, 1·04
Model 2†	1	0·98	0·75, 1·28	1·08	0·82, 1·42	0·94	0·72, 1·25	0·77	0·98	0·93, 1·04
Model 3‡	1	1·00	0·76, 1·31	1·09	0·83, 1·43	0·94	0·71, 1·25	0·74	0·98	0·93, 1·03
Model 4§	1	1·01	0·77, 1·33	1·16	0·88, 1·52	0·98	0·74, 1·30	0·98	0·98	0·93, 1·04

Table 2. *Continued*

	Intake quartile									
	Quartile 1 (*n* 583)	Quartile 2 (*n* 583)		Quartile 3 (*n* 583)		Quartile 4 (*n* 583)			Per 5 g increase	
	HR	HR	95 % CI	HR	95 % CI	HR	95 % CI	P_{trend}	HR	95 % CI
Protein from fish										
Median intake (g/d)	0·01	3·2		8·2		17·5				
Number of events, incidence rate/1000 PY	106, 9·26	110, 9·70		115, 10·14		101, 9·36				
Model 1*	1	1·02	0·77, 1·33	1·07	0·82, 1·40	1·03	0·78, 1·35	0·82	1·02	0·97, 1·07
Model 2†	1	0·98	0·75, 1·29	1·05	0·80, 1·38	0·99	0·75, 1·30	0·99	1·01	0·96, 1·06
Model 3‡	1	1·00	0·76, 1·31	1·05	0·79, 1·38	0·98	0·73, 1·32	0·94	1·01	0·96, 1·07
Model 4§	1	0·90	0·68, 1·19	0·89	0·67, 1·17	0·84	0·62, 1·13	0·31	0·98	0·93, 1·04
Protein from eggs										
Median intake (g/d)	1·1	2·3		3·9		6·6				
Number of events, incidence rate/1000 PY	124, 11·85	120, 10·67		95, 8·16		93, 8·04				
Model 1*	1	0·84	0·65, 1·09	0·64	0·49, 0·84	0·65	0·50, 0·85	0·001	0·76	0·63, 0·93
Model 2†	1	0·87	0·67, 1·13	0·66	0·50, 0·86	0·68	0·52, 0·90	0·003	0·78	0·65, 0·95
Model 3‡	1	0·87	0·66, 1·14	0·66	0·49, 0·90	0·67	0·44, 1·00	0·03	0·79	0·56, 1·12
Model 4§	1	0·98	0·75, 1·28	0·75	0·55, 1·02	0·74	0·49, 1·13	0·10	0·82	0·58, 1·18
Protein from dairy products										
Median intake (g/d)	17·2	25·2		31·6		40·5				
Number of events, incidence rate/1000 PY	105, 9·33	103, 9·10		89, 7·84		135, 12·27				
Model 1*	1	0·96	0·73, 1·26	0·82	0·62, 1·09	1·34	1·04, 1·74	0·04	1·04	1·00, 1·09
Model 2†	1	1·03	0·78, 1·36	0·83	0·63, 1·11	1·41	1·09, 1·82	0·02	1·05	1·01, 1·10
Model 3‡	1	0·98	0·73, 1·30	0·79	0·57, 1·08	1·25	0·88, 1·78	0·30	1·03	0·97, 1·09
