

Fatty acid consumption and incident type 2 diabetes: an 18-year follow-up in the female E3N (Etude Epidémiologique auprès des femmes de la Mutuelle Générale de l'Education Nationale) prospective cohort study

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Abstract

We evaluated the association between dietary estimates of fatty acid (FA) consumption and type 2 diabetes (T2D) risk in the French E3N (Etude Epidémiologique auprès des femmes de la Mutuelle Générale de l'Education Nationale) cohort. In total, 71 334 women without diabetes at baseline were followed up from 1993 to 2011. Diabetes was identified using questionnaires and drug-reimbursement claims, and incident cases were validated. FA consumption in 1993 was estimated from a validated dietary questionnaire. Cox regression estimated hazard ratios (HR) and 95% CI of diabetes risk, comparing the upper tertile group with the lowest. High *n*-3 PUFA consumption was associated with T2D even after adjustment for confounders, including other FA and BMI (HR 1.26; 95% CI 1.13, 1.41; upper tertile compared with lowest). Upon stratification by overweight (BMI \geq 25 kg/m²)/non-overweight, a positive association between total PUFA consumption and T2D was observed, but it was restricted to non-overweight women (HR 1.22; 95% CI 1.05, 1.42), whereas *n*-3 PUFA consumption was associated with increased T2D risk in both BMI strata (BMI $<$ 25 kg/m²: HR 1.19; 95% CI 1.01, 1.40 and BMI \geq 25 kg/m²: HR 1.38; 95% CI 1.20, 1.59). Within the *n*-3 PUFA, high DPA (HR 1.41; 95% CI 1.23, 1.63) and α -linolenic acid (ALA) intakes were associated with increased T2D risk, but the effects of ALA were restricted to overweight women (HR 1.17; 95% CI 1.01, 1.36). Within the *n*-6 PUFA, only arachidonic acid (AA) intake was associated with T2D risk (HR 1.49; 95% CI 1.33, 1.66). The associations with DPA and AA persisted even after adjustment of their principal source in this cohort, the consumption of meat. The effects of PUFA are heterogeneous within the FA group. Intake of DPA and AA may contribute to T2D development.

Key words: Fatty acids: Diabetes: *n*-3 PUFA: *n*-6 PUFA: Study cohorts: Dietary intakes: French women

The incidence of type 2 diabetes (T2D) is influenced by many modifiable risk factors, such as overweight or obesity, physical inactivity, smoking and a poor diet, whose management could prevent 83–91% of cases in women^(1,2). One important risk factor is diet; however, focus has been placed heavily on carbohydrate consumption, although fats are responsible for a large part of energy intake and also have strong metabolic effects. Fatty acids (FA), the constituents of fat, are vital sources of energy and are required for normal growth and development⁽³⁾. Nevertheless, excessive intake of some FA may have negative health effects and their consumption should be limited^(4,5).

Current evidence concerning the association between FA consumption and the risk for T2D is sparse and controversial. Some studies have found no association between total fat intake and incidence of T2D^(6–8); yet there is evidence to suggest that FA groups, and even individual FA within these groups, can have different effects on T2D risk^(6–10). Meta-analyses also suggest that the effects of *n*-3 PUFA intake differ by geographical region, with high intake associated with an increased risk for T2D in Western countries, no association in European countries and a decreased risk in Asian countries^(11–13). Moreover, *n*-3 and *n*-6 PUFA are almost always studied in their groups, and the effects of the individual PUFA rest scarcely

Abbreviations: AA, arachidonic acid; ALA, α -linolenic acid; E3N, Etude Epidémiologique auprès des femmes de la Mutuelle Générale de l'Education Nationale; FA, fatty acid; HR, hazard ratio; T2D, type 2 diabetes; TFA, *trans*-unsaturated fatty acids.

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examined despite the present *n*-3 PUFA health craze. In addition, most studies take the intake of other macronutrients into account when examining FA; however, many do not control for the consumption of the remaining FA, which is important, as groups and individual FA are never ingested in isolation and influence the metabolism of each other⁽¹⁴⁾.

The objective of this study was therefore to examine the associations between the main dietary FA intakes and the incidence of T2D. In particular, we focused on the relationship between *n*-3 and *n*-6 PUFA while adjusting for the concomitant intake of other FA.

Methods

Study cohort

E3N (Etude Epidémiologique auprès des femmes de la Mutuelle Générale de l'Éducation Nationale) is a prospective French cohort study of 98 995 females born between 1925 and 1950 and initiated in 1990. It is the French constituent of the large European Prospective Investigation into Cancer and Nutrition (EPIC), and the EPIC sub-study devoted to diabetes: interaction of genetic and lifestyle factors on the incidence of type 2 diabetes (InterAct). Data are available from mailed questionnaires that participants have returned every 2–3 years, in addition to a drug-reimbursement claims database that has been available since 2004 from the participant's medical records (Mutuelle Générale de l'Éducation Nationale). The average response rate for each of the eleven questionnaire cycles has been 83%, and to date the total loss to follow-up since 1990 has been 3% (women who either never answered a questionnaire, withdrew from the study, or whose contact information was lost). All women signed letters of informed consent, in compliance with the French National Commission for Computerized Data and Individual Freedom. From the 98 995 women in the cohort, women who did not complete the dietary questionnaire (*n* 24 473), who had pre-existing diabetes (*n* 859), did not complete any questionnaires after the dietary questionnaire (*n* 890), or who had extreme values for the ratio between energy intake and required energy (i.e. the 1st and 99th percentiles of the distribution in the population) (*n* 1439) were excluded. Thus, the analysis included 71 334 women, of whom 2610 had a validated T2D diagnosis during follow-up (June 1993–December 2011).

