

The prospective association between total and type of fish intake and type 2 diabetes in 8 European countries: EPIC-InterAct Study^{1–3}

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ABSTRACT

Background: Epidemiologic evidence of an association between fish intake and type 2 diabetes (T2D) is inconsistent and unresolved.

Objective: The objective was to examine the association between total and type of fish intake and T2D in 8 European countries.

Design: This was a case-cohort study, nested within the European Prospective Investigation into Cancer and Nutrition (EPIC) study, with 3.99 million person-years of follow-up, 12,403 incident diabetes cases, and a random subcohort of 16,835 individuals from 8 European countries. Habitual fish intake (lean fish, fatty fish, total fish, shellfish, and combined fish and shellfish) was assessed by country-specific dietary questionnaires. HRs were estimated in each country by using Prentice-weighted Cox regression models and pooled by using a random-effects meta-analysis.

Results: No overall association was found between combined fish and shellfish intake and incident T2D per quartile (adjusted HR: 1.00; 95% CI: 0.94, 1.06; *P*-trend = 0.99). Total fish, lean fish, and shellfish intakes separately were also not associated with T2D, but fatty fish intake was weakly inversely associated with T2D: adjusted HR per quartile 0.97 (0.94, 1.00), with an HR of 0.84 (0.70, 1.01), 0.85 (0.76, 0.95), and 0.87 (0.78, 0.97) for a comparison of the second, third, and fourth quartiles with the lowest quartile of intake, respectively (*P*-trend = 0.06).

Conclusions: These findings suggest that lean fish, total fish, and shellfish intakes are not associated with incident diabetes but that fatty fish intake may be weakly inversely associated. Replication of these findings in other populations and investigation of the mechanisms underlying these associations are warranted. Meanwhile, current public health recommendations on fish intake should remain unchanged. *Am J Clin Nutr* 2012;95:1445–53.

INTRODUCTION

Epidemiologic evidence from prospective cohort studies for the association between self-reported fish and shellfish intake and the risk of type 2 diabetes (T2D)⁴ is inconsistent and unresolved. For instance, the Finnish Mobile Clinical Health Examination Survey (FMCHES) Study and the Adventist Health Study, USA, showed null associations between fish intake and risk of T2D (1,

2), whereas the European Prospective Investigation into Cancer and Nutrition (EPIC)–Norfolk Study, United Kingdom, observed an inverse association between total fish intake and a positive association between shellfish intake and T2D risk (3). More recently, several prospective studies observed positive associations between fish intake and incident diabetes (4–6), whereas the Cardiovascular Healthy Study observed no association between fish intake and diabetes risk (7). Conversely, the Shanghai Women’s Health Study found an inverse association between total fish and shellfish intake and diabetes risk, whereas

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the Shanghai Men's Health Study observed an inverse association with shellfish intake but no association with total fish intake and diabetes risk (8). Thus, the nature of the association for diabetes risk is currently unclear and requires further investigation.

Lack of heterogeneity in fish and shellfish intake may be one plausible explanation for the discrepancies observed in the studies reported thus far. Previous analyses based on total and types of fish and shellfish intakes derived by 24-h recall in representative samples within EPIC cohorts ($n = 36,000$) have shown substantial variability in fish and shellfish intake in Europe (9). Thus, given the 6- to 7-fold variation in total fish and shellfish intake in European populations, for instance with highest intake in Spain and the lowest intake in Germany and the Netherlands, the large EPIC-InterAct study of incident diabetes provides a unique opportunity for examining the association between dietary fish and shellfish intake and the risk of diabetes (10). Specifically, our aim was to investigate the prospective association between total and types of fish and shellfish intakes and T2D within 8 European countries participating in the EPIC Study.

SUBJECTS AND METHODS

Study population and setting

The InterAct Project was initiated to investigate the role of diet, lifestyle, genetic factors, and their possible interaction on the risk of T2D (10). The EPIC-InterAct Study is a case-cohort study (10) nested within the existing EPIC Study (11). In brief, EPIC-InterAct includes adults who were resident in geographically defined areas within 26 centers in 8 European countries (Denmark, France, Germany, Italy, Netherlands, Spain, Sweden, and the United Kingdom). Each EPIC center has individual written informed consent and local ethical approval.

Case-cohort design and case ascertainment

The recruitment frame for EPIC-InterAct ($n = 340,234$) was initially sampled from 8 of 10 countries of the EPIC Study ($n = 455,680$), excluding those without stored blood ($n = 109,625$) or reported diabetes status ($n = 5821$). Of this 340,234-participant

sample with 3.99 million person-years of follow-up, a subcohort of 16,835 individuals was randomly selected from those with available stored blood and buffy coat, stratified by center. After the exclusion of 548 individuals with prevalent diabetes, 129 individuals without information on reported diabetes status, and 4 individuals with postcensuring diabetes, 16,154 subcohort individuals were included. Because of the random selection, this subcohort also included a random set of 778 individuals who had developed incident T2D during follow-up.

Described in detail previously, incident diabetes cases were ascertained and verified by each participating EPIC center within the InterAct Project (10). New cases occurring up until 31 December 2007 were ascertained by using multiple data sources: self-report of doctor-diagnosed diabetes or diabetes-specific medication, linkage to primary or secondary care registers, linkage to pharmacy databases, hospital admissions data, or mortality data. Verification of incident diabetes was undertaken, for participants with <2 independent sources of information, by individual medical record checking, or confirmation from another independent source of information. Follow-up was censored at the date of diagnosis, 31 December 2007, or date of death, whichever occurred first. In total, 12,403 incident diabetes cases were identified, of which 778 were within the subcohort, as also described above.

