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The relationship between fermented food intake and mortality risk in the European Prospective Investigation into Cancer and Nutrition-Netherlands cohort

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Abstract

The objective of the present study was to investigate the relationship between total and subtypes of bacterial fermented food intake (dairy products, cheese, vegetables and meat) and mortality due to all causes, total cancer and CVD. From the European Prospective Investigation into Cancer and Nutrition-Netherlands cohort, 34 409 Dutch men and women, aged 20–70 years who were free from CVD or cancer at baseline, were included. Baseline intakes of total and subtypes of fermented foods were measured with a validated FFQ. Data on the incidence and causes of death were obtained from the national mortality register. Cox proportional hazards models were used to analyse mortality in relation to the quartiles of fermented food intake. After a mean follow-up of 15 (SD 2·5) years, 2436 deaths occurred (1216 from cancer and 727 from CVD). After adjustment for age, sex, total energy intake, physical activity, education level, hypertension, smoking habit, BMI, and intakes of fruit, vegetables and alcohol, total fermented food intake was not found to be associated with mortality due to all causes (hazard ratio upper *v.* lowest quartile ($HR_{Q4\,v.\,Q1}$) 1·00, 95 % CI 0·88, 1·13), cancer ($HR_{Q4\,v.\,Q1}$ 1·02, 95 % CI 0·86, 1·21) or CVD ($HR_{Q4\,v.\,Q1}$ 1·04, 95 % CI 0·83, 1·30). Bacterial fermented foods mainly consisted of fermented dairy foods (78 %) and cheese (16 %). None of the subtypes of fermented foods was consistently related to mortality, except for cheese which was moderately inversely associated with CVD mortality, and particularly stroke mortality ($HR_{Q4\,v.\,Q1}$ 0·59, 95 % CI 0·38, 0·92, $P_{trend} = 0·046$). In conclusion, the present study provides no strong evidence that intake of fermented foods, particularly fermented dairy foods, is associated with mortality.

Key words: Mortality: Cancer: CVD: Fermented foods: Fermented dairy foods

Conventional fermented foods represent about one-third of the foods in the human diet[1]. These foods have been subject to fermentation, a chemical process caused by micro-organisms, such as bacteria, that are either present in or added to the food. The goal of fermentation in these products is to improve the preservation, taste, structure or nutritional value of the food[1]. Besides the use of micro-organisms for fermentation, specific live bacteria, known as probiotics, have been added to foods during the previous decades, because of their suggested, but debatable, role in health improvement, such as reduced risk of cancer and lowering of blood cholesterol levels[2]. Even though the micro-organisms in conventional fermented foods are not particularly used in foods for health improvement, the results from two trials have shown that conventional fermented foods may enhance the immune system[3,4]. A trial in which the diets of thirty subjects were deprived from all fermented food products has shown an adverse effect on both the gut microbiome

Abbreviations: EPIC-NL, European Prospective Investigation into Cancer and Nutrition-Netherlands; HR, hazard ratio; ICD, International Classification of Diseases; MORGEN, Monitoring Project on Risk Factors for Chronic Diseases.

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and the immune system[4]. Another trial has shown that regular intake of yogurt and yogurt enriched with probiotics has similar positive effects on immunological reaction[3]. Observational studies specifically investigating the health effects of conventional fermented foods are scarce. A few studies have differentiated between fermented and non-fermented soya[5,6] or dairy[7–11] foods, and have investigated their associations with the risk of chronic disease incidence and mortality. In one meta-analysis, it has been shown that fermented soya intake is not associated with the occurrence of prostate cancer[6], while another meta-analysis has found an increased risk of gastric cancer compared with non-fermented soya intake[5]. Conversely, several studies have shown that a high intake of fermented dairy foods is associated with a decreased risk of cancer[10,12], while others have shown a weak[9] or no association[13]. Observational studies have reported no association between fermented dairy foods and mortality due to stroke[7,11] or heart disease[7,11,14]. However, two of these studies have found that fermented dairy foods are inversely associated with all-cause mortality[11,14]. In the present study, we investigated the relationship between total and subtypes of bacterial fermented food intake (dairy products, cheese, vegetables and meat) and mortality due to all causes, cancer and CVD in the prospective European Prospective Investigation into Cancer and Nutrition-Netherlands (EPIC-NL) cohort.

Methods

Study population and design

The EPIC-NL cohort is the Dutch contribution to the EPIC and consists of the Prospect-EPIC and Monitoring Project on Risk Factors for Chronic Diseases (MORGEN-EPIC) cohorts[15]. The Prospect-EPIC study included 17 357 women aged 49–70 years, living in Utrecht and vicinity, who participated in the nationwide Dutch breast cancer screening programme. The MORGEN-EPIC cohort consisted of 22 654 men and women aged 20–65 years selected from random samples of the Dutch population in three Dutch towns. Participants were recruited in both studies from 1993 to 1997[15]. At baseline, a general questionnaire and a FFQ were administered to the participants, and a medical examination was performed for blood pressure measurements, anthropometry and blood sampling. All subjects gave informed consent before inclusion. The study complied 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 the Netherlands Organisation for Applied Scientific Research (TNO) Nutrition and Food Research (MORGEN).