Assessment of fatty acid consumption

In June 1993, a validated 208-item dietary questionnaire was sent to participants to assess the habitual diet of the previous year. Food and drink consumption throughout the day took French meal patterns into account, as questions regarding each meal occasion, as well as snacks and appetisers, were also included. Divided into two sections, the first section of the questionnaire included habitual frequency and portion size questions, based on sixty-six food types and items grouped by meals. To quantify frequency, eleven categories were used: never or less than once per month; 1, 2 or 3 times/month; or 1–7 times/week. Portion sizes were indicated with the aid of a photo booklet. The second section had a detailed qualitative description of each food

consumed from each food group and the frequency of its consumption (never; 1–3 times/month; or 1–7 times/week). After weighing the data from the first section by that from the second section, the average daily consumption of 208 types of foods and drinks was evaluated. The validity and reproducibility of the questionnaire have been previously described⁽¹⁵⁾.

FA consumption was then estimated using the database of food nutritional composition published by French Agency for Food, Environmental, and Occupational Health & Safety. For each woman, intakes of the following principal FA were obtained: total SFA, total MUFA, total PUFA (including total *n*-3 PUFA and total *n*-6 PUFA), and total *trans*-unsaturated fatty acids (TFA) (g/d). In addition, we also estimated the consumptions of *n*-6 PUFA – linoleic acid (LA; 18:2*n*-6) and arachidonic acid (AA; 20:4*n*-6) – and *n*-3 PUFA – α -linolenic acid (ALA; 18:3*n*-3), EPA (20:5*n*-3), DPA (22:5*n*-3) and DHA (22:6*n*-3).

Ascertainment of diabetes

Cases of T2D were first identified either in follow-up questionnaires (with declaration of at least one of the following: T2D; a self-reported, physician-recommended diabetic diet; use of diabetic medication; or hospitalisation due to diabetes) or they were identified as receiving diabetic drug reimbursements from health insurance records at least once between January 2004 and March 2012. Those identified through both methods were considered validated. All women were mailed a diabetes-specific questionnaire that included questions on the circumstances of the diagnosis (year, symptoms, biological exams, etc.), management (diabetic diet, physical activity, medications) and results of their most recent concentrations of fasting glucose and HbA1c. Cases were validated if one of the following was met: fasting plasma glucose ≥ 7.0 mmol/l; random glucose ≥ 11.1 mmol/l at diagnosis; report of diabetic medication use; or last values of fasting glucose or HbA1c concentrations ≥ 7.0 mmol/l or $\geq 7\%$, respectively. Prevalent and non-validated cases were excluded from analyses. Consequently, 2610 validated cases of incident diabetes and 68 724 non-cases were analysed in the present study.

Statistical analysis

For time-independent variables with <5% of values missing during follow-up, missing values were imputed with the median of the study population (quantitative variables) or the mode (qualitative variables). In the case of $\geq 5\%$ of missing values, a 'missing' category was created. For time-dependent variables presenting with a missing value during follow-up, the value was imputed by the preceding provided value. If the missing value was the baseline value, it was imputed with the first available value.

Because of the high correlation between energy intake and nutrient intake, FA consumption was energy adjusted using the residual method to allow analyses independent of total energy intake⁽¹⁶⁾. Each FA group was then divided into tertile groups of consumption, with women in the first tertile group of intake systematically employed as the reference category. Cox multi-variable regression models with age as the timescale were used

to estimate hazard ratios (HR) and 95% CI of T2D risk. All variables were found to satisfy the proportional hazards assumption. The time at entry was the age at the start of follow-up and the exit time was the age when participants were diagnosed with diabetes, died, were lost to follow-up, or were censored at the end of the follow-up period, whichever occurred first. The univariate model included only the FA in question and was followed by model 1, which was adjusted for daily energy intake (kJ/d or kcal/d), alcohol consumption (grams of ethanol per 100 g/d) and non-dietary variables such as level of education (undergraduate *v.* postgraduate), smoking status (never *v.* current/former), hypertension (yes *v.* no), hypercholesterolaemia (yes *v.* no), family history of diabetes (yes *v.* no) and physical activity (metabolic equivalents (MET)-h/week). Model 2 additionally adjusted for all types of FA besides the primary considered, allowing for the examination of the independent effects of specific FA subtypes. Finally, model 3 was adjusted for BMI (<20, 20–25, 25–30 or >30 kg/m²).

In addition, stratification by BMI (<25 and ≥25 kg/m²) was performed to determine whether the effects of FA groups varied between overweight and non-overweight women. Models were adjusted in the same manner as in the analyses for the whole population, first for non-dietary variables, alcohol consumption and energy intake, and subsequently for other FA groups. All statistical analyses used SAS 9.2 software (PHREG procedure for Cox models; SAS Institute Inc.). All statistical tests were two-sided and considered significant at $P < 0.05$.