Model 4§	1	1·04	0·78, 1·39	0·85	0·62, 1·18	1·35	0·95, 1·91	0·14	1·04	0·98, 1·11
Protein from non-fermented dairy products										
Median intake (g/d)	6·6	13·7		20·0		29·3				
Number of events, incidence rate/1000 PY	112, 9·70	99, 8·81		106, 9·30		115, 10·71				
Model 1*	1	0·90	0·69, 1·18	0·96	0·73, 1·26	1·17	0·90, 1·52	0·18	1·04	0·99, 1·09
Model 2†	1	0·87	0·66, 1·15	0·94	0·72, 1·24	1·12	0·85, 1·47	0·30	1·03	0·99, 1·08
Model 3‡	1	0·85	0·64, 1·12	0·86	0·65, 1·14	0·94	0·69, 1·27	0·78	1·00	0·94, 1·06
Model 4§	1	0·88	0·67, 1·17	0·88	0·66, 1·17	0·97	0·72, 1·31	0·90	1·01	0·96, 1·07
Protein from milk										
Median intake (g/d)	5·8	12·9		19·3		28·8				
Number of events, incidence rate/1000 PY	111, 9·56	106, 9·48		101, 8·86		114, 10·64				
Model 1*	1	0·99	0·76, 1·30	0·93	0·71, 1·22	1·19	0·91, 1·54	0·24	1·04	0·99, 1·09
Model 2†	1	0·92	0·70, 1·20	0·89	0·67, 1·17	1·12	0·85, 1·46	0·41	1·03	0·98, 1·08
Model 3‡	1	0·88	0·66, 1·16	0·80	0·60, 1·07	0·93	0·69, 1·26	0·62	0·99	0·94, 1·05
Model 4§	1	0·92	0·70, 1·22	0·81	0·61, 1·09	0·95	0·70, 1·29	0·67	1·01	0·95, 1·07
Protein from fermented dairy products										
Median intake (g/d)	1·3	7·0		12·6		22·3				
Number of events, incidence rate/1000 PY	101, 9·25	109, 9·58		108, 9·66		114, 9·97				
Model 1*	1	1·00	0·76, 1·31	1·01	0·77, 1·32	1·03	0·79, 1·35	0·81	1·01	0·97, 1·07
Model 2†	1	1·08	0·82, 1·42	1·16	0·88, 1·53	1·13	0·86, 1·48	0·38	1·03	0·98, 1·08
Model 3‡	1	1·06	0·81, 1·41	1·13	0·86, 1·50	1·08	0·82, 1·44	0·57	1·02	0·97, 1·08
Model 4§	1	1·11	0·84, 1·46	1·18	0·89, 1·56	1·13	0·85, 1·50	0·42	1·02	0·97, 1·08
Protein from cheese										
Median intake (g/d)	−0·1	1·9		5·7		12·7				
Number of events, incidence rate/1000 PY	115, 10·68	106, 9·55		102, 8·90		109, 9·41				
Model 1*	1	0·84	0·64, 1·10	0·76	0·58, 1·00	0·81	0·62, 1·06	0·19	0·94	0·87, 1·02
Model 2†	1	0·82	0·62, 1·08	0·81	0·62, 1·07	0·87	0·66, 1·15	0·59	0·97	0·89, 1·04