Population for current analysis

Of the 27,779 participants [12,403 cases, of which 778 were within the 16,154-participant subcohort (778 cases and 15,376 noncases)] in EPIC-InterAct, we made further exclusions for baseline self-report of history of heart attack, angina, or stroke ($n = 1374$); being in the top 1% or bottom 1% of the ratio of energy intake to basal metabolic rate ($n = 568$); or for missing information in the main exposures or covariates used in the statistical analyses ($n = 1024$). This left a final sample of 24,813 participants for inclusion in this analysis with 10,740 cases and 14,746 in the subcohort, including 673 cases in the subcohort.

Dietary assessment

The EPIC dietary data were assessed at baseline by means of a quantitative dietary questionnaire with individual average portion sizes (in France, Spain, Netherlands, Germany, and Italy, except Naples) or a semiquantitative food-frequency questionnaire [in Denmark, Naples (Italy), Sweden, and the United Kingdom], which were developed and validated locally (12, 13). Single 24-h dietary-recall data were also collected from a representative sample of 8% of each EPIC center. Dietary intake data from each EPIC cohort were standardized by using the EPIC Nutrient Database, which was developed to provide standardized information on food pattern, food items, and individual nutrients across EPIC countries (14). For this study analysis, we used the following categories of fish and shellfish intake derived by the EPIC Nutrient Database: lean fish intake, fatty fish intake, total fish intake (sum of lean and fatty fish), shellfish intake (including seafood such as prawn, crab, mussels), and combined fish and shellfish intake, which was defined as the sum of intake of lean fish, fatty fish, shellfish, and "other" types of fish. The "other" categories of fish included fish products/fish in crumbs and nonspecific or combined fish, which formed a minor part of intake

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⁴Abbreviations used: EPIC, European Prospective Investigation into Cancer and Nutrition; FMCHES, Finnish Mobile Clinical Health Examination Survey; NHS, Nurses' Health Study; T2D, type 2 diabetes.

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(intake of “other” categories constitutes <11.4% of combined fish and shellfish intake in the subcohort) or had limited availability in some countries. There was limited information on cooking or preparation methods from the dietary questionnaires; thus, each category of fish and shellfish intake included fresh, canned, salted, smoked, or fried fish. Previously published data in a subsample of the EPIC study ($n = 35,644$), in which cooking methods of fish were established by 24-h recall, suggest that ~20% of fish is fried in the 8 European cohorts used in this study (15).

Nondietary assessment

Standardized information on health and lifestyle exposures was assessed by questionnaire at baseline (12). Anthropometric information (height, weight, and waist circumference) was measured by trained staff in standardized health checks at baseline in all centers, except in a proportion of Oxford (United Kingdom) and France, where self-reported data were obtained, and Umea (Sweden), where waist circumference was not measured. Questionnaires ascertained education level (none, primary, technical/professional, secondary, or university), occupational and leisure-time physical activity [derived into the validated 4-scale Cambridge physical activity index (16): inactive, moderately inactive, moderately active, or active], smoking status (never, former, or current), and history of previous illness.

Statistical analysis

The median and IQR of dietary-questionnaire-derived intakes of total fish and types of fish and shellfish intake were calculated. Baseline characteristics according to quartiles of combined fish and shellfish intake (in the subcohort) were summarized by using means (\pm SDs) for symmetrically distributed continuous variables, medians (IQRs) for continuous variables with a skewed distribution, and percentages and frequencies for categorical variables in the subcohort population ($n = 14,746$). To estimate the association between fish intake and the hazard of T2D, Prentice-weighted Cox proportional hazards analysis was used, with age as the underlying time scale (17). Combined fish and shellfish intake, total fish intake, lean fish intake, fatty fish intake, and shellfish intake were categorized by using quartiles (based on the distributions in the subcohort). HRs of diabetes (comparing quartiles of fish intake) and corresponding 95% CIs were estimated within each country and then combined by using random-effects meta-analysis. The I^2 statistic for between-cohort heterogeneity was also calculated. The effect of per-quartile change of each type of fish intake was also calculated. Three models were fitted with different levels of adjustment for potential confounders: model 1 included adjustment for nondietary factors [center, sex, smoking status (3 categories), education level (3 categories), and physical activity (4 categories)]. Center was included in model 1 to control for center effects such as different follow-up procedures and questionnaire types. Model 2 included further adjustment for dietary risk factors, including total energy intake (kcal/d), alcohol intake (g/d), and fruit and vegetable intake (g/d). Model 3 was further adjusted for BMI (continuous). Family history of diabetes was not included in model 1 because there was incomplete or missing information in some centers on this variable.

Interaction and sensitivity analyses

Interactions of combined fish and shellfish intake (per quartile change) with sex and BMI (continuous) were tested within each country and pooled using random-effects Prentice-weighted Cox regression analysis described above. In sensitivity analyses we examined the association between combined fish and shellfish intake (quartiles) and hazard of diabetes after the exclusion of those with a date of diabetes diagnosis within 2 y of baseline. In addition, because one center (Umea) did not record waist circumference, this variable was added only to model 3 in sensitivity analyses. The potential confounding effect of other variables—including waist circumference and BMI (4 categories) and intakes (g/d) of red and processed meat, fiber, fat, SFA, MUFA, cholesterol, and protein—was assessed by adding each covariate in turn to model 3. In addition, we examined the independent effects of types of fish intake (lean, fatty, and shellfish) and incident diabetes by mutual adjustment for one another. We also re-analyzed the associations between types of fish intake recategorized as nonconsumers (referent) and tertiles of consumers, and incident diabetes, to address the issue of fish nonconsumption.