Sensitivity analyses

In order to test for potential reverse-causation, analyses were performed on a subpopulation excluding women who developed diabetes in the first 5 years after inclusion in the study. Individual *n*-3 and *n*-6 PUFA models were also adjusted for meat consumption, to account for residual confounding related to a major source of the FA, as it has already been reported in our cohort that a high intake of processed red meat was associated with an increased T2D risk⁽¹⁷⁾. Scores adhering to two dietary patterns, 'Mediterranean/Prudent' (characterised by a high diet quality, with a high consumption of fruit, vegetables, seafood, olive oil and sunflower oil) and 'Western' (characterised by a lower dietary quality, with a high consumption of meat, French fries, rice, pasta, potatoes, alcohol, butter, eggs, etc.), were also included in the models. These dietary quality scores were derived using principal component analysis and have been previously described in published work from our cohort⁽¹⁸⁾.

Results

Baseline characteristics

In total, 2610 cases of incident T2D were diagnosed during 1 158 131 person-years between June 1993 and December 2011. The incidence of T2D was 225/100 000 person-years. Median follow-up was 18 years (ranging from 29 d to 18 years) for non-cases and 11 years (ranging from 18 d to 18 years) for cases. Mean age of the study participants was 52.9 (SD 6.7) (Table 1). The majority (66%) of women had a healthy BMI (20–24.9 kg/m²),

whereas 19.5% had an excess weight, including 3.3% obese women. Hypertension was declared by 37.1% of women and hypercholesterolaemia by 7.1%. In total, 11.1% of women reported a family history of diabetes. The average daily energy intake was 8916 (SD 2276) kJ/d (2131 (SD 544) kcal/d) and the average physical activity was 49.4 (SD 50.5) MET-h/week. Mean FA group consumptions were as follows: 36.4 (SD 9.9) for SFA, 31.4 (SD 9.9) for MUFA, 1.6 (SD 0.7) for TFA, 14.2 (SD 5.4) for PUFA, 1.5 (SD 0.5) for *n*-3 PUFA, and 12.6 (SD 5.0) for *n*-6 PUFA g/d.

SFA

SFA consumption above the upper tertile was associated with an increased risk for T2D in the univariate analysis (HR 1.13; 95% CI 1.02, 1.24), and in the multivariable model adjusted for non-dietary factors, energy intake and alcohol (model 1: HR 1.17; 95% CI 1.06, 1.28) (Table 2), when compared with the first tertile group. Further adjustment for other FA groups slightly decreased the association, rendering it non-significant (HR 1.13; 95% CI 0.97, 1.31).

MUFA

MUFA consumption above the first tertile was associated with a higher risk for T2D in the unadjusted model and model 1 (third tertile group: HR 1.15; 95% CI 1.04, 1.27 and HR 1.32; 95% CI 1.20, 1.46, respectively). Additional adjustment for FA groups in model 2 decreased the strength of the association in the second tertile group (HR 1.10; 95% CI 0.99, 1.22), whereas the association in the third tertile group became statistically non-significant after adjustment for BMI (model 3: HR 1.06; 95% CI 0.95, 1.19).

PUFA

PUFA intake was associated with a 29% increased risk for T2D in women above the third tertile of intake after adjustment for other types of FA in model 2 (HR 1.29; 95% CI 1.17, 1.43). However, there was no longer an association after adjusting for BMI (model 3: HR 1.06; 95% CI 0.96, 1.17). Positive associations were observed for both *n*-3 and *n*-6 PUFA in model 1, but after adjusting for other FA and BMI only the association above the third tertile of *n*-3 PUFA intake remained (HR 1.26; 95% CI 1.13, 1.41).

Trans-unsaturated fatty acids

The second tertile group of TFA intake was associated with a lower T2D risk in the univariate analysis (HR 0.87; 95% CI 0.79, 0.96) and in model 2 (HR 0.88; 95% CI 0.78, 0.98), adjusted for other FA, but not in model 1 (HR 0.93; 95% CI 0.85, 1.02). After adjustment for BMI, high TFA intake showed a borderline statistically significant association with T2D (HR 0.90; 95% CI 0.80, 1.00).

Stratification by BMI

After stratification by BMI, in the fully adjusted models, neither SFA nor MUFA nor TFA were associated with T2D risk in either BMI stratum (Fig. 1, online Supplementary Table S1). Regarding total PUFA, a high intake was associated with increased T2D

Table 1. Baseline characteristics of the study population (E3N (Etude Epidémiologique auprès des femmes de la Mutuelle Générale de l'Education Nationale) cohort data; *n* 71 334 women) (Mean values and standard deviations; numbers and percentages)