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Table 2. *Continued*

	Intake quartile									
	Quartile 1 (*n* 583)	Quartile 2 (*n* 583)		Quartile 3 (*n* 583)		Quartile 4 (*n* 583)			Per 5 g increase	
	HR	HR	95 % CI	HR	95 % CI	HR	95 % CI	P_{trend}	HR	95 % CI
Model 3‡	1	0·83	0·63, 1·10	0·81	0·61, 1·07	0·87	0·65, 1·15	0·52	0·96	0·89, 1·04
Model 4§	1	0·87	0·66, 1·15	0·87	0·65, 1·15	0·98	0·74, 1·30	0·83	0·98	0·91, 1·06
Protein from other fermented dairy products¶										
Median intake (g/d)	– 0·4	1·3		5·5		14·2				
Number of events, incidence rate/1000 PY	108, 9·59	96, 8·59		111, 9·76		117, 10·53				
Model 1*	1	0·83	0·62, 1·12	0·96	0·73, 1·27	1·06	0·81, 1·39	0·23	1·07	1·01, 1·14
Model 2†	1	0·85	0·63, 1·14	1·03	0·78, 1·36	1·12	0·85, 1·47	0·11	1·08	1·01, 1·14
Model 3‡	1	0·85	0·63, 1·15	1·00	0·75, 1·33	1·12	0·84, 1·49	0·17	1·08	1·01, 1·16
Model 4§	1	0·88	0·65, 1·20	1·00	0·75, 1·34	1·12	0·83, 1·50	0·21	1·06	0·99, 1·14
Plant protein										
Median intake (g/d)	19·6	23·8		27·2		32·2				
Number of events, incidence rate/1000 PY	119, 10·89	123, 11·21		107, 9·45		83, 7·10				
Model 1*	1	1·00	0·77, 1·28	0·81	0·62, 1·06	0·59	0·44, 0·78	<0·001	0·84	0·77, 0·92
Model 2†	1	1·02	0·79, 1·33	0·88	0·66, 1·15	0·62	0·46, 0·84	0·001	0·86	0·78, 0·94
Model 3‡	1	1·04	0·78, 1·38	0·88	0·63, 1·23	0·65	0·42, 1·00	0·04	0·83	0·71, 0·99
Model 4§	1	1·01	0·76, 1·34	0·89	0·64, 1·25	0·71	0·46, 1·10	0·10	0·85	0·72, 1·01
Protein from grain products										
Median intake (g/d)	14·6	18·6		21·8		26·7				
Number of events, incidence rate/1000 PY	112, 10·33	121, 10·88		114, 10·02		85, 7·34				
Model 1*	1	1·01	0·78, 1·31	0·90	0·69, 1·17	0·64	0·48, 0·85	0·001	0·87	0·80, 0·95
Model 2†	1	1·05	0·81, 1·36	0·96	0·73, 1·26	0·68	0·50, 0·92	0·01	0·89	0·81, 0·98
Model 3‡	1	1·06	0·80, 1·41	0·98	0·72, 1·35	0·73	0·49, 1·11	0·13	0·93	0·80, 1·07
Model 4§	1	1·07	0·81, 1·42	0·99	0·72, 1·36	0·83	0·55, 1·25	0·33	0·95	0·82, 1·09
Protein from non-grain plant protein sources										
Median intake (g/d)	3·0	4·4		5·6		7·8				
Number of events, incidence rate/1000 PY	119, 10·95	120, 10·82		97, 8·42		96, 8·39				
Model 1*	1	0·98	0·76, 1·26	0·74	0·56, 0·97	0·75	0·57, 0·98	0·01	0·73	0·59, 0·91
Model 2†	1	1·06	0·82, 1·37	0·77	0·59, 1·02	0·79	0·60, 1·04	0·03	0·75	0·60, 0·94
Model 3‡	1	1·09	0·84, 1·42	0·83	0·63, 1·11	0·90	0·67, 1·20	0·26	0·84	0·65, 1·07
Model 4§	1	1·13	0·87, 1·47	0·81	0·61, 1·08	0·91	0·68, 1·22	0·26	0·84	0·66, 1·06

PY, person-years.
* Model 1 adjusted for age, examination year and energy intake.
† Model 2 adjusted for model 1 and marital status, income, use of hypertension medication, family history of diabetes, pack-years of smoking, education, leisure-time physical activity, serum ferritin and alcohol intake.
‡ Model 3 adjusted for model 2 and glycaemic index, and dietary intakes of fibre, Mg, coffee, cholesterol, and SFA, MUFA, PUFA and *trans*-fatty acids.
§ Model 4 adjusted for model 3 and BMI, fasting plasma glucose and fasting serum insulin.
‖ Total meat includes red meat, white meat and offal.
¶ Other fermented dairy products include sour milk, yogurt, curdled milk, quark, sour cream and crème fraiche.

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factors (models 2, 3 and 4). Protein from eggs was associated with a decreased risk of T2D (model 1), and although the association was attenuated after multivariable adjustments, it remained statistically significant (absolute risk in the lowest quartile 21·3 %, absolute risk reduction in the highest quartile 7·1 %, model 3). On the basis of observed associations between proteins and BMI, plasma glucose and serum insulin (online Supplementary Table S4), we tested them as possible effect mediators. Inclusion of these factors into the model further slightly attenuated the association between egg protein intake and risk of T2D (HR in the highest *v.* lowest intake quartile 0·74; 95 % CI 0·49, 1·13). Total dairy product protein intake was associated with an increased risk of T2D in the models adjusted for non-dietary factors (models 1 and 2, Table 2), but in the multivariable-adjusted models total dairy product protein or protein from any dairy product subtype was not associated with T2D risk (models 3 and 4).

Plant protein intake was associated with a decreased risk of T2D (model 1, Table 2), and this association remained statistically significant after multivariable adjustments (absolute risk in the lowest quartile 20·4 %, absolute risk reduction in the highest quartile 7·2 %, model 3). Each 5 g higher plant protein intake was associated with 17 % (HR 0·83; 95 % CI 0·71, 0·99) lower risk of T2D. Adjustment for the potential effect mediators slightly attenuated the associations (model 4, Table 2). Proteins from both grain products and from non-grain plant products showed non-significant associations towards lower risk of T2D.