Measurement error

The potential effect of measurement error in the assessment of combined fish and shellfish intake by dietary questionnaire was assessed by using data from the 8% of participants of the subcohort who were included in representative samples in the EPIC subcalibration study, conducted between 1995 and 2000 ($n = 2347$), to calibrate dietary measurements by using a single 24-h recall (18). The calibration exercise is based on several assumptions: 1) 24-h recall provides an unbiased measure of fish intake, 2) the error in assessing fish intake by 24-h recall is random, and 3) the error in assessing fish intake by 24-h recall is uncorrelated with the error in assessing fish intake by dietary questionnaire.

We used a multivariate error correction method described by Wood et al (19) in the Fibrinogen Studies Collaboration. The method involved regressing 24-h recall-derived total fish and shellfish intakes (100 g/wk) ($n = 2137$ after exclusions, see Subjects and Methods) on the questionnaire-derived fish and shellfish intake (100 g/wk) with adjustment for center, sex, education level, smoking status, physical activity, total energy intake, alcohol intake, fruit and vegetable intake, BMI, day of recall (weekday compared with weekend), and season within each country to estimate regression dilution ratios. The regression dilution ratios were then used to correct the HRs (95% CIs) by multiplying the regression dilution ratios by the HRs before the Prentice-weighted Cox regression random-effects meta-analysis. All statistical analyses were performed by using STATA/SE 11.1 (StataCorp). All P values were based on 2-sided tests.

RESULTS

Pattern of fish and shellfish intake and baseline characteristics of the subcohort population

The median (IQR) estimated combined fish and shellfish intake from dietary questionnaires in the subcohort was 239 (119, 427) g/wk in men and 188 (94, 330) g/wk in women. For total fish intake, the corresponding estimated intakes were 138 (30, 296) and 92 (16, 223) g/wk in men and women, respectively. The

dietary-questionnaire-derived fish and shellfish intake varied markedly across the cohorts in various European countries in a similar manner as previously described in detail, when measured by the 24-h recall method (9). Overall, the highest estimated intake of combined fish and shellfish was reported in Spain and the lowest estimated intake in the Netherlands and Germany (*see* Supplementary Table 1 under “Supplemental data” in the online issue). Spain and Sweden, respectively, had the highest and lowest reported intakes of lean fish. For fatty fish, men in Spain and women in Denmark had the highest intake, whereas men in the Netherlands and women in Sweden reported the lowest intake. In general, shellfish intakes were low, ranging from 0 to 30.3 g/wk overall.

Baseline characteristics by quartiles of combined fish and shellfish intake in the subcohort population are presented in **Table 1**. High consumers of fish and shellfish were more likely

to be older, be current smokers, be less physically active, be obese (higher BMI and waist circumference), have a lower education level, and have higher intakes of other dietary variables (total energy, fat as a percentage of energy, fiber, and protein as a percentage of energy, and alcohol, fruit and vegetable, red meat, and processed meat intakes).

Association between fish and shellfish intake and risk of diabetes

The pooled HRs (95% CIs) for diabetes across quartiles of combined fish and shellfish intake and also intakes of different types of fish are shown in **Table 2**. A higher combined fish and shellfish intake was not associated with risk of diabetes in analyses adjusted for risk factors for diabetes, dietary risk factors, and BMI [quartile 4 compared with quartile 1: 0.99 (0.86,

TABLE 1

Baseline characteristics of the study population according to quartiles of combined fish and shellfish intake estimated from dietary questionnaires (in the subcohort): EPIC-InterAct Study¹