Variables	Study population (<i>n</i> 71 334)	SD or (%)	Diabetes non-cases (<i>n</i> 68 724)	SD or (%)	Incident diabetes cases (<i>n</i> 2610)	SD or (%)
Age (years)	52.9	6.7	5.8	6.7	54.4	6.7
BMI (kg/m ²) (<i>n</i> (%))						
<20	10 307	(14.5)	10 239	(14.9)	68	(2.6)
20–24.9	47 101	(66.0)	46 073	(67.0)	1028	(39.4)
25–30	11 554	(16.2)	10 572	(15.4)	982	(37.6)
>30	2372	(3.3)	1840	(2.7)	532	(20.4)
Birth cohort (<i>n</i> (%))						
Before 1930	5437	(7.6)	5186	(7.6)	251	(9.6)
1930–1934	9175	(12.9)	8702	(12.7)	473	(18.1)
1935–1939	13 530	(19.0)	12 991	(18.9)	539	(20.7)
1940–1944	17 142	(24.0)	16 494	(24.0)	648	(24.8)
After 1944	26 050	(36.5)	25 351	(36.9)	699	(26.8)
Education (<i>n</i> (%))						
Undergraduate	7913	(11.1)	7493	(10.9)	420	(16.1)
Graduate/postgraduate	63 421	(88.9)	61 231	(89.1)	2190	(83.9)
Smoking status (<i>n</i> (%))						
Never	38 504	(54.0)	37 115	(54.0)	1389	(53.2)
Current/former	32 830	(46.0)	31 609	(46.0)	1221	(46.8)
Hypertension (<i>n</i> (%))						
No	44 857	(62.9)	43 773	(63.7)	1084	(41.5)
Yes	26 477	(37.1)	24 951	(36.3)	1526	(58.5)
Hypercholesterolaemia treatment (<i>n</i> (%))						
No	66 297	(92.9)	64 085	(93.3)	2212	(3.1)
Yes	5037	(7.1)	4639	(6.8)	398	(15.3)
Family history of T2D (<i>n</i> (%))						
No	63 432	(88.9)	61 451	(89.4)	1981	(75.9)
Yes	7902	(11.1)	7273	(10.6)	629	(24.1)
Physical activity (MET-h/week)	49.4	50.5	49.3	50.1	50.0	59.2
Alcohol consumption (g of ethanol per 100 g/d)	11.6	13.9	11.6	13.8	12.1	15.9
Daily energy intake (kJ/d)	8914.4	2274.8	8906.8	2266.1	9114.8	2481.5
Daily energy intake (kcal/d)	2130.6	543.7	2128.8	541.6	2178.5	593.1
Average consumption (g/d)						
SFA	36.4	13.0	36.4	12.9	37.6	14.1
MUFA	31.3	9.9	31.3	9.8	32.6	10.8
TFA	1.6	0.7	1.6	0.7	1.6	0.7
PUFA	14.2	5.3	14.1	5.3	15.2	5.9
<i>n</i> -3 PUFA	1.5	0.5	1.5	0.5	1.6	0.6
<i>n</i> -6 PUFA	12.6	5.0	12.6	5.0	13.6	5.6

T2D, type 2 diabetes; MET, metabolic equivalents; TFA, *trans*-unsaturated fatty acids.

risk in non-overweight women (HR 1.22; 95% CI 1.05, 1.42), but not in overweight women (HR 1.02; 95% CI 0.90, 1.16). *n*-6 PUFA were not associated with T2D risk in either BMI stratum (HR 1.13; 95% CI 0.96, 1.32 and HR 0.95; 95% CI 0.83, 1.08, respectively, for non-overweight and overweight women), whereas high *n*-3 PUFA consumption was associated with an increased risk for T2D in both strata in fully adjusted models (HR 1.19; 95% CI 1.01, 1.40 and HR 1.38; 95% CI 1.20, 1.59, respectively, for non-overweight and overweight women).

n-6 Fatty acids

LA was not significantly associated with T2D risk in the whole population (HR 0.97; 0.87, 1.07) or in either BMI stratum (Table 3). Conversely, a high consumption of AA was associated with an almost 50% increased risk for T2D (HR 1.49; 95% CI 1.33, 1.66). Within BMI strata, AA intake above the third tertile was associated with T2D risk in non-overweight women (HR 1.50; 95% CI 1.28, 1.76) and intake above the second tertile

was associated with T2D risk in overweight women (HR 1.74; 95% CI 1.49, 2.03).

n-3 Fatty acids

In the full population, neither ALA intake nor intake of long-chain *n*-3 PUFA (EPA or DHA) was associated with T2D in the fully adjusted models (HR 1.03; 95% CI 0.92, 1.15; HR 0.88; 95% CI 0.67, 1.15; HR 1.11; 95% CI 0.85, 1.44, respectively). However, high DPA intake above the second tertile was associated with an increased risk for T2D (HR 1.41; 95% CI 1.23, 1.63). There was no association with EPA or DHA in models stratified on BMI. However, high ALA intake was associated with an increased T2D risk in women with excess weight (HR 1.17; 95% CI 1.01, 1.36) for the highest tertile group, but not in the normal-weight women (HR 0.90; 95% CI 0.75, 1.07). High DPA intake was associated with an increased risk for T2D in both BMI strata (HR 1.45; 95% CI 1.17, 1.80 and HR 1.54; 95% CI 1.27, 1.85, respectively).