In the substitution models, replacing 1 E% from carbohydrates with an equal amount of energy coming from total protein was associated with a 5 % (HR 1·05; 95 % CI 1·00, 1·11) increased risk of T2D, whereas the replacement with plant protein was associated with an 18 % (HR 0·82; 95 % CI 0·69, 0·98) decreased risk (Fig. 1). Replacing carbohydrates with protein from other sources was not associated with the risk (Fig. 1). When the models were adjusted for fibre intake, replacing total or high-quality carbohydrates with protein was no longer statistically significantly associated with an increased risk of T2D (HR 1·01; 95 % CI 0·95, 1·07 and HR 1·01; 95 % CI 0·94, 1·07, respectively), and the inverse association of replacing total or high-quality carbohydrates with plant protein was also attenuated (HR 0·89; 95 % CI 0·74, 1·07 and HR 0·86; 95 % CI 0·71, 1·05). After additional adjustment for BMI, the HR for replacing total or high-quality carbohydrates with protein were 0·99 (95 % CI 0·94, 1·05) and 0·99 (95 % CI 0·93, 1·05), respectively, and for replacing total or high-quality carbohydrates with plant protein 0·85 (95 % CI 0·70, 1·02) and 0·83 (95 % CI 0·68, 1·02), respectively.

Replacing 1 E% coming from any animal protein, except for protein from eggs, with energy from plant protein was associated with a 14–20 % decreased risk of T2D, although not all associations reached statistical significance (Fig. 2). However, almost all associations were slightly stronger after further adjustment for BMI (online Supplementary Table S5).

Associations of dietary protein sources with risk of type 2 diabetes

In the secondary analyses with the protein sources, total meat intake was associated with a markedly increased risk of T2D after multivariable adjustments (online Supplementary Table S4, absolute risk in the lowest quartile 16·1 %, absolute risk increase in the highest quartile 7·3 %, model 3). Processed red meat intake showed a borderline statistically significant association with a higher risk (P_{trend} 0·06, model 3). Intakes of total red meat or unprocessed red meat did not associate with the risk of T2D (online Supplementary Table S6). Higher dairy product intake, especially fermented dairy product intake from sources other than cheese, was associated with borderline increased risk of T2D (online Supplementary Table S6). Intake of major plant protein sources was associated with a decreased risk of T2D (models 1–3, online Supplementary Table S6).