From the total cohort (n 40 011), participants who gave no permission for linkage with both vital status and causes of death registries (n 2369) were excluded, as well as participants with missing questionnaires (n 177) or with an implausible energy intake as defined by the extreme percentiles of the ratio of reported energy intake:estimated BMR (n 347), and those with prevalent cancer or CVD at baseline (n 2709), leaving a final sample of 34 409 participants for the analysis.

Dietary assessment

Information on (fermented) food intake was collected by a FFQ, which assessed the average consumption frequency of seventy-nine main food items during the preceding year and allowed for the estimation of the habitual consumption of 178 foods. Portion sizes were estimated by using photographs of several food items. Based on consumption frequencies and portion sizes, the mean daily intake in g/d was calculated for each subject individually[16]. Before the start of the study, the FFQ was validated against twelve 24 h dietary recalls and biomarkers measured in 24 h urine and serum among 121 men and women. Spearman's correlations for relative validity were good for milk and milk products (r 0·71 for men and r 0·79 for women), moderate for cheese (r 0·64 for men and r 0·38 for women) and meat (r 0·47 for men and r 0·70 for women), but weak for vegetables (r 0·38 for men and r 0·31 for women). As the most commonly used micro-organisms in probiotics are bacteria[17], we only focused on fermented food products with bacteria as the most important starter cultures[18,19] that are commonly consumed by the Dutch population. Products fermented with yeast as the main starter culture (bread, wine, beer and alcoholic drinks)[18] or by endogenous enzymes or micro-organisms (cocoa, coffee and tea)[18] were excluded. Thus, 'fermented food' in the remaining part of the present study refers to bacterial fermented foods.

The total fermented food intake comprised fermented dairy foods (yogurt, buttermilk and quark, but no cheese), cheese, fermented meat (dried sausage), fermented vegetables (i.e. sauerkraut, pickles and olives), fermented soya (tempeh) and vinegar. Cheese was treated as a separate subgroup because of the relatively high content of saturated fat and salt compared with other fermented dairy foods. An overview of all the included foods is presented in online Supplementary Table S1.

The FFQ contained three separate items on fermented dairy foods and two on cheese. All other fermented products were components of one or more aggregated items. Subjects could indicate the consumption frequency of food items as times per d, per week, per month or per year, or as never. Additional questions on the frequency of consuming a specific subtype included four multiple-choice categories, i.e. always/mostly, often, sometimes and seldom/never. To convert these relative frequencies to absolute frequencies, 'always/mostly' was defined as 90 % of the absolute frequency of the food item referred to. The categories often, sometimes and seldom/never were defined as 65, 35 and 10 %, respectively. These percentages were defined with the response categories in the FFQ. Frequencies per d were calculated and multiplied with portion sizes to obtain g/d for each food item[16].

Outcome assessment

Vital status was obtained through digital record linkage with the municipal administration registries, and causes of death were provided by Statistics Netherlands (Central Agency for Statistics). Causes of death were coded according to the

Ninth Revision of the International Classification of Diseases (ICD-9) for deaths until 1996 and according to the Tenth Revision (ICD-10) for deaths thereafter. For cause-specific analysis, cause of death was further specified into death from cancer (ICD-9 140–239; ICD-10 C00–D48) and death from stroke (ICD-9 430–434, 436; ICD-10 I60–I66), CHD (ICD-9 410–414, 427.5, 798.1, 798.2, 798.9; ICD-10 I20–I25, I46, R96) and overall CVD (including ICD codes for stroke, CHD and ICD-9 428, 415.1, 443.9, 430–438, 440–442, 444; ICD-10 I26, G45, I60–I67, I69, I70–I74, I50). Additionally, mortality due to other causes than CVD and cancer was used. Follow-up data were complete up to 1 January 2011, and at this time, 2·2 % (*n* 763) of the study population was lost to follow-up.