Table 2. Cox proportional hazards ratios for the risk for incident type 2 diabetes by fatty acid consumption (g/d) in the E3N (Etude Epidémiologique auprès des femmes de la Mutuelle Générale de l'Education Nationale) study cohort (*n* 71 334 women)(Hazard ratios (HR) and 95% confidence intervals)

Variables (g/d)	No. of cases/ non-cases	Univariate		Model 1*		Model 2†		Model 3‡	
		HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI
SFA									
<33.3	843/22 698	1 (Ref.)		1 (Ref.)		1 (Ref.)		1 (Ref.)	
33.3–38.9	847/22 692	1.04	0.95, 1.14	1.10	1.00, 1.21	1.11	0.99, 1.25	1.06	0.95, 1.19
≥38.9	920/23 334	1.13	1.02, 1.24	1.17	1.06, 1.28	1.13	0.97, 1.31	1.07	0.92, 1.25
<i>P</i> _{for trend}		0.01		0.001		0.14		0.40	
MUFA									
<28.7	785/22 755	1 (Ref.)		1 (Ref.)		1 (Ref.)		1 (Ref.)	
28.7–33.3	839/22 702	1.11	1.01, 1.23	1.15	1.04, 1.27	1.10	0.99, 1.22	1.01	0.91, 1.12
≥33.3	986/23 267	1.31	1.19, 1.44	1.32	1.20, 1.46	1.23	1.10, 1.38	1.06	0.95, 1.19
<i>P</i> _{for trend}		<0.0001		<0.0001		<0.0001		0.25	
PUFA									
<12.0	720/22 821	1 (Ref.)		1 (Ref.)		1 (Ref.)		1 (Ref.)	
12.0–15.3	835/22 704	1.17	1.06, 1.29	1.14	1.03, 1.26	1.13	1.02, 1.25	1.03	0.93, 1.14
≥15.3	1055/23 199	1.46	1.33, 1.61	1.34	1.21, 1.47	1.29	1.17, 1.43	1.06	0.96, 1.17
<i>P</i> _{for trend}		<0.0001		<0.0001		<0.0001		0.24	
<i>n</i>-6 PUFA									
<10.5	734/22 807	1 (Ref.)		1 (Ref.)		1 (Ref.)		1 (Ref.)	
10.5–13.7	840/22 700	1.15	1.04, 1.27	1.13	1.02, 1.25	1.07	0.96, 1.18	1.01	0.91, 1.12
≥13.7	1036/23 217	1.41	1.28, 1.55	1.29	1.17, 1.42	1.15	1.04, 1.27	1.00	0.90, 1.10
<i>P</i> _{for trend}		<0.0001		<0.0001		<0.01		0.94	
<i>n</i>-3 PUFA									
<1.3	665/22 876	1 (Ref.)		1 (Ref.)		1 (Ref.)		1 (Ref.)	
1.3–1.6	819/22 720	1.25	1.13, 1.38	1.23	1.11, 1.37	1.18	1.06, 1.31	1.10	0.99, 1.22
≥1.6	1126/23 128	1.68	1.52, 1.84	1.60	1.45, 1.76	1.47	1.32, 1.64	1.26	1.13, 1.41
<i>P</i> _{for trend}		<0.0001		<0.0001		<0.0001		<0.0001	
TFA									
<1.4	920/22 621	1 (Ref.)		1 (Ref.)		1 (Ref.)		1 (Ref.)	
1.4–1.7	794/22 745	0.87	0.79, 0.96	0.93	0.85, 1.02	0.88	0.78, 0.98	0.90	0.80, 1.00
≥1.7	896/23 358	0.98	0.89, 1.07	1.03	0.94, 1.13	0.93	0.81, 1.07	1.02	0.89, 1.17
<i>P</i> _{for trend}		<0.01		0.79		0.25		0.85	

Ref., referent values; TFA, *trans*-unsaturated fatty acids.

* Model 1: adjusted for daily energy intake, alcohol consumption and non-dietary factors such as level of education, family history of diabetes, physical activity, hypertension, hypercholesterolaemia and smoking status.

† Model 2: model 1 + tertile groups of remaining fatty acid groups.

‡ Model 3: model 2 + BMI.

