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Systematic Review with Meta-analysis

Association of fish and *n*-3 fatty acid intake with the risk of type 2 diabetes: a meta-analysis of prospective studies

Yunping Zhou, Changwei Tian and Chongqi Jia*

Department of Epidemiology and Health Statistics, Shandong University, Jinan 250012, Shandong, People's Republic of China

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Abstract

Results from observational studies on the association of fish and *n*-3 fatty acid consumption with type 2 diabetes mellitus (T2DM) risk are conflicting. Hence, a meta-analysis was performed to investigate this association from cohort studies. A comprehensive search was then conducted to identify cohort studies on the association of fish and/or *n*-3 fatty acid intake with T2DM risk. In the highest *v.* lowest categorical analyses, the fixed or random-effect model was selected based on the homogeneity test among studies. Linear and non-linear dose–response relationships were also assessed by univariate and bivariate random-effect meta-regression with restricted maximum likelihood estimation. In the highest *v.* lowest categorical analyses, the pooled relative risk (RR) of T2DM for intake of fish and *n*-3 fatty acid was 1·146 (95 % CI 0·975, 1·346) and 1·076 (95 % CI 0·955, 1·213), respectively. In the linear dose–response relationship, the pooled RR for an increment of one time (about 105 g)/week of fish intake (four times/month) and of 0·1 g/d of *n*-3 fatty acid intake was 1·042 (95 % CI 1·026, 1·058) and 1·057 (95 % CI 1·042, 1·073), respectively. The significant non-linear dose–response associations of fish and *n*-3 fatty acid intake with T2DM risk were not observed. The present evidence from observational studies suggests that the intake of both fish and *n*-3 fatty acids might be weakly positively associated with the T2DM risk. Further studies are needed to confirm these results.

Key words: **Fish: *n*-3 Fatty acids: Type 2 diabetes: Meta-analyses**

Diet is widely believed to play an important role in the development of type 2 diabetes mellitus (T2DM)[1,2]. Among dietary components, fish, an ideal source of *n*-3 PUFA, has been documented to be associated with T2DM risk, by experimental research and observational studies. Experimental research suggested that *n*-3 fatty acids could lower glucose utilisation and increase glucagon-stimulated C-peptide[3] or hepatic gluconeogenesis[4] with increasing uptake and oxidation of NEFA in the liver[5]. Therefore, fish intake and *n*-3 fatty acid consumption may increase T2DM risk by increasing circulating concentrations of glucose[6]. Vessby *et al.*[7] also reported that fasting glucose increased significantly after consumption of fish. Besides, *n*-3 fatty acids may cause oxidative stress and subsequent increase in pro-inflammatory products known to promote T2DM[8]. Moreover, recent studies have suggested that environmental contaminants such as dioxins[9] and methyl mercury, found in fish, might raise T2DM risk[10]. Furthermore, mouse models showed that elevated blood mercury levels may interrupt insulin signalling pathways, and decrease plasma insulin and elevate blood glucose levels[11]. A cross-sectional study also suggested that serum concentrations of persistent organic pollutants were strongly associated with diabetes prevalence[9]. However, an ecological study reported that populations with a high consumption of fish and marine animals have a lower prevalence of T2DM than do other populations[12], and *n*-3 fatty acid supplementation may increase insulin sensitivity[13] in animal models. Besides, cross-sectional studies showed inverse[14,15], no[16,17], and positive[18] associations between fish consumption and glycaemic status. Prospective studies reported that fish intake is either positively[6,19] or not associated[20] with T2DM risk.

Prospective cohort studies are assumed to provide better evidence than case–control studies, since they are not biased by recall of past dietary habits after T2DM has been diagnosed. Therefore, we decided to focus this meta-analysis on results from prospective cohort studies to: (1) assess the

Abbreviations: REML, restricted maximum likelihood; RR, relative risk; SFFQ, semiquantitative FFQ; T2DM, type 2 diabetes mellitus.

* **Corresponding author:** C. Jia, email jiachongqi@sdu.edu.cn

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effects and evaluate the dose–response relationship between fish and *n*-3 fatty acid consumption with T2DM risk; (2) evaluate the potential heterogeneity among studies; and (3) explore the potential publication bias.

Methods

Search strategy

A comprehensive search was performed for relevant articles published between January 1990 and July 2011 using the following databases: (1) PubMed; (2) Web of Science (ISI); (3) China Biology Medical literature database (CBM); (4) Database of Chinese Scientific and Technical Periodicals (VIP) and (5) China National Knowledge Infrastructure (CNKI). Search terms included 'fish', 'ω-3 fatty acid', '*n*-3 fatty acid' and 'diabetes'. Moreover, we identified studies not captured by our database by reviewing reference lists from retrieved articles to search for further relevant articles.