Assessment of covariates

The general questionnaire contained questions on demographic characteristics, presence of chronic diseases and risk factors for chronic diseases. Smoking status was categorised into never, former and current smoker. Education level was classified into three categories: low (primary education up to completing intermediate vocational education); intermediate (up to higher secondary education); high (higher vocational education and university). Physical activity was assessed through a validated questionnaire, and the Cambridge Physical Activity Index[20] was calculated and used to categorise physical activity. We could not calculate the total physical activity score for 14 % of the participants because of missing data. Therefore, we imputed missing scores by single linear regression modelling (SPSS Missing Value Analysis procedure)[21]. During the baseline physical examination screening, systolic and diastolic blood pressure measurements were made twice in supine position on the right arm using a Boso Oscillomat (Bosch & Son; Prospect-EPIC) or on the left arm using a random zero sphygmomanometer (MORGEN-EPIC), from which the mean was taken. Hypertension was considered as present when one or more of the following criteria were met: systolic blood pressure >140 mmHg; diastolic blood pressure >90 mmHg; self-reported use of antihypertensive medication use; self-report of physician-diagnosed hypertension. Height and weight were measured, and BMI was calculated as weight divided by height squared (kg/m^2). Total and HDL-cholesterol concentrations were measured using standardised enzymatic methods. Alcohol consumption was categorised as follows: 0, 0·1–6·0, 6·1–12·0, 12·1–24·0 and >24 g/d for women and 0, 0·1–6·0, 6·1–12·0, 12·1–24·0, 24·1–60·0 and >60 g/d for men[22].

Data analysis

Baseline characteristics of all the participants were calculated as means and standard deviations, medians and interquartile ranges or percentages. Intakes of total and subtypes of fermented foods were adjusted for total energy intake according to the residual method[23]. Based on the distribution of energy-adjusted intake, quartiles were constructed for each fermented food intake variable. The duration of follow-up was calculated as the interval between the date of study entry and death, loss to follow-up, or 1 January 2011, whichever came first.

Cox regression was used to estimate hazard ratios (HR) and 95 % CI for the relationship between total fermented food intake and all-cause mortality or cause-specific mortality, using the lowest quartile of intake as reference. The analyses were repeated for the different subtypes of fermented foods, such as dairy products, cheese, vegetables and meat, and additionally yogurt. Vinegar and fermented soya foods were not analysed separately, because intakes were too low and the percentage of non-consumers was too high (47 and 75 % for vinegar and fermented soya, respectively). All analyses were stratified for cohort (Prospect or MORGEN). To adjust for potential confounders, three models were constructed. In model 1, HR were adjusted for age, sex and total energy intake (model 1). In model 2, additional adjustment was made for physical activity (inactive, moderately inactive, moderately active and active), education level (low, intermediate and high), hypertension at baseline (yes/no), smoking habit (non-smoker, former smoker and current smoker) and BMI. In model 3, further adjustment was made for energy-adjusted intakes of fruit (continuous), vegetables (continuous) and alcohol (six categories). Instead of adjustment for total vegetable intake, the HR for the association between fermented vegetables and mortality were adjusted for intake of total vegetables excluding fermented vegetables. To test for linear trends, fermented food intake values for each participant were replaced by the median values of the quartile to which they belonged and included in the model as a continuous covariate. To test for non-linear trends, quadratic terms of fermented food intake variables were added to the continuous models. When *P* values for these terms were <0·05, we subsequently constructed restricted cubic splines with four knots. The proportional hazards assumption was checked by calculating Schoenfeld residuals and visual inspection of log–log plots, showing no significant deviations.

In sensitivity analyses, we divided total fermented dairy food intake into low-fat food (<2 % fat) and high-fat food (≥2 % fat), and calculated HR for their associations with mortality. Also, we excluded BMI from the final model to examine whether this variable was an intermediate factor in our analyses. Because of the high salt content of fermented food products, except for dairy products, we included Na intake as a potential confounder in the final model. Since (fermented) food intake was only measured at baseline, misclassification of subjects may have occurred as a result of the changes in dietary behaviour during the follow-up. Therefore, we repeated the analyses for the first 5 years of follow-up by censoring all subjects alive after that time. Because the number of fatal stroke events during the first 5 years was too low (*n* 4), we also repeated the analysis for the first 8 years of follow-up. All analyses were carried out using SAS 9.2 (SAS Institute, Inc.), and results were considered significant when *P*<0·05 (two-sided).

Results

Table 1 presents the baseline characteristics of the 34 409 men and women participating in the EPIC-NL cohort. Of the total population, 74 % were women. The average age of women and men differed and was 51 (SD 12) and 43 (SD 11) years, respectively.

The contribution of fermented food intake to total food intake was 6·4 %. The median intake of total fermented food intake was 128 g/d (interquartile range 70–261) and its main contributors were dairy foods (78 %) and cheese (16 %) (see online Supplementary Table S1). The increase in the energy-adjusted intake of total fermented foods across the quartiles was mainly attributable to the increased intakes of dairy foods and cheese. However, the median intakes of fermented meat and fermented vegetables were relatively low and constant across all the quartiles. On average, subjects who reported a high intake of fermented foods were more likely to be women, older, physically active and hypertensive, but were less likely to smoke. In addition, they reported a higher consumption of fruit and vegetables than subjects in the lower quartiles.