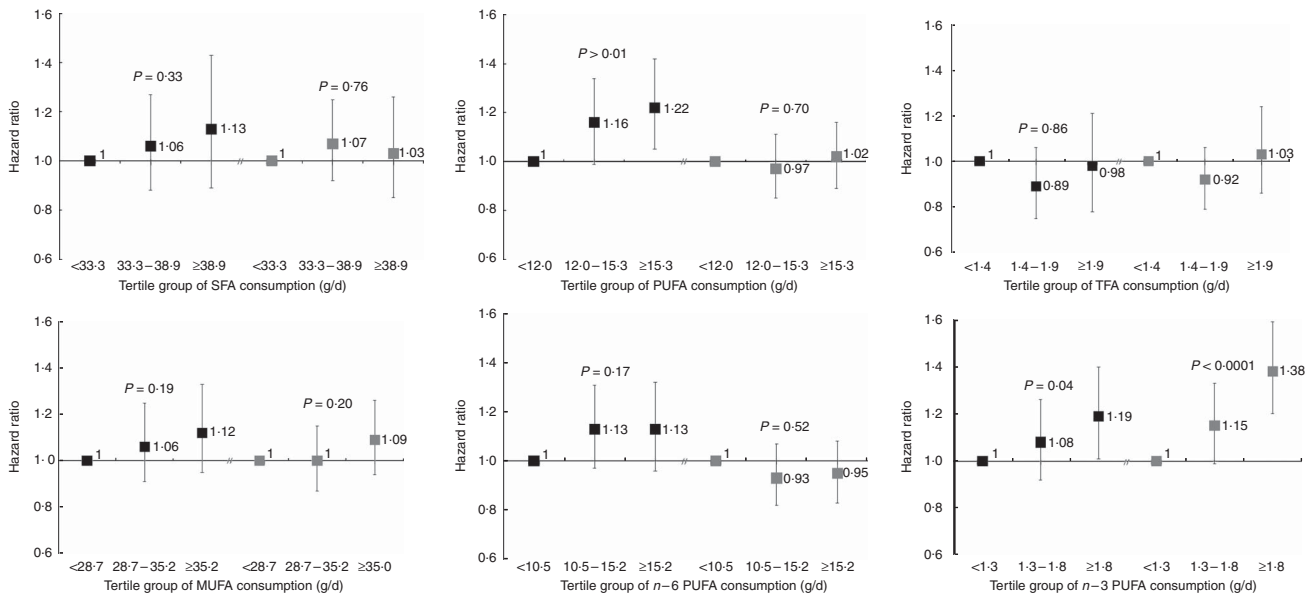


Fig. 1. BMI-stratified Cox proportional hazard ratios (95% CI) of type 2 diabetes by tertile group of fatty acid consumption (g/d). Models adjusted for daily energy intake, alcohol consumption, level of education, family history of diabetes, physical activity, hypertension, hypercholesterolaemia, smoking status and tertile groups of remaining fatty acid groups. TFA, *trans*-unsaturated fatty acids. ■, BMI < 25 kg/m²; □, BMI ≥ 25 kg/m².

Table 3. Cox proportional hazards ratios of the risk for incident type 2 diabetes by *n*-3 and *n*-6 PUFA consumption (g/d) with BMI stratification in the E3N (Etude Epidémiologique auprès des femmes de la Mutuelle Générale de l'Education Nationale) study cohort (*n* 71 334) (Hazard ratios (HR) and 95% confidence intervals)

Variables (g/d)	No. of cases/ non-cases	<i>n</i> 71 334		No. of cases/ non-cases	BMI < 25 kg/m ² (<i>n</i> 57 408)		No. of cases/ non-cases	BMI ≥ 25 kg/m ² (<i>n</i> 13 926)	
		HR	95% CI		HR	95% CI		HR	95% CI
ALA									
<0.90	757/22 784	1 (Ref.)		358/19 213	1 (Ref.)		399/3571	1 (Ref.)	
0.90–1.14	843/22 696	1.00	0.90, 1.12	365/18 649	0.93	0.79, 1.09	478/4047	1.08	0.94, 1.25
≥1.14	1010/23 244	1.03	0.92, 1.15	373/18 450	0.90	0.75, 1.07	637/4794	1.17	1.01, 1.36
<i>P</i> _{for trend}			0.62			0.24			0.04
EPA									
<0.09	711/22 830	1 (Ref.)		329/19 275	1 (Ref.)		382/3555	1 (Ref.)	
0.09–0.20	823/22 716	0.88	0.73, 1.06	344/18 694	0.86	0.65, 1.14	479/4022	0.87	0.67, 1.12
≥0.20	1076/23 178	0.88	0.67, 1.15	423/18 343	0.79	0.53, 1.19	653/4835	0.93	0.65, 1.32
<i>P</i> _{for trend}			0.51			0.32			0.96
DPA									
<0.05	610/22 931	1 (Ref.)		307/19 912	1 (Ref.)		303/3019	1 (Ref.)	
0.05–0.08	805/22 734	1.15	1.02, 1.30	364/18 723	1.23	1.04, 1.47	441/4011	1.15	0.98, 1.36
≥0.08	1195/23 059	1.41	1.23, 1.63	425/17 677	1.45	1.17, 1.80	770/5382	1.54	1.27, 1.85
<i>P</i> _{for trend}			<0.0001			<0.01			<0.0001
DHA									
<0.19	702/22 839	1 (Ref.)		327/19 243	1 (Ref.)		375/3596	1 (Ref.)	
0.19–0.38	839/22 701	1.15	0.95, 1.38	344/18 743	1.06	0.80, 1.41	495/3958	1.23	0.96, 1.58
≥0.38	1069/23 184	1.11	0.85, 1.44	425/18 326	1.24	0.83, 1.86	644/4858	1.01	0.71, 1.43
<i>P</i> _{for trend}			0.65			0.27			0.62
LA									
<10.3	740/22 801	1 (Ref.)		330/19 348	1 (Ref.)		410/3453	1 (Ref.)	
10.3–13.5	838/22 702	0.98	0.89, 1.08	382/18 764	1.10	0.94, 1.28	456/3938	0.90	0.78, 1.03
≥13.5	1032/23 221	0.97	0.87, 1.07	384/18 200	1.08	0.92, 1.26	648/5021	0.91	0.80, 1.04
<i>P</i> _{for trend}			0.52			0.39			0.25
AA									
<0.19	579/22 961	1 (Ref.)		327/20 068	1 (Ref.)		252/2893	1 (Ref.)	
0.19–0.25	750/22 790	1.11	0.99, 1.24	329/18 869	1.05	0.90, 1.23	421/3921	1.28	1.09, 1.50
≥0.25	1281/22 973	1.49	1.33, 1.66	440/17 375	1.50	1.28, 1.76	841/5598	1.74	1.49, 2.03
<i>P</i> _{for trend}			<0.0001			<0.0001			<0.0001

ALA, α-linolenic acid (18:3*n*-3); Ref., referent values; EPA, 20:5*n*-3; DPA, 22:5*n*-3; DHA, 22:6*n*-3; LA, linoleic acid (18:2*n*-6); AA, arachidonic acid (20:4*n*-6).