Eligibility criteria

Each identified study was independently reviewed by two investigators to determine whether an individual study was eligible for inclusion in this meta-analysis. The inclusion criteria were as follows: (1) cohort study; (2) the exposure of interest was the frequency of fish intake or *n*-3 fatty acid consumption; (3) the outcome of interest was T2DM and (4) multivariate adjusted relative risk (RR) estimates or hazard ratios with 95 % CI relating to each category of fish or *n*-3 fatty acid consumption. If there was disagreement between the two investigators about eligibility of the article, it was resolved by consensus with a third reviewer.

Data extraction

The following data were collected from all studies: the first author's name, year of publication, country where the study was performed, sex, participant age at baseline, sample size, duration of follow-up, number of cases, methods for measurement and range of exposure, variables adjusted for in the analysis, as well as multivariate adjusted RR and 95 % CI for the highest *v.* lowest categories of fish and *n*-3 fatty acid intake or for each category of fish or *n*-3 fatty acid. For studies that reported results from various covariate analyses, we abstracted the estimates based on the model that included the most potential confounders. For fish consumption, measurement of fish intake varied among studies (grams, servings or times consumed per d, week, or month), and we used times/month as a standard measure of fish intake using the following equivalence: 105 g/time[21]. As the levels of fish consumption were often given by a range, the value of exposure was assigned as the midpoints of the ranges of the reported categories of fish intake. When the lowest category was open-ended, we set the lower boundary to 0. When the highest category was open-ended, we assumed the values as 1·2 times the lower bound[22]. For *n*-3 fatty acid intake, we used g/d as a standard measure, and the median value of each category was extracted as reported in the original studies. If results were reported for both total fish and the type of fish (lean and fatty), as in one study[23], we used the results for total fish in the main analysis. Of the relevant studies, one[24] was excluded because it had only two levels of fish intake. The study quality was assessed using the nine-star Newcastle–Ottawa Scale[25].

Statistical analysis

A pooled measure was calculated as the inverse variance-weighted mean of the natural logarithm of multivariate adjusted RR with 95 % CI for the highest *v.* lowest levels to assess the association of fish and *n*-3 fatty acid intake with T2DM risk. The *Q* test and I^2 of Higgins & Thompson[26] were used to assess heterogeneity among studies. I^2 describes the proportion of total variation attributable to between-study heterogeneity as opposed to random error or chance. In the presence of substantial heterogeneity ($I^2 > 50\,\%$)[27], the DerSimonian and Laird random-effect model was adopted as the pooling method; otherwise, the fixed-effect model was used as the pooling method. Meta-regression with restricted maximum likelihood (REML) estimation was performed to assess the potentially important covariate exerting substantial impact on between-study heterogeneity. The 'leave one out' sensitivity analysis[28] was carried out using $I^2 > 50\,\%$ as the criterion to evaluate the key studies with substantial impact on between-study heterogeneity. Publication bias was estimated using Egger's regression asymmetry test[29]. An analysis of influence was conducted[30], which describes how robust the pooled estimator is to the removal of individual studies. An individual study is suspected of excessive influence, if the point estimate of its omitted analysis lies outside the 95 % CI of the combined analysis.

In the dose–response analysis about the relationship between fish and *n*-3 fatty acid intake and T2DM risk, the between-study heterogeneity was taken into account. The method proposed by Greenland & Longnecker[31] and Orsini *et al.*[32] was used to calculate the study-specific slopes (linear trend) and their standard errors from the correlated natural logarithm of RR and their CI across categories of fish and *n*-3 fatty acid intake, and then the univariate random-effect meta-regression with REML estimation was performed to synthesise the study-specific slopes. The non-linear dose–response association of fish and *n*-3 fatty acid intake with T2DM risk was assessed by bivariate random-effect meta-regression with REML estimation[33] used to pool the study-specific two trend components generated by generalised least squares[31,32] based on the restricted cubic spline model[25,34] with three knot values at percentiles of 10, 50 and 90 % in the dose distribution. The potential non-linearity was tested on the coefficient of the second spline[25]. The adequacy of the bivariate random-effects model with respect to the linear one is evaluated by comparing the Akaike's information criteria between the two models. The results for both linear and non-linear models were reported. All statistical analyses were performed with STATA version 11.2 (Stata Corporation). All reported probabilities (*P* values) were two-sided, with $P < 0{\cdot}05$ considered statistically significant.