During an average follow-up time of 15 (SD 2·5) years, 2436 deaths occurred, of which 1216 (49·9 %) were caused by cancer and 727 (29·8 %) by CVD. The estimated HR for total fermented food intake and its subtypes in relation to

Table 1. Baseline characteristics and dietary food intakes of 34 409 men and women from the European Prospective Investigation into Cancer and Nutrition-Netherlands cohort according to the quartiles of the energy-adjusted intake of total fermented foods

(Medians and interquartile ranges (IQR); mean values and standard deviations; number of participants and percentages)

	Q1		Q2		Q3		Q4	
	Median	IQR	Median	IQR	Median	IQR	Median	IQR
Subjects (*n*)	8603		8601		8602		8603	
Male								
n	3902		2611		1537		851	
%	45·4		30·4		17·9		9·9	
Age at recruitment (years)								
Mean	45·1		47·1		50·1		53·4	
SD	12·1		12		11·7		10·3	
BMI (kg/m^2)								
Mean	25·5		25·5		25·6		25·9	
SD	4		3·9		4		4·1	
High physical activity level								
n	3474		3552		3712		3781	
%	40·4		41·3		43·2		44	
High education level								
n	1554		1843		1924		1780	
%	18·1		21·5		22·5		20·8	
Current smoker								
n	3651		2746		2151		1806	
%	42·5		32		25·1		21·1	
Hypertension								
n	2880		2966		3126		3604	
%	33·5		34·5		36·3		41·9	
Total energy intake (kcal)								
Mean	2256		2136		1980		1866	
SD	679		615		558		498	
Total energy intake (kJ)								
Mean	9464		8961		8310		7837	
SD	2844		2577		2340		2090	
Dietary food intake*								
Total fermented foods (g/d)	46	33–58	95	82–100	173	148–210	399	322–539
Fermented dairy foods (g/d)	12·4	40–25·5	54·3	33·5–72·6	129	102–165	352	277–492
Low fat (g/d)	4·1	1·4–7·4	23·1	16·3–31·9	83·4	60–119	305	233–444
High fat (g/d)	1·4	0·4–2·7	9·1	6·4–12·3	28·2	21·8–36·6	80	61·4–109
Yogurt (g/d)	3·8	1·0–7·6	26·6	19·2–34·1	62·9	52·2–75·7	144·5	111·7–215·8
Cheese (g/d)	15·8	6·5–26·3	26·8	15·5–42·7	29·2	17·8–46·3	30·2	19·0–45·6
Fermented meat (g/d)	4·5	1·8–9·2	4·7	1·8–10·1	4·3	1·5–10·0	3·9	1·3–9·5
Fermented vegetables (g/d)	2·6	1·5–4·3	3	1·8–4·6	3·1	1·9–4·9	3·3	2·0–5·0
Fruit (g/d)								
Mean	215		242		277		306	
SD	173		156		159		159	
Vegetables (g/d)								
Mean	125		133		141		151	
SD	53		51		54		57	
Ethanol†	5·2	0·8–16	4·4	0·8–13·1	4·3	0·7–13·2	4·7	0·8–14·7

* All foods were adjusted for total energy intake.
† Not adjusted for total energy intake.

all-cause mortality are presented in Table 2. Compared with a low intake of total fermented foods, a high intake was significantly associated with a reduced risk of all-cause mortality after adjustment for model 1 ($HR_{Q4\,v.\,Q1}$ 0·83, 95 % CI 0·74, 0·94). This association attenuated after further adjustment for model 2 ($HR_{Q4\,v.\,Q1}$ 0·97, 95 % CI 0·86, 1·09), and after full adjustment for model 3, no association was observed at all ($HR_{Q4\,v.\,Q1}$ 1·00, 95 % CI 0·88, 1·13).

Similar results were found for most subtypes of fermented foods. Only fermented vegetable intake was associated with a reduced risk of all-cause mortality. After full adjustment, HR for all-cause mortality were 0·98 (95 % CI 0·87, 1·09), 0·86 (95 % CI 0·77, 0·97) and 0·88 (95 % CI 0·78, 1·00) in the second, third and fourth quartiles, respectively (P_{trend} = 0·033). This association was mainly attributable to an inverse association between fermented vegetables and mortality due to other causes than cancer and CVD ($HR_{Q4\,v.\,Q1}$ 0·69, 95 % CI 0·53, 0·90, P_{trend} = 0·003). Other subtypes were not associated with mortality due to causes other than cancer and CVD, except for yogurt, which was related to a lower risk ($HR_{Q4\,v.\,Q1}$ 0·74, 95 % CI 0·58, 0·95, P_{trend} = 0·025).