Sensitivity analysis

In a sensitivity analysis excluding the 388 women who developed diabetes during the first 5 years of follow-up, thus on a population of 70 946 women with 2222 incident cases of diabetes, the associations were similar to those in the full analysis (online Supplementary Table S2).

Meat was the food group that contributed most to both AA and DPA intakes (42.7 and 31.3%, respectively). Dietary ALA primarily came from dairy products (11.9%), followed by fats (8.2%), olives (7.0%), and meat, processed meats and offal (6.9%). After additional adjustment for total meat consumption, including processed meats and offal, ALA intake was no longer associated with T2D risk (HR 1.03; 95% CI 0.92, 1.15) (online Supplementary Table S3), whereas high intakes of DPA and AA remained associated with T2D risk (HR 1.21; 95% CI 1.03, 1.42 and HR 1.40; 95% CI 1.23, 1.59, respectively). After stratification by BMI, high DPA intake was associated with T2D risk only in women with BMI ≥ 25 kg/m² (HR 1.33; 95% CI 1.08, 1.64), whereas high AA intake increased T2D risk in the non-overweight strata (HR 1.31; 95% CI 1.09, 1.59) and all intakes above the reference tertile group increased T2D risk in the overweight BMI strata (HR 1.68; 95% CI 1.41, 2.00, third tertile group compared with first tertile group).

Adjustment for the overall dietary pattern using scores adhering to 'Mediterranean/Prudent' or 'Western' dietary patterns yielded similar results, with the exception of a modest increase in risk for high TFA intake (results not shown).

Discussion

In this prospective cohort of middle-aged women, high total *n*-3 PUFA intake was associated with T2D risk, irrespective of the BMI. Yet, within the *n*-3 PUFA, only DPA and ALA (ALA in overweight women only) were associated with increased T2D risk. Total *n*-6 PUFA intake was not associated with T2D risk, but AA intake was associated with increased T2D risk. The other groups of FA (SFA, MUFA, TFA) were not associated with T2D risk.

SFA

The absence of an association between SFA intake and T2D risk has already been reported^(7,8,19–21). However, biomarkers of very long-chain SFA and odd-chain SFA have been associated with decreased T2D risk, and even-chain SFA with increased risk, either individually or combined^(10,21,22). Biomarkers of odd-chain SFA are considered representative of dietary

intake⁽¹⁰⁾, whereas even-chain SFA are also endogenously produced⁽²³⁾. It can thus be suggested that the absence of an association in our study could be due to heterogeneous effects within SFA; yet this phenomenon should also be estimated using dietary intake of SFA.

MUFA

Our findings that MUFA intake was not associated with incident T2D are in agreement with other studies^(7,8,21). Yet, some studies have found extra virgin olive oil, an important source of MUFA⁽²⁴⁾, to be beneficial for T2D risk^(25,26). However, in our cohort, dairy and meat intakes are the primary sources of MUFA (13.4 and 11.9%, respectively) and prospective studies suggest either an inverse or no association of dairy intake with T2D risk^(27,28) and a negative association of meat intake with T2D risk^(17,28), offering an explanation why we observed no association.

PUFA

Our findings contrast other findings in which PUFA intake was associated with decreased T2D risk^(7,8). However, PUFA are a group of FA likely to have different effects on T2D risk. Our results support this hypothesis as individual FA within the *n-3* and *n-6* PUFA groups had differing effects on T2D risk.

To our knowledge, only an Australian case-cohort study examined the relationship between the dietary intake of individual *n-6* PUFA and T2D and found no association with any of the *n-6* PUFA⁽²⁹⁾. Contrary to us, studies using plasma measurements of *n-6* PUFA reported no association with AA, and have mixed results concerning LA, as one found no association and two reported an inverse association with T2D^(19,21,29).

Few studies have examined the associations of individual *n-3* PUFA on T2D risk; yet, available evidence concerning the dietary intake of ALA contradicts our results and suggests the absence of an association with T2D risk^(11,13,29). However, ALA may be differentially associated with T2D in populations, with a decreased risk in Asian countries but no association in Western countries⁽¹¹⁾. The marine *n-3* PUFA (EPA, DPA and DHA) are often grouped together and rarely analysed on their own. However, the Australian case-cohort study did look at each individually and did not find any associations with T2D risk⁽²⁹⁾. But average intakes of DPA and AA were lower in their population than in the E3N cohort (0.03 (SD 0.03) and 0.07 (SD 0.03) g/d, respectively, for DPA and 0.04 (SD 0.03) and 0.22 (SD 0.09) g/d, respectively, for AA)⁽²⁹⁾. In addition, although meat is the primary contributor to AA intake in the Australian population (70.2%), fish and meat provided nearly equal contributions to the consumption of DPA (50.4 and 49.2%, respectively)⁽³⁰⁾. In meta-analyses stratified by sex, intake of marine *n-3* PUFA was not associated with T2D risk in the whole population or in men, but was associated with an increased T2D risk in women⁽¹²⁾. As the Australian case-cohort differed in the amounts and dietary sources of certain PUFA and included both men and women, this could explain the discrepancies with our findings.