	Quartile of combined fish and shellfish intake			
	1 (n = 3690)	2 (n = 3683)	3 (n = 3686)	4 (n = 3687)
Median intake of combined fish and shellfish (g/wk)	51.2 (21.5, 76.9) ²	148.9 (123.5, 175.5)	269.4 (237.0, 309.6)	515.3 (428.0, 662.2)
Sociodemographic characteristics				
Age (y)	50.7 ± 9.8 ³	52.3 ± 9.0	52.9 ± 8.7	52.7 ± 8.4
Men [n (%)]	1118 (30.3)	1281 (34.8)	1353 (36.7)	1714 (46.5)
Education level [n (%)]				
None	121 (3.3)	201 (5.5)	316 (8.6)	503 (13.6)
Primary school completed	1072 (29.1)	1143 (31.0)	1282 (34.8)	1375 (37.3)
Technical/professional	963 (26.1)	937 (25.4)	806 (21.9)	697 (18.9)
Secondary school	715 (19.4)	600 (16.3)	515 (14.0)	423 (11.5)
Longer education	819 (22.2)	802 (21.8)	767 (20.8)	689 (18.7)
Anthropometric characteristics				
BMI (kg/m ²)	25.3 ± 4.1	25.7 ± 4.1	26.2 ± 4.2	26.9 ± 4.2
Waist circumference (cm) ⁴				
Men	92.8 ± 10.2	94.8 ± 10.2	94.9 ± 9.5	97.0 ± 9.3
Women	79.8 ± 10.8	80.4 ± 10.9	81.3 ± 11.0	82.8 ± 11.4
Lifestyle characteristics				
Smoking status [n (%)]				
Never	1748 (47.4)	1789 (48.6)	1784 (48.4)	1648 (44.7)
Former	1021 (27.7)	984 (26.7)	959 (26.0)	976 (26.5)
Current	921 (25.0)	910 (24.7)	943 (25.6)	1063 (28.8)
Physical activity [n (%)]				
Active	860 (23.3)	689 (18.7)	709 (19.2)	696 (18.9)
Moderately active	891 (24.2)	877 (23.8)	785 (21.3)	817 (22.2)
Moderately inactive	1190 (32.3)	1296 (35.2)	1280 (34.7)	1213 (32.9)
Inactive	749 (20.3)	821 (22.3)	912 (24.7)	961 (26.1)
Dietary characteristics				
Total energy intake (kcal/d)	1956.0 ± 594.5	2049.7 ± 585.7	2169.8 ± 597.6	2378.3 ± 673.2
Fat intake (% of energy)	34.4 ± 6.1	34.6 ± 5.7	35.0 ± 5.7	35.4 ± 5.8
Carbohydrate (% of energy)	46.3 ± 7.3	44.9 ± 6.7	43.5 ± 6.5	41.2 ± 6.8
Protein intake (% of energy)	15.7 ± 2.8	16.4 ± 2.8	17.3 ± 2.9	18.7 ± 3.1
Fiber intake (g/d)	20.2 (15.9, 25.1)	20.8 (16.7, 25.3)	22.1 (17.8, 27.5)	24.6 (19.7, 30.5)
Alcohol intake (g/d)	4.0 (0.6, 13.2)	6.0 (1.0, 16.4)	6.8 (0.9, 18.3)	9.3 (1.1, 25.1)
Fruit and vegetable intake (g/d)	292.1 (192.1, 437.5)	336.5 (218.5, 486.4)	396.0 (258.5, 559.8)	483.2 (320.9, 675.4)
Red meat (g/d)	31.1 (13.7, 57.0)	35.4 (18.0, 60.2)	41.4 (23.1, 67.2)	43.7 (23.2, 71.9)
Processed meat (g/d)	26.1 (12.9, 46.1)	28.7 (15.5, 49.4)	29.0 (15.4, 49.4)	29.9 (15.3, 52.2)

¹ n = 14,746; EPIC, European Prospective Investigation into Cancer and Nutrition.

² Median; IQR in parentheses (all such values).

³ Mean ± SD (all such values).

⁴ Umea center excluded because of missing data [n = 953; total no. of missing waist circumference values in the subcohort = 1001 (n = 481 men and n = 520 women)].

TABLE 2HRs (95% CIs) for incident diabetes from a comparison of quartiles of total and types of fish and shellfish intakes in men and women combined: EPIC-InterAct Study¹

Type of fish and shellfish	HR (95% CI) for quartile of fish and shellfish intake				P-trend
	1 (reference)	2	3	4	
Combined fish and shellfish					
No. of cases ²	2451	2474	2799	3016	
Range of intake (g/wk)	≤104.7	>104.7 to ≤203.2	>203.2 to ≤362.4	>362.4	
Model 1 ³	1	0.95 (0.88, 1.03)	1.07 (0.96, 1.19)	1.12 (0.99, 1.27)	0.04
Model 2 ⁴	1	0.95 (0.87, 1.03)	1.05 (0.94, 1.19)	1.10 (0.97, 1.25)	0.08
Model 3 ⁵	1	0.91 (0.82, 1.00)	0.96 (0.81, 1.13)	0.99 (0.86, 1.15)	0.99
Total fish					
No. of cases	2566	2502	2732	2940	
Range of intake (g/wk)	≤19.8	>19.8 to ≤110.1	>110.1 to ≤244.4	>244.4	
Model 1 ³	1	0.96 (0.87, 1.05)	0.96 (0.86, 1.07)	1.03 (0.91, 1.16)	0.11
Model 2 ⁴	1	0.95 (0.87, 1.04)	0.95 (0.86, 1.06)	1.01 (0.89, 1.15)	0.21
Model 3 ⁵	1	0.95 (0.85, 1.07)	0.98 (0.86, 1.11)	1.06 (0.92, 1.22)	0.31
Lean fish^{6,7}					
No. of cases	2661	1169	2598	2925	
Range (g/wk)	0	>0 to ≤38.1	>38.1 to ≤139.7	>139.7	
Model 1 ³	1	0.97 (0.81, 1.16)	0.94 (0.85, 1.05)	1.05 (0.90, 1.23)	0.29
Model 2 ⁴	1	0.98 (0.81, 1.17)	0.94 (0.84, 1.05)	1.04 (0.89, 1.20)	0.34
Model 3 ⁵	1	0.99 (0.81, 1.22)	0.96 (0.84, 1.09)	1.04 (0.88, 1.23)	0.57
Fatty fish					
No. of cases	2684	2474	2734	2848	
Range of intake (g/wk)	≤4.1	>4.1 to ≤41.0	>41.0 to ≤102.6	>102.6	
Model 1 ³	1	0.95 (0.81, 1.11)	0.93 (0.84, 1.02)	0.99 (0.84, 1.16)	0.87
Model 2 ⁴	1	0.95 (0.81, 1.12)	0.92 (0.84, 1.02)	0.97 (0.83, 1.13)	0.44
Model 3 ⁵	1	0.84 (0.70, 1.01)	0.85 (0.76, 0.95)	0.87 (0.78, 0.97)	0.06
Shellfish⁶					
No. of cases	2253	1710	2623	2767	
Range of intake (g/wk)	0	>0 to ≤7.3	>7.3 to ≤30.3	>30.3	
Model 1 ³	1	1.01 (0.86, 1.18)	1.00 (0.91, 1.09)	1.12 (1.03, 1.22)	0.01
Model 2 ⁴	1	1.01 (0.86, 1.20)	1.00 (0.92, 1.10)	1.12 (1.03, 1.22)	0.01
Model 3 ⁵	1	0.96 (0.76, 1.22)	0.96 (0.80, 1.15)	1.05 (0.92, 1.20)	0.32

¹ HRs (and 95% CIs) were first estimated separately by country, and then the country-specific estimates were combined by using random-effects meta-analysis. HRs were derived from Prentice-weighted Cox regression with age as the underlying time scale. Fish intake was assessed by dietary questionnaires, and values are the minimum and maximum values for each of its corresponding quartiles. EPIC, European Prospective Investigation into Cancer and Nutrition.