Separate analyses for cancer mortality (Table 3) showed no association with the intake of total fermented food ($HR_{Q4\,v.\,Q1}$ 1·02, 95 % CI 0·86, 1·21) or any of its subtypes. Total fermented food intake was also not associated with CVD mortality ($HR_{Q4\,v.\,Q1}$ 1·03, 95 % CI 0·83, 1·29). However, cheese consumption in quartiles 2 and 4 was associated with a significantly reduced risk of CVD mortality compared with the lowest quartile ($HR_{Q4\,v.\,Q1}$ 0·80, 95 % CI 0·65, 0·99), but no linear trend was observed (P=0·12). For all the other fermented subtypes, no consistent significant associations with CVD mortality were observed.

CVD mortality was further divided into CHD mortality (n 253) and stroke mortality (n 159) (Table 3). No associations were observed between the intake of total fermented food or any of its subtypes and CHD mortality. For stroke mortality,

Table 2. Association of the intakes of total fermented foods and fermented food groups (in quartiles) with all-cause mortality among 34 409 subjects of the European Prospective Investigation into Cancer and Nutrition-Netherlands cohort (Hazard ratios (HR) and 95 % confidence intervals)

	Q1	Q2		Q3		Q4		
	HR	HR	95 % CI	HR	95 % CI	HR	95 % CI	P_{trend}
Total fermented foods								
Median intake (g/d)	46·1	95·4		173·5		398·6		
No. of deaths	563	579		602		692		
Model 1*	1	0·97	0·86, 1·09	0·85	0·76, 0·96	0·83	0·74, 0·94	0·002
Model 2†	1	1·05	0·94, 1·19	0·99	0·88, 1·11	0·97	0·86, 1·09	0·3
Model 3‡	1	1·06	0·94, 1·20	1·00	0·89, 1·13	1·00	0·88, 1·13	0·6
Fermented dairy foods								
Median intake (g/d)	8·8	52·2		128		351		
No. of deaths	625	524		587		700		
Model 1	1	0·86	0·76, 0·96	0·79	0·71, 0·89	0·80	0·71, 0·89	0·003
Model 2	1	0·95	0·84, 1·07	0·92	0·82, 1·04	0·94	0·84, 1·06	0·5
Model 3	1	0·95	0·84, 1·07	0·94	0·83, 1·05	0·97	0·86, 1·09	0·9
Yogurt								
Median intake (g/d)	3·8	26·2		62·9		144·5		
No. of deaths	673	540		564		659		
Model 1	1	0·84	0·75, 0·94	0·78	0·70, 0·88	0·79	0·71, 0·88	<0·001
Model 2	1	0·94	0·84, 1·06	0·93	0·83, 1·05	0·94	0·84, 1·06	0·5
Model 3	1	0·95	0·85, 1·06	0·94	0·84, 1·06	0·95	0·85, 1·07	0·6
Cheese								
No. of deaths	555	606		621		654		
Median intake (g/d)	6·6	19·6		31·8		53·2		
Model 1	1	0·92	0·82, 1·04	0·90	0·80, 1·01	0·93	0·83, 1·04	0·3
Model 2	1	0·96	0·86, 1·08	0·95	0·85, 1·07	0·99	0·88, 1·11	0·9
Model 3	1	0·97	0·86, 1·09	0·96	0·86, 1·08	1·00	0·89, 1·12	0·9
Fermented vegetables								
No. of deaths	649	629		570		588		
Median intake (g/d)	1·1	2·4		3·7		6·4		
Model 1	1	0·91	0·82, 1·02	0·80	0·71, 0·89	0·80	0·72, 0·90	<0·001
Model 2	1	0·96	0·86, 1·07	0·84	0·75, 0·94	0·85	0·76, 0·95	0·002
Model 3§	1	0·98	0·87, 1·09	0·86	0·77, 0·97	0·88	0·78, 1·00	0·034
Fermented meat								
No. of deaths	533	561		628		714		
Median intake (g/d)	0·6	3·1		6·8		15·9		
Model 1	1	1·04	0·92, 1·17	1·09	0·97, 1·22	1·12	1·00, 1·26	0·048
Model 2	1	1·01	0·90, 1·14	1·02	0·91, 1·15	1·01	0·90, 1·13	0·9
Model 3	1	1·02	0·90, 1·15	1·02	0·91, 1·14	1·00	0·89, 1·12	0·9

* Model 1 was adjusted for age, sex and total energy intake.
† Model 2 was model 1 and further adjusted for smoking habit, BMI, physical activity, education level and hypertension at baseline.
‡ Model 3 was model 2 and further adjusted for intakes of alcohol and energy-adjusted intakes of fruit and vegetables.
§ Adjusted for total vegetable intake without fermented vegetable intake.

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no significant associations were observed, except for a significant inverse association with intake of cheese ($HR_{Q4\,v.\,Q1}$ 0·59, 95 % CI 0·38, 0·92, P_{trend} = 0·046).