Trans-unsaturated fatty acids

Total dietary intake of TFA was not associated with incident T2D, supporting the findings of other prospective studies^(8,19,31) and contrasting with one study⁽⁷⁾. Residual dietary confounding could explain the inconsistency in results, as the mentioned cohorts all found significant associations with TFA until adjustment for other dietary factors^(8,19,31), or TFA may also be a heterogeneous group. Plasma measurements of two TFA have been associated with an increased T2D risk⁽³¹⁾, while evidence from a prospective cohort suggests that *trans*-palmitoleate (*trans*-16:1*n*-7), a naturally occurring dairy TFA, is associated with decreased T2D risk⁽³²⁾.

Potential biological pathways

One of the mechanisms through which FA are believed to affect the development of T2D is through their effects on cell membranes. The FA tails of the phospholipid (PL) bilayer can differ in both length and saturation (with double bonds reducing their ability to pack closely together); thus the FA composition of the cell membrane affects its fluidity, membrane protein incorporation, enzyme activities and receptor functions⁽³³⁾. As recent studies have demonstrated that the FA composition of the membrane is at least partially determined by the diet, this may have important health implications^(34,35). Participants randomly assigned to a diet with a high proportion of SFA later showed higher proportions of SFA in skeletal muscle PL, compared with individuals consuming a high-proportion MUFA diet⁽³⁴⁾. Increasing ratios of SFA:PUFA in the skeletal muscle cell membrane have been observed to decrease glucose effectiveness and insulin sensitivity in humans, which could theoretically lead to T2D^(36,37). However, both the proportion and absolute amount of FA can affect plasma concentrations, and thus both may be important in determining skeletal muscle PL composition as well, and could explain why we did not observe a similar relationship with SFA in our study⁽³⁵⁾. In addition, as the food preparation methods could alter the FA content differentially by FA group and type of food, this offers another theory to the discrepancy in our results with the biological studies⁽³⁸⁾.

FA may also exert their effects through gene expression, as they act as signalling molecules of genes coding for metabolic enzymes⁽³⁹⁾. Studies have shown that persistent exposure of pancreatic islet cells to high levels of free FA prevented transcription of acetyl-CoA carboxylase, an enzyme required for glucose-stimulated insulin release from the pancreas⁽³⁹⁾. A few studies have also suggested that AA could stimulate insulin secretion^(40,41) and suggested that the plasma concentration of AA could be a key determinant of insulin resistance⁽⁴²⁾. However, the relationship between AA and insulin is not yet clear, as studies also suggest that in the case of diabetes, insulin may activate enzymes responsible for AA synthesis⁽⁴³⁾. Pure DPA has been difficult to isolate and was not readily accessible; hence, there is no literature on the biological relationship between DPA and T2D⁽⁴⁴⁾.

Strengths and limitations

Potential limitations should be considered. The dietary estimates are based on a single questionnaire at baseline; thus,

misclassification of exposure is possible. However, as the study is prospective, any effects are likely to be non-differential and would lead to an attenuation of the true association. T2D cases were carefully identified and validated; yet it is possible there was an under-ascertainment of T2D cases, especially in asymptomatic women. With our large sample size, small deviations could lead to small *P*-values when comparing the characteristics between cases and non-cases, but these would not affect the *P*-values of the Cox models. For this reason, the mean values between cases and non-cases were used to interpret our results in Table 1 and the *P*-values were not displayed. Finally, we were not able to adjust for contaminants such as Hg (found in fish) and persistent organic pollutants (POP, the major sources of which are fish, meat and dairy products) that have been associated with incident T2D in some studies^(45–47). However, epidemiological evidence on POP has been considered inconsistent enough that studies suggest that the potential hazards of the low levels of exposure present in seafood do not outweigh the benefits of seafood consumption. In addition, meta-analyses in East Asian countries where fish consumption is high even demonstrate a decreased risk for T2D associated with a high fish consumption^(48,49). Yet, interpretation of the findings should be cautious in the absence of adjustment for contaminants. The strengths of this study include the prospective design that minimises recall and selection biases and the large study population. The high rate of follow-up allowed the validation of many cases of incident T2D, and the dietary questionnaire was validated and specific for this French population. Extensive information on potential confounders was collected, minimising residual confounding. We also controlled for other FA groups besides the one in question in our analyses. This is important, as the fate of FA depends heavily on the overall FA profile of the diet, likely due to competition between FA for enzymes⁽¹⁴⁾.

Conclusion

Despite the large body of evidence suggesting that PUFA, and specifically *n*-3 PUFA, are beneficial for cardiovascular health⁽⁵⁰⁾, based on our results we could not recommend a high consumption of either as beneficial for diabetes prevention, as the effects of PUFA appear heterogeneous within the group and even within subgroups. High AA and DPA intakes were associated with an increased risk for incident T2D, independently of meat consumption, the primary food group contributor. The association could be even more pronounced in the general population, as our population is relatively homogeneous. Further investigation into the associations of individual dietary *n*-3 and *n*-6 PUFA intake and the risk for T2D is warranted.

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The authors declare that there are no conflicts of interest.

Supplementary materials

For supplementary material/s referred to in this article, please visit <https://doi.org/10.1017/S0007114516003883>

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