² Includes cases from the subcohort; total no. of cases = 10,740, except for lean fish and shellfish ($n = 9353$).

³ Model 1: adjusted for center, sex, education level, smoking status, and physical activity.

⁴ Model 2: adjusted as for model 1 plus total energy intake, alcohol intake, and fruit and vegetable intake.

⁵ Model 3: adjusted as for model 2 plus BMI.

⁶ Data on intake not available in Germany ($n = 1387$ cases).

⁷ Data on intake not available in quartile 4 for the Netherlands because of low intake.

1.15), P -trend = 0.99], with no significant heterogeneity (I^2 22.8%, $P = 0.25$). Total fish (lean and fatty fish) intake and lean fish intake were also not associated with incident diabetes (Table 2), with no evidence of significant heterogeneity. However, fatty fish intake was inversely associated with incident diabetes across consecutive quartiles in model 3, which included adjustment for BMI. Compared with the first quartile, HRs (95% CIs) for T2D were 0.84 (0.70, 1.01), 0.85 (0.76, 0.95), and 0.87 (0.78, 0.97) (P -trend = 0.06), with no evidence of heterogeneity (quartile 4 compared with quartile 1: $I^2 = 0\%$, $P = 0.65$) in model 3. Shellfish intake was not significantly associated with diabetes in an adjusted analysis (model 3). The HR (95% CI) for incident diabetes per quartile change in fish and shellfish intake, with an overall null association for combined fish and shellfish intake (HR: 1.00; 95% CI: 0.94, 1.06) and for subtypes (HRs: all 1.02), except for a modest inverse association with fatty fish

intake (HR: 0.97; 95% CI: 0.94, 1.00) per quartile, are shown in **Figure 1A–D**.

The exclusion of participants who developed diabetes in <2 y after baseline did not alter our findings. Similarly, none of the other sensitivity analyses (*see* Subjects and Methods) had a major effect on the reported HRs. No interaction between combined fish and shellfish (per quartile change) with BMI (continuous) or sex was observed (P -interaction = 0.16 and 0.46, respectively) when country specific interaction terms were pooled by using a random-effects meta-analysis as above.

Calibration of combined fish and shellfish intake with dietary 24-h recall data by using the multivariate error correction method did not affect the observed associations. Estimated regression dilution ratios were <1 in all countries and ranged from 0.44 in Sweden to 0.48 in Spain and the United Kingdom (*see* Supplementary Table 2 under “Supplemental data” in the online issue).