Overall, no significant non-linear trends were observed (*P* values between 0·10 and 0·98), except for a borderline significant inverse association between stroke mortality and fermented dairy foods (P_{trend} = 0·06) and low-fat fermented dairy foods (P_{trend} = 0·04).

In sensitivity analyses, the results for high-fat and low-fat fermented dairy foods did not differ from the results for total dairy food intake, except for a significantly lower risk of mortality from non-CVD and non-cancer causes in the third and fourth quartiles of high-fat dairy food intake ($HR_{Q4\,v.\,Q1}$ 0·75, 95 % CI 0·58, 0·94, P_{trend} = 0·11; see online Supplementary Table S2). HR were unaffected by exclusion of BMI or additional adjustment for Na intake as measured with the FFQ (see online Supplementary Table S3).

Analysis of the first 5 years of follow-up only altered HR for the association between cheese intake and CVD mortality ($HR_{Q4\,v.\,Q1}$ 0·95, 95 % CI 0·42, 2·13, P_{trend} = 0·8) and for the associations between non-CVD/non-cancer mortality and intake of yogurt ($HR_{Q4\,v.\,Q1}$ 0·30, 95 % CI 0·08, 1·14, P_{trend} = 0·14) and fermented vegetables ($HR_{Q4\,v.\,Q1}$ 1·39, 95 % CI 0·40, 4·82, P_{trend} = 0·7). Analysis for the first 8 years of follow-up showed no significant associations for any of the subtypes either. However, the HR for the association between cheese intake and stroke mortality did not alter, but was

Table 3. Fully adjusted hazard ratios (HR)* for the association of the intakes of total fermented foods and fermented food subtypes (in quartiles) with cancer and CVD mortality among 34 409 subjects of the European Prospective Investigation into Cancer and Nutrition-Netherlands cohort

(Hazard ratios and 95 % confidence intervals)

	Q1	Q2		Q3		Q4		
	HR	HR	95 % CI	HR	95 % CI	HR	95 % CI	P_{trend}
Cancer mortality								
No. of deaths‡	274	286		308		348		
Total fermented foods	1	1·06	0·89, 1·25	1·03	0·87, 1·23	1·02	0·86, 1·21	0·9
Fermented dairy foods	1	0·97	0·82, 1·15	0·96	0·81, 1·14	0·99	0·84, 1·17	0·9
Yogurt	1	0·94	0·80, 1·11	0·97	0·83, 1·15	1·02	0·86, 1·20	0·6
Cheese	1	1·04	0·88, 1·23	0·98	0·83, 1·16	1·11	0·94, 1·30	0·3
Fermented vegetables†	1	1·03	0·88, 1·21	0·92	0·78, 1·09	0·93	0·77, 1·11	0·3
Fermented meat	1	1·01	0·86, 1·20	1·12	0·95, 1·32	0·99	0·84, 1·17	0·8
CVD mortality								
No. of deaths‡	164	184		174		204		
Total fermented foods	1	1·20	0·97, 1·49	1·03	0·82, 1·29	1·04	0·83, 1·30	0·7
Fermented dairy foods	1	0·98	0·79, 1·22	0·98	0·79, 1·22	0·98	0·79, 1·22	0·9
Yogurt	1	1·09	0·88, 1·33	1·09	0·88, 1·35	1·08	0·87, 1·34	0·6
Cheese	1	0·79	0·64, 0·98	0·88	0·72, 1·09	0·80	0·65, 0·99	0·1
Fermented vegetables†	1	0·98	0·80, 1·21	0·89	0·72, 1·11	1·05	0·83, 1·32	0·7
Fermented meat	1	0·94	0·75, 1·18	0·94	0·75, 1·17	1·17	0·95, 1·44	0·034
CHD mortality								
No. of deaths‡	70	66		68		49		
Total fermented foods	1	1·16	0·82, 1·64	1·23	0·86, 1·75	0·82	0·55, 1·22	0·2
Fermented dairy foods	1	1·00	0·71, 1·41	1·03	0·73, 1·46	0·78	0·53, 1·15	0·2
Yogurt	1	1·13	0·80, 1·58	1·11	0·77, 1·59	1·02	0·70, 1·49	0·9
Cheese	1	0·89	0·63, 1·28	1·01	0·71, 1·43	0·97	0·68, 1·39	0·9
Fermented vegetables†	1	0·97	0·69, 1·36	0·88	0·60, 1·27	1·01	0·68, 1·49	0·9
Fermented meat	1	0·87	0·59, 1·30	1·02	0·70, 1·49	1·29	0·90, 1·84	0·045
Stroke mortality								
No. of deaths‡	35	41		34		49		
Total fermented foods	1	1·07	0·68, 1·70	0·72	0·44, 1·17	0·82	0·51, 1·30	0·3
Fermented dairy foods	1	1·05	0·66, 1·69	0·86	0·54, 1·38	0·89	0·56, 1·41	0·5
Yogurt	1	1·27	0·80, 2·02	1·02	0·64, 1·64	0·96	0·60, 1·52	0·5
Cheese	1	0·60	0·39, 0·93	0·62	0·41, 0·96	0·59	0·38, 0·92	0·046
Fermented vegetables†	1	1·15	0·75, 1·78	0·65	0·39, 1·08	1·05	0·65, 1·70	0·9
Fermented meat	1	1·14	0·72, 1·82	1·01	0·63, 1·61	1·07	0·68, 1·68	0·9
Non-CVD/non-cancer mortality								
No. of deaths‡	143	132		134		161		
Total fermented foods	1	0·97	0·76, 1·24	0·89	0·70, 1·15	0·94	0·73, 1·20	0·7
Fermented dairy foods	1	0·89	0·70, 1·13	0·80	0·63, 1·03	0·94	0·74, 1·19	0·9
Yogurt	1	0·85	0·67, 1·07	0·76	0·60, 0·96	0·74	0·58, 0·94	0·025
Cheese	1	1·09	0·85, 1·39	1·09	0·85, 1·38	1·03	0·81, 1·32	0·9
Fermented vegetables†	1	0·95	0·76, 1·18	0·76	0·59, 0·97	0·69	0·53, 0·90	0·003
Fermented meat	1	1·08	0·85, 1·36	0·95	0·75, 1·21	0·85	0·66, 1·08	0·068