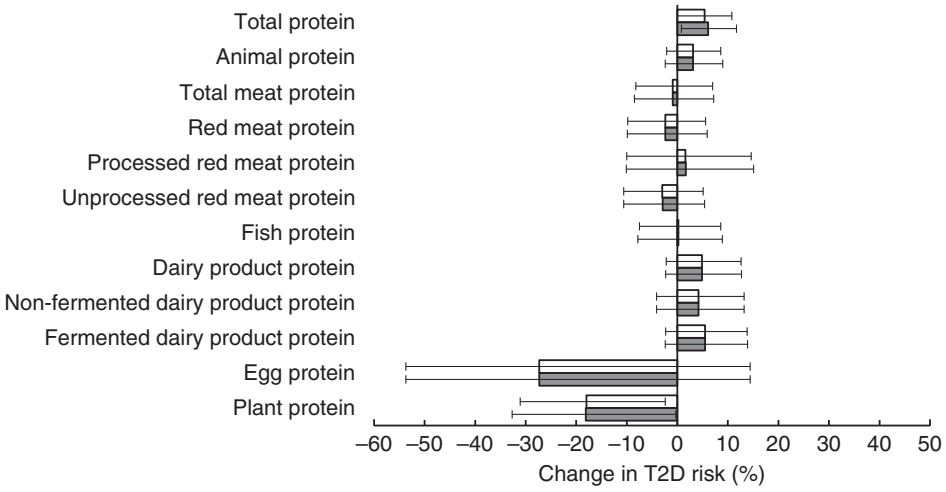

Fig. 1. Change in risk of type 2 diabetes (T2D) with isoenergetic replacement of 1 % of energy from total carbohydrates (□) or high-quality carbohydrates (■) with equal amount of energy from different proteins. Values are hazard ratios and 95 % CI derived from the Cox proportional hazards regression models. Adjusted for age, examination year, energy intake, marital status, income, use of hypertension medication, family history of diabetes, pack-years of smoking, education, leisure-time physical activity, serum ferritin, and intakes of alcohol, Mg, coffee, cholesterol, and SFA, MUFA, PUFA and *trans*-fatty acids. High-quality carbohydrates include carbohydrates from whole-grain products, legumes, nuts, seeds, mushrooms, fruits, berries and vegetables (excluding potatoes). Total meat includes red meat, white meat and offal.

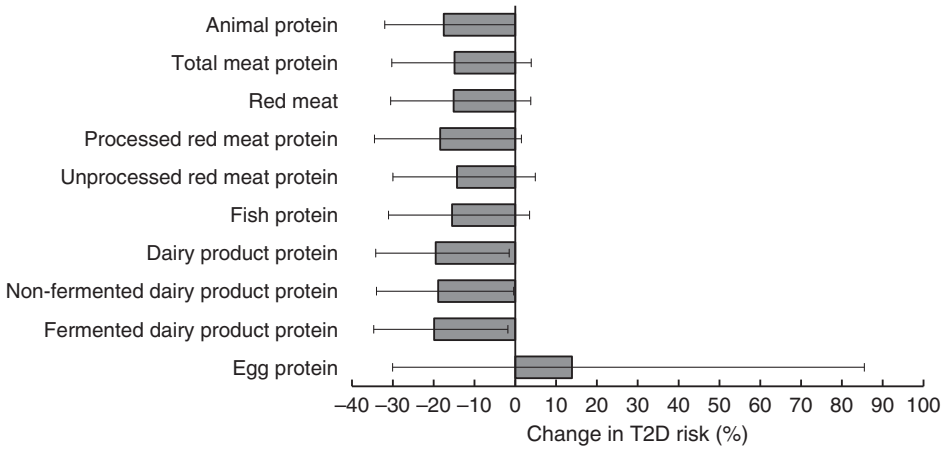

Fig. 2. Change in risk of type 2 diabetes (T2D) with isoenergetic replacement of 1 % of energy from different animal proteins with energy from plant protein. Values are hazard ratios and 95 % CI derived from the Cox proportional hazards regression models. Adjusted for age, examination year, energy intake, marital status, income, use of hypertension medication, family history of diabetes, pack-years of smoking, education, leisure-time physical activity, serum ferritin, alcohol intake, glycaemic index, and intakes of fibre, Mg, coffee, cholesterol, and SFA, MUFA, PUFA and *trans*-fatty acids. Total meat includes red meat, white meat and offal.

This association was markedly attenuated after inclusion of nutrients to the model (model 4).

In the substitution models, replacing 50 g of total meat, total red meat, processed red meat, fish or dairy products with plant protein sources were all associated with a decreased risk of T2D (online Supplementary Fig. S2). These associations were, however, not statistically significant after inclusion of BMI into the models (online Supplementary Table S7). Replacement of 50 g of processed red meat (HR 0·72; 95 % CI 0·57, 0·91), unprocessed red meat (HR 0·77; 95 % CI 0·60, 0·97), fish (HR 0·72; 95 % CI 0·57, 0·91) or dairy products (HR 0·76; 95 % CI 0·61, 0·94) with an equal amount of eggs was also associated with a decreased risk of T2D. Further adjustments for BMI had little impact on these associations (online Supplementary Table S8).

Sensitivity analyses

We tested effect modification by BMI, age, and physical activity. Interactions were not statistically significant ($P_{interactions} > 0{\cdot}05$), except for the intake of protein from non-grain plant sources and BMI (below the median BMI: HR/5 g intake 0·98; 95 % CI 0·59, 1·62; above the median BMI: HR 0·78; 95 % CI 0·56, 1·07 (model 3); $P_{interaction}$ 0·05) and for non-fermented dairy products and age (below the median age: HR/100 g intake 1·00; 95 % CI 0·95, 1·04; above the median age: HR 1·04; 95 % CI 0·99, 1·09 (model 3); $P_{interaction}$ 0·04).