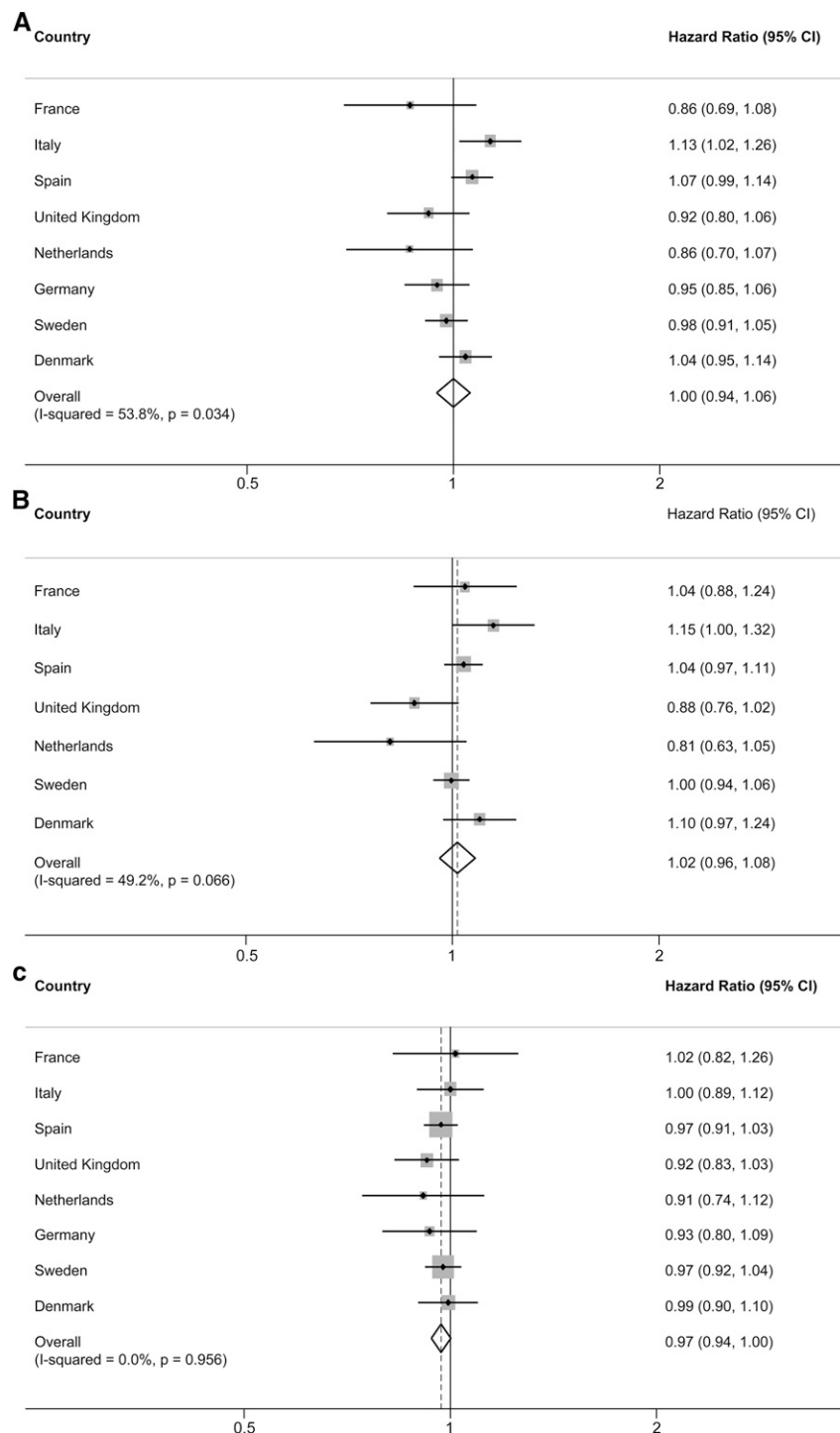


FIGURE 1. HRs (95% CIs) for incident diabetes per quartile change in combined fish and shellfish intake (A), lean fish intake (B), fatty fish intake (C), and shellfish intake (D). Estimates are derived per country, and the pooled estimate is based on random-effects meta-analysis by using Prentice-weighted Cox regression analysis (with time as the underlying time scale and adjusted for center, education level, smoking status, physical activity, total energy intake, alcohol intake, fruit and vegetable intake, and BMI. European Prospective Investigation into Cancer and Nutrition-InterAct Study: $n = 10,740$ cases, except for lean fish and shellfish intakes ($n = 9353$ cases because of no fish intake information from Germany).

DISCUSSION

Main findings in the context of other available evidence

In this large European prospective nested case-cohort study of 24,813 men and women, including 10,740 diabetes cases from

among 340,234 participants with 3.99 million years of follow-up, there was no association between combined fish and shellfish intake and incident diabetes and limited evidence of heterogeneity between countries. We found no association of diabetes with intakes of total fish, lean fish, or shellfish in adjusted models,

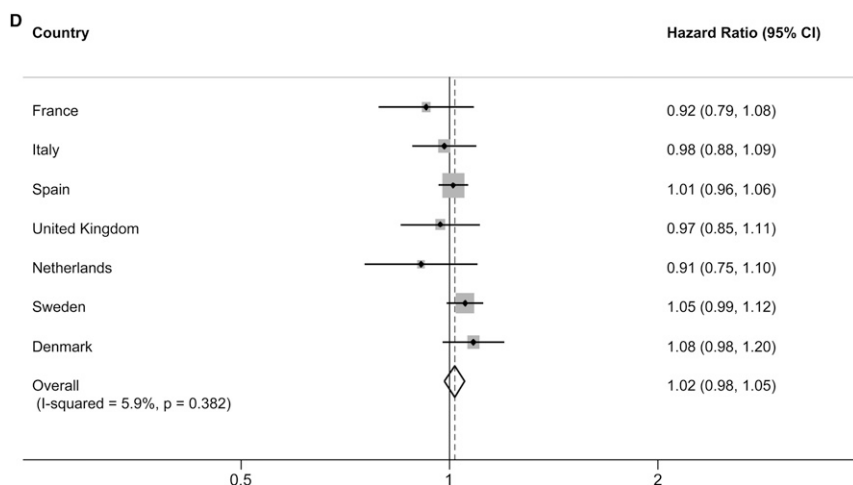


FIGURE 1. (continued)

but we observed a suggestion of a modest inverse association for fatty fish intake.

Our findings of a null association between combined fish and shellfish intake and risk of T2D are in contrast with the positive associations with increasing intake observed in the Rotterdam Study (RR: 1.32; 95% CI: 1.02, 1.70) in a comparison between the highest fish consumers (28 g/d) and the nonfish consumers (4); a pooled analysis of the Nurses' Health Study (NHS) I, NHS II, and the Health Professionals Follow-Up Study (RR: 1.22; 95% CI: 1.08, 1.39) in a comparison between ≥ 5 times/wk and < 1 time/mo (5); and the Women's Health Study (HR: 1.49; 95% CI: 1.30, 1.70) in a comparison of the highest (3.93 servings/wk) with the lowest quintile (0.47 servings/wk) of intake (6). Like us, null associations between fish intake and incident diabetes were observed in the FMCHES Study (1), the Adventist Health Study (2), and the Cardiovascular Health Study (7). Conversely, the Shanghai Women's Health Study observed an inverse association between total fish intake and diabetes risk. Two (5, 6) of the 3 studies reporting positive associations between fish intake and T2D risk were examined in the United States; thus, it may be reasonable to speculate that other characteristics of these cohorts and/or the composition of fish sources as determined by geographic location, could explain the discrepancy between the US findings and those reported in this study and others (1–3, 8). We speculated that it is unlikely that differences in the absolute amount of fish intake can explain the discrepancies between our study and others, because positive associations with diabetes risk were observed in studies with both lower (Rotterdam Study) (4) and higher (NHS I, NHS II, and the Health Professionals Follow-Up Study) (5) intakes of fish, and given the fact we observed low heterogeneity in our study between countries with high and low intakes of fish. The types of fish included in the total fish intake category may also contribute to the discrepant results. However, only a few studies have examined total as well as types of fish intake and incident diabetes, with varying inclusion of types of fish (1, 3, 4). In the EPIC-Norfolk Study (3), shellfish intake was positively associated with diabetes risk, whereas in the Shanghai Women's and Shanghai Men's studies shellfish intake was inversely associated with diabetes risk (8). This could reflect different preparation and cooking method