* Adjusted for age, sex, total energy intake, smoking habit, BMI, physical activity, education level, hypertension at baseline, intakes of alcohol and energy-adjusted intakes of fruit and vegetables.
† Adjusted for total vegetable intake without fermented vegetable intake.
‡ Number of deaths across quartiles of total fermented food intake.

no longer significant ($HR_{Q4\,v.\,Q1}$ 0·59, 95 % CI 0·21, 1·62, P_{trend} = 0·3; see online Supplementary Table S3).

Discussion

In the present prospective cohort study consisting of 34 409 Dutch participants, we observed no associations between the intake of total fermented food or any of its subtypes and mortality due to all causes, cancer or CVD.

The strengths of the present study include its prospective design with 15 years of follow-up, large sample size and availability of a wide range of potential confounding factors. However, as in any observational study, we cannot exclude unknown or unmeasured confounding. For instance, salt intake was assessed through a FFQ instead of 24 h urinary Na excretion. Therefore, salt intake was underestimated. However, we would expect that correct adjustment for salt intake would result in the attenuation of the HR, and thus this does not explain the null findings. Another limitation in the present study is the fact that the FFQ was not specifically designed to measure the intake of fermented foods. As a result, information on intakes of particular products was not available. For instance, salami and chorizo were not included in the FFQ, except for salami as pizza topping. An open-ended question on 'which types of cold cuts do you usually eat?' was included. We evaluated the answers to this question by manually checking the FFQ for half of the cohort. The percentage of people who reported intake of salami was very low (<2 %); therefore, salami intake was excluded from the fermented meat analysis. Therefore, we most probably underestimated the intake of fermented meat. Second, tools such as the FFQ are prone to non-differential measurement error, which may have attenuated true associations. The usual daily quantity of consumption of most fermented products, except for dairy foods and cheese, was estimated based on a relative frequency of consumption on a four-point scale (always/mostly, often, sometimes and seldom/never) instead of a quantitative intake per d, per week, per month or per year. Finally, Spearman's correlations for relative validity were good for milk and milk products (0·7 and 0·8 for men and women, respectively), moderate for cheese (0·6 and 0·4 for men and women, respectively) and meat (0·5 and 0·7 for men and women, respectively), but weak for vegetables (0·4 for both men and women)[24]. This may have diluted the relationship of vegetable intake with mortality risk. Finally, since food intake was assessed at baseline only, the consequences of subjects subsequently changing their pattern of fermented food consumption are uncertain. Such changes may have resulted in subject misclassification and explain the null findings in the present study. In contrast, assessment of the long-term reproducibility of the FFQ in the EPIC-Heidelberg cohort, after a mean follow-up of 5·7 years, showed a fairly high correlation between measurements at baseline and at follow-up (correlation coefficient 0·41–0·77)[25]. Moreover, although analyses for a shorter follow-up period of 5 and 8 years altered the HR, no significant associations between fermented foods and mortality were found either.