Because BMI is a risk factor for T2D and related to intake of energy and most nutrients, including protein, BMI might also be a confounder instead of a mediator. Adjustment for BMI did not attenuate the statistical significance of the protein associations observed in model 2, but slightly attenuated the association with egg protein in the model 3. After the additional adjustment for BMI, the extreme-quartile HR for the intake of egg protein in model 3 was 0·70 (95 % CI 0·46, 1·06). In the secondary analyses, the extreme-quartile HR for intakes of total meat, processed red meat and major plant protein sources after the additional adjustment for BMI in model 3 were 1·32 (95 % CI 0·99, 1·77), 1·15 (95 % CI 0·87, 1·53) and 0·74 (95 % CI 0·53, 1·04), respectively.

Because the long follow-up time may attenuate associations with the exposures that were assessed only at baseline, we also tested the associations of proteins and protein sources with the risk of T2D after 10 years of follow-up (seventy-two cases). The associations were generally similar, but only the association of egg protein intake with lower risk of T2D was statistically significant (HR 0·17; 95 % CI 0·06, 0·49; model 3). For example, the extreme-quartile HR in model 3 for total, animal and plant protein intakes were 1·07 (95 % CI 0·43, 2·71), 0·98 (95 % CI 0·38, 2·49) and 0·42 (95 % CI 0·13, 1·29), respectively. We also excluded the T2D cases that occurred during the first 2 years of follow-up (n 3), but this did not change the associations.

Discussion

In this population-based cohort study in middle-aged and older Finnish men, total or animal protein intakes were not independently associated with the risk of T2D, but plant and egg protein intakes were associated with a decreased risk. In the substitution models, replacement of energy from carbohydrates with energy from protein was associated with an increased risk of T2D. Replacing animal or dairy product protein or carbohydrates with plant protein was associated with a decreased risk of T2D. Results of food substitution models showed similar beneficial associations of replacing typical animal protein foods with foods rich in plant protein.

Previous studies have observed inconclusive results, with many[10–14], but not all[15–17], suggesting that either total or animal protein intake associates with an increased risk of T2D. Only one previous epidemiological study found plant protein to associate with a decreased risk of T2D[13]. Discrepancy between the results may be due to differences in both quality and quantity of protein and carbohydrates. For example, in our study the total protein intake was only moderate in the highest

quartile (18·6 E%) compared with some other cohorts, in which the intake has been >20 E% in the highest intake group[10,13]. Also, although higher total protein intake was associated with lower carbohydrate intake, the difference was modest (2·4 E% between the lowest and highest quartiles) compared with the majority of studies, which have observed total protein intake to associate with the risk of T2D[10–13].

In our study, replacing both total and high-quality carbohydrates with protein was associated with an increased risk of T2D, whereas a study from the USA indicated a risk increase only when high-quality carbohydrates were replaced[13]. This difference could be explained by the more fundamental role of whole grains in the Finnish diet compared with the American diet[41], given that high whole-grain intake has been associated with a decreased risk of T2D[2,41]. The importance of carbohydrate quality is also emphasised by the finding that replacing carbohydrates with protein was not associated with a risk of T2D after adjustment for fibre.

Our results indicate that fibre intake may not be the only benefit of favouring plant protein sources, as the association of plant protein with a lower risk of T2D remained after adjustment for fibre. Also, replacement of animal protein with plant protein was statistically significant despite fibre adjustment. Thus, the plant protein in particular may be of importance in T2D prevention. In the analyses with diabetes risk factors, plant protein intake was associated with lower plasma glucose concentrations, suggesting that plant protein could affect T2D risk via glucose metabolism. Although trials have indicated that replacing animal protein with plant protein could improve glycaemic control[42], more investigations are needed to confirm these benefits. Furthermore, other factors related to plant protein intake, such as polyphenols, might partly explain the association between plant protein intake and a decreased risk of T2D[32,43].