practices in different locations, such as in the United Kingdom, shellfish such as prawns are often consumed with rich sauces such as garlic butter or mayonnaise or are deep fried. The reasons for the observed positive association between lean fish and T2D risk in the Rotterdam Study (4), and contrasting null associations for types of fish and T2D in the FMCHES Study (1), currently remain unclear, but could be due to differences in preparation methods. Studies reporting on fatty fish intake previously reported null associations with incident diabetes (3, 4).

Interpretation

The weak inverse association between fatty fish intake and incident diabetes we observed is suggestive, but nonconclusive, and should be further examined in other populations and with improved methods of dietary assessment, including objective biomarkers of fish intake such as marine-derived fatty acids, vitamin D, and toxins, where appropriate. The modest inverse association we observed may be explained by the potential effect of long-chain omega-3 (n-3) PUFAs, including EPA and DHA, in large quantities in fatty fish. Omega-3 PUFAs may have beneficial effects on insulin action through several mechanisms, including by improving membrane fluidity, production of anti-inflammatory eicosanoids, affecting key proteins in signal transduction pathways and modulation in expression of peroxisome proliferator-activated receptor γ (20). Whether or not omega-3 fatty acids mediate a beneficial effect on diabetes risk has yet to be confirmed, because other components within fish—such as toxins or contaminants—may also act adversely on pathways leading to T2D (21, 22). Such contaminants include methyl mercury, polychlorinated biphenyls, and persistent organic pollutants, which may be present to varying extents in different geographic locations (23) and may help to explain the inconsistencies reported in the association between fatty fish and T2D. Another possible mechanism by which fatty fish may exert its protective effects could be through vitamin D in the diet, which is mainly acquired from fatty fish. Whether vitamin D has a causal association with reduced risk of T2D is currently not established, but epidemiologic evidence suggests an inverse association (24).

Strengths and limitations

The major strengths of this study include the large number of incident diabetes cases and the large variation in fish and shellfish intake in 8 European countries with standardized information of diet and lifestyle exposures. These factors combine to enhance the power to detect associations if they truly exist. We excluded those with prevalent cardiovascular disease and diabetes who may change or report their diet differently as a result of diagnosis. However, we cannot exclude the possibility that other factors associated with fish intake or contained within fish affected our findings. We adjusted for a range of other potential confounders, including dietary factors and physical activity, with no effect on our findings. Nonetheless, measurement error in these variables could leave residual confounding effects in the fish-diabetes association. A limitation of our study included the measurement error in the assessment of estimated fish and shellfish intake by self-reported questionnaires. We attempted to account for this by re-analyzing the association with calibrated fish and shellfish intake assessed by 24-h recall using an established method, but the results were largely unchanged. However, the findings from these calibration exercises should be interpreted with caution, because a single 24-h recall estimate of fish intake may not reflect true habitual fish intake, and its validity may vary by country depending on the frequency of consumption. We could not examine the absolute incidence of T2D by fish intake status in our study, but T2D incidence by country was reported previously (10). Last, we examined the effect of quantity of fish intake (in g/d), but could not examine the frequency of fish intake (servings/d) because of the different types of dietary questionnaires and portion sizes used in each country.

Conclusions

Overall, our results do not support the hypothesis that self-reported combined fish and shellfish intake is associated with incident diabetes. However, fatty fish intake may be modestly inversely associated with diabetes risk, but our findings are merely suggestive and need to be confirmed. Additional studies should examine types of fish and shellfish intake and use objective biomarkers of fish intake, including marine-derived long-chain fatty acids and toxins. This may improve our ability to detect and understand the association between total and types of fish intake and the risk of T2D. Meanwhile, the current public health recommendations to consume fish regularly for better health should be upheld, and no change to this recommendation is warranted on the basis of our findings.

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The authors' responsibilities were as follows—PSP, NGF, and SJS: had access to all data for this study and take responsibility for the manuscript's contents. All authors contributed to the conception and design of the study, the interpretation of the data, the critical revision of the article for important intellectual content, and final approval of the version to be published. None of the authors declared a conflict of interest.