We observed no associations between total fermented food intake and all-cause mortality. The vast majority of fermented foods in the present study population were dairy foods (78 %) and cheese (16 %). In contrast to two previous observational prospective studies[11,14], we did not find an association between fermented dairy food intake and all-cause mortality in the present study. In the Whitehall II study, authors reported a significantly reduced all-cause mortality risk of 35 % for the highest tertile of fermented dairy food intake compared with the lowest tertile[14]. In *post hoc* analysis, they found that this association was attributable to cancer mortality (HR upper tertile *v.* lowest tertile 0·59, 95 % CI 0·39, 0·91) rather than CVD mortality (HR 0·69, 95 % CI 0·35, 1·36). In the present study, we could not confirm either of these associations. The range of intake in the present study was higher (median 83·7 g/d) than that in the Whitehall II study (median 41 g/d), which may explain this discrepancy. In contrast, fermented dairy foods in the present study (yogurt, buttermilk and quark) contained other products than those used in the previous study (soft and hard cheese and yogurt), which makes these studies difficult to compare. In the Netherlands Cohort Study[11], fermented full-fat milk intake was inversely associated with all-cause mortality in men and women, but no associations were found between low-fat fermented milk and all-cause mortality. Separating low-fat from high-fat fermented dairy foods in the present study did not result in other HR for all-cause mortality risk compared with those for total fermented dairy foods. However, high intakes of high-fat fermented dairy foods and yogurt were inversely associated with mortality due to other causes than cancer and CVD. Nevertheless, both associations attenuated in sensitivity analysis in the first 5 and 8 years of follow-up, and since non-cancer/non-CVD mortality comprises a wide variety of causes and aetiologies, they are unlikely to share one particular underlying mechanism that could explain these associations.

No former studies have investigated the associations between total fermented food intake and cancer mortality. However, the relationship between fermented milk and cancer incidence was investigated earlier. Published results of the relationship between fermented dairy foods and cancer have been found to differ per cancer type. Fermented milk intake has been associated with a reduced risk of breast cancer in a Dutch case–control study[12]. A cohort study has observed a reduced risk of bladder cancer only for a low intake of fermented milk[9], and another study has shown that yogurt intake is associated with a lower risk of colorectal cancer[10]. However, a recent meta-analysis of five cohort studies has found no significant associations between fermented dairy foods or fermented milk and colorectal cancer[13]. In the present study, we observed no relationship between the intake of fermented dairy foods and total cancer mortality, which comprises a group of diseases with widely differing aetiologies. Due to insufficient events per type, the present study lacked power to differentiate between specific types of cancer; however, our null findings for total cancer do not preclude that associations may exist with mortality due to particular types of cancer.

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In the present study, we found an inverse association between cheese intake and CVD mortality. A previous Swedish cohort study[26] has found an inverse association between cheese consumption and CVD incidence, with a magnitude similar to that reported in the present study. They found HR of 0·82, 0·83, 0·82 and 0·90 for quintiles 2, 3, 4 and 5, respectively. The association with CVD mortality in the present study was mainly attributable to an inverse association between cheese consumption and stroke mortality. The underlying mechanism for this observed association is not clear. For instance, in previous studies, plasma markers of dairy fat intake were inversely associated with both blood pressure[27] and stroke incidence[28], which could explain our results. Another nutrient present in cheese is menaquinone-n, which was inversely associated with CVD risk in several previous cohort studies[29,30]. However, a previous study in the EPIC-NL cohort has found no association between menaquinone-n intake and stroke incidence[31], so we find it very unlikely that this micronutrient explains our association. Moreover, the present results are not consistent with those of recent observational studies that reported no consistent associations between cheese intake and stroke incidence[11,32,33], and a previous analysis in the EPIC-NL cohort has shown no association between cheese intake and stroke incidence[7]. The discrepancy with this latter study may be the result of the longer follow-up in this analysis and the outcome of mortality compared with a combination of non-fatal events and mortality. Still, the lack of a plausible underlying mechanism in combination with incomparable results from former cohort studies leads us to believe that the inverse association found in the present study may also be a chance finding. The previous study in the EPIC-NL cohort has observed a trend towards reduced stroke risk for intake of fermented dairy foods[7]. Similarly, restricted cubic splines in the present study showed a trend towards a non-linear inverse association between stroke mortality and fermented dairy foods ($P_{trend} = 0{\cdot}06$), and particularly low-fat fermented dairy foods ($P_{trend} = 0{\cdot}04$).

In the present study population, fermented meat and fermented vegetables represented 4 and 2 % of the total fermented foods, respectively. Even though we observed a borderline significant association between fermented vegetables and all-cause mortality risk, intakes were too low (median 3 g/d, interquartile range 0–36·3) to make any inferences about the risk associated with higher consumption. In combination with the null association that we observed after a shorter follow-up time of 5 years, it is very likely that the association between fermented vegetables and mortality is simply a chance finding, and we cannot draw definitive conclusions for this subtype.

In conclusion, intakes of fermented dairy foods and cheese were not associated with mortality due to all causes, cancer or CVD in this Dutch cohort. Whether other fermented foods in the Dutch diet play an important role in mortality is unlikely based on the results of the present study.

Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S0007114514003766

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