Our results support the previous observations that the associations between protein intake and T2D risk may be partly mediated by BMI[10–13]. In our cohort, both total and animal protein intakes were associated with higher BMI, and many of the protein–T2D associations were slightly attenuated after adjustment for BMI. However, it is hard to disentangle whether BMI is a mediator or a confounder, and the slight attenuation of the associations after inclusion of BMI into the models might also be due to reduced confounding. Importantly, the advantages of replacing animal proteins with plant protein remained after adjustment for BMI, suggesting that this association was not significantly affected by the weight status.

When animal protein intake was divided into more specific categories, multivariable-adjusted protein models did not show statistically significant associations, except for the association of egg protein with a lower risk of T2D. We have earlier reported in this study population that egg intake was associated with a significantly decreased risk of T2D[44]. Because egg protein intake is highly correlated with egg intake, we cannot conclude whether the beneficial association was due to the whole egg intake or egg protein itself. In addition to being of high quality, egg proteins are suggested to have bioactive functions, such as anti-inflammatory properties[45].

In the models with protein sources, high total meat intake and high total and fermented dairy product intakes indicated an association with an increased risk of T2D. The association between high total meat intake and an increased risk of T2D remained after adjustments for SFA, cholesterol, serum ferritin and BMI, which have been suggested to explain the association between meat intake and risk of T2D[23,40]. High exposure to advanced glycation end products, trimethylamine *N*-oxide, branched chain amino acids, nitrites and Na could thus be more potential factors[23,39,40]. Whereas meta-analyses have generally indicated the most robust association for processed red meat intake[21–23], in our study, total meat intake was the strongest predictor of T2D risk, whereas processed red meat intake showed only a borderline association. Similarly, a more pronounced association for total meat intake was observed in another Finnish study[46]. Interestingly, the association between processed red meat and an increased risk of T2D appears to be stronger in the studies conducted in the USA or in Britain than in studies from other European countries[23]. Differences in the typically consumed meats and preparation methods and in lifestyle factors associated with meat intake could explain these results. For example, intake of bacon, which appears to be especially strongly associated with T2D[22,23], is rare in our population.

Meta-analyses have indicated either inverse[25] or neutral association[26] between total dairy product intake and the risk of T2D. The suggestion for a harmful association in our population may be due to the exceptionally high dairy product intake. The median intake was 689 g, whereas the recent meta-analysis reported medians between 111 and 400 g[25]. Very high dairy product or meat intakes may be markers of an unbalanced diet, which could explain the increased risk of T2D. The indicative association between fermented dairy products and an increased risk of T2D is also contradictory to meta-analyses that have shown fermented dairy product intake, especially yogurt intake, to associate with lower risk of T2D[25,26]. Only 14 % of our study population consumed yogurt, whereas other types of fermented dairy products, such as sour milk, were more typical. Thus, further comparisons of different types of dairy products are essential.

The strengths of this study include the long follow-up and comprehensive information about dietary protein sources and possible confounding factors. Although the 4-d food record provides detailed information about diet and is not prone to memory errors, it may not be the best method for capturing foods that are consumed occasionally. The long follow-up time may have attenuated the relationships between dietary proteins and T2D; however, the associations were not markedly different in the analyses with a shorter follow-up time. Despite extensive adjustments, we cannot exclude the possibility of residual confounding. In free-living people, many dietary factors tend to correlate with each other. Thus, it is hard to disentangle whether the observed associations are due to specific nutrients or foods, or whether the associations rather represent a healthy diet as a whole. For example, high plant protein intake was strongly associated with a healthier lifestyle, which may partly explain its association with a lower T2D risk. The study population included Caucasian middle-aged men, so results may not be generalisable to women and other age groups or to other populations. Finally, the median intake of protein was 18·6 E% in the highest consumption quartile; thus,

the results may not be comparable with high-protein diets that usually provide at least 20 E% of protein[31].

In conclusion, our results suggest that comparatively high protein intake does not independently associate with risk of T2D, but the quality of both protein and carbohydrates modify the risk when protein is consumed in place of carbohydrates. Favouring protein from plant sources and eggs over other animal sources may be beneficial in the prevention of T2D. Mechanisms behind the distinct associations of dietary proteins with T2D risk require further investigation. Long-term interventions comparing diets with different macronutrient composition are also expected to shed more light on the potentiality of higher protein consumption in T2D prevention[3,47].

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The authors declare that there are no conflicts of interest.

Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114517000745

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