REFERENCES

- Montonen J, Jarvinen R, Heliövaara M, Reunanen A, Aromaa A, Knekt P. Food consumption and the incidence of type II diabetes mellitus. *Eur J Clin Nutr* 2005;59:441–8.
- Vang A, Singh PN, Lee JW, Haddad EH, Brinegar CH. Meats, processed meats, obesity, weight gain and occurrence of diabetes among adults: findings from Adventist Health Studies. *Ann Nutr Metab* 2008;52:96–104.
- Patel PS, Sharp SJ, Luben RN, Khaw KT, Bingham SA, Wareham NJ, Forouhi NG. Association between type of dietary fish and seafood intake and the risk of incident type 2 diabetes: the European prospective investigation of cancer (EPIC)-Norfolk cohort study. *Diabetes Care* 2009;32:1857–63.
- van Woudenberg GJ, van Ballegooijen AJ, Kuijsten A, Sijbrands EJ, van Rooij FJ, Geleijnse JM, Hofman A, Witteman JC, Feskens EJ. Eating fish and risk of type 2 diabetes: a population-based, prospective follow-up study. *Diabetes Care* 2009;32:2021–6.
- Kaushik M, Mozaffarian D, Spiegelman D, Manson JE, Willett WC, Hu FB. Long-chain omega-3 fatty acids, fish intake, and the risk of type 2 diabetes mellitus. *Am J Clin Nutr* 2009;90:613–20.
- Djoussé L, Gaziano JM, Buring JE, Lee IM. Dietary omega-3 fatty acids and fish consumption and risk of type 2 diabetes. *Am J Clin Nutr* 2011;93:143–50.
- Djoussé L, Biggs ML, Lemaitre RN, King IB, Song X, Ix JH, Mukamal KJ, Siscovick DS, Mozaffarian D. Plasma omega-3 fatty acids and incident diabetes in older adults. *Am J Clin Nutr* 2011;94:527–33.
- Villegas R, Xiang YB, Elasy T, Li HL, Yang G, Cai H, Ye F, Gao YT, Shyr Y, Zheng W, et al. Fish, shellfish, and long-chain n-3 fatty acid consumption and risk of incident type 2 diabetes in middle-aged Chinese men and women. *Am J Clin Nutr* 2011;94:543–51.
- Welch AA, Lund E, Amiano P, Dorronsoro M, Brustad M, Kumle M, Rodriguez M, Lasheras C, Janzon L, Jansson J, et al. Variability of fish consumption within the 10 European countries participating in the European Investigation into Cancer and Nutrition (EPIC) study. *Public Health Nutr* 2002;5(6B):1273–85.
- Langenberg C, Sharp S, Forouhi NG, Franks PW, Schulze MB, Kerrison N, Ekelund U, Barroso I, Panico S, Tormo MJ, et al. Design and cohort description of the InterAct Project: an examination of the interaction of genetic and lifestyle factors on the incidence of type 2 diabetes in the EPIC Study. *Diabetologia* 2011;54:2272–82.
- Riboli E, Kaaks R. The EPIC Project: rationale and study design. *European Prospective Investigation into Cancer and Nutrition. Int J Epidemiol* 1997;26(suppl 1):S6–14.
- Riboli E, Hunt KJ, Slimani N, Ferrari P, Norat T, Fahey M, Charrondiere UR, Hemon B, Casagrande C, Vignat J, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. *Public Health Nutr* 2002;5(6B):1113–24.
- Margetts BM, Pietinen P. European Prospective Investigation into Cancer and Nutrition: validity studies on dietary assessment methods. *Int J Epidemiol* 1997;26(suppl 1):S1–5.
- Slimani N, Deharveng G, Unwin I, Southgate DA, Vignat J, Skeie G, Salvini S, Parpinel M, Moller A, Ireland J, et al. The EPIC nutrient database project (ENDB): a first attempt to standardize nutrient databases across the 10 European countries participating in the EPIC study. *Eur J Clin Nutr* 2007;61:1037–56.
- Rohrmann S, Linseisen J, Becker N, Norat T, Sinha R, Skeie G, Lund E, Martinez C, Barricarte A, Mattisson I, et al. Cooking of meat and fish in Europe—results from the European Prospective Investigation into Cancer and Nutrition (EPIC). *Eur J Clin Nutr* 2002;56:1216–30.
- Wareham NJ, Jakes RW, Rennie KL, Schuit J, Mitchell J, Hennings S, Day NE. Validity and repeatability of a simple index derived from the short physical activity questionnaire used in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. *Public Health Nutr* 2003;6:407–13.
- Prentice R. A case-cohort design for epidemiologic cohort studies and disease prevention trials. *Biometrika* 1986;73:1–11.
- Slimani N, Ferrari P, Ocke M, Welch A, Boeing H, Liere M, Pala V, Amiano P, Lagiou A, Mattisson I, et al. Standardization of the 24-hour diet recall calibration method used in the European prospective investigation into cancer and nutrition (EPIC): general concepts and preliminary results. *Eur J Clin Nutr* 2000;54:900–17.
- Fibrinogen Studies Collaboration. Correcting for multivariate measurement error by regression calibration in meta-analyses of epidemiological studies. *Stat Med* 2009;28:1067–92.
- Calder PC. n–3 Polyunsaturated fatty acids, inflammation, and inflammatory diseases. *Am J Clin Nutr* 2006;83(suppl):1505S–19S.

21. Chen YW, Huang CF, Tsai KS, Yang RS, Yen CC, Yang CY, Lin-Shiau SY, Liu SH. Methylmercury induces pancreatic beta-cell apoptosis and dysfunction. *Chem Res Toxicol* 2006;19:1080-5.
22. Everett CJ, Frithsen I, Player M. Relationship of polychlorinated biphenyls with type 2 diabetes and hypertension. *J Environ Monit* 2011;13:241-51.
23. Lee DH, Jacobs DR Jr. Inconsistent epidemiological findings on fish consumption may be indirect evidence of harmful contaminants in fish. *J Epidemiol Community Health* 2010;64:190-2.
24. Mitri J, Muraru MD, Pittas AG. Vitamin D and type 2 diabetes: a systematic review. *Eur J Clin Nutr* 2011;65:1005-15.