Results

Study characteristics

Overall, ten publications with thirteen cohort studies[6,19,23,35–41] were identified in the analysis for the association of fish and *n*-3 fatty acid consumption with risk of T2DM (Fig. 1). Of the ten articles, one study[6] included three independent cohorts, and another one[41] reported two independent cohorts; seven of the publications were conducted in the USA[6,19,35–38,40], one in the Netherlands[23] and two in Asia[39,41]. General characteristics in the published articles included in this meta-analysis are shown in Tables 1 and 2. Data on dietary assessment were collected by using FFQ (seven articles[19,23,35,37,38,40,41]) and semiquantitative FFQ (SFFQ) (three articles[6,36,39]). The range of follow-up period was from 4 to 15 years. All studies included met quality criteria ranging from 6 to 7 stars. For studies on *n*-3 fatty acids, four articles[6,35,37,41] reported long-chain *n*-3 fatty acids and three articles[19,36,39] reported *n*-3 fatty acids. Most studies provided risk estimates that were adjusted for smoking, alcohol consumption, physical activity (or exercise) and age.

Fish

High v. low analysis

Overall, six publications with nine cohort studies[6,19,23,38,40,41] including 367 757 subjects were included in the analysis on the association of fish intake with T2DM risk. The pooled RR was 1·146 (95 % CI 0·975, 1·346) with substantial between-study heterogeneity ($P_{\text{heterogeneity}} < 0{\cdot}001$, $I^2 = 79{\cdot}0\,\%$) (Fig. 2).

Sources of heterogeneity and sensitivity analysis

To explore the heterogeneity, we performed meta-regression for covariate, and sensitivity analysis for individual results. However, the univariate meta-regression analysis, with the covariates publication year, sex (male, female, both sexes), sample size, methods of dietary assessment (FFQ, SFFQ), duration of follow-up, and study quality, showed that no covariate had a significant impact on between-study heterogeneity. In the sensitivity analysis, two studies conducted by Djousse *et al.*[19] and Villegas *et al.*[41] for the Shanghai Women's Health Study were found to be the key contributors to the between-study heterogeneity. After excluding these two studies, no substantial between-study heterogeneity was observed among the seven cohorts left ($P_{\text{heterogeneity}} = 0{\cdot}198$, $I^2 = 30{\cdot}1\,\%$) and the pooled RR was 1·157 (95 % CI 1·051, 1·274).

No significant influence and publication bias were observed before and after the sensitivity analysis.

Dose–response meta-analysis

Overall, three publications with five cohort studies[6,23,40] were available to evaluate the dose–response association of fish intake with T2DM risk. For the linear trend analysis, the pooled RR for an increment of one time (about 105 g)/week of fish intake (four times/month) was 1·042 (95 % CI

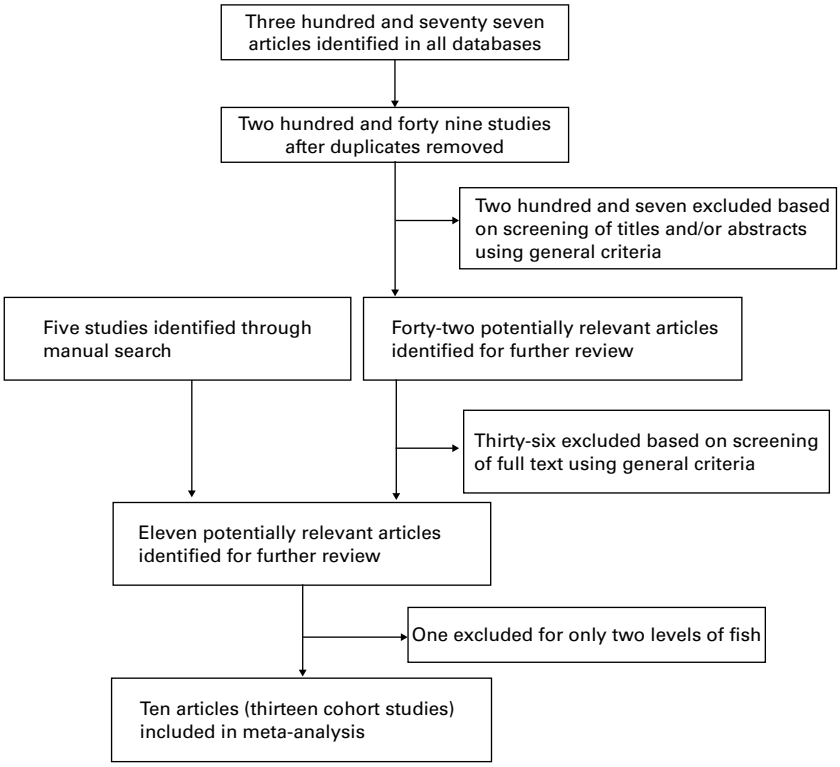

Fig. 1. Selection of studies for inclusion in meta-analysis.

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Table 1. Characteristics of prospective studies on fish intake and type 2 diabetes
(Relative risks (RR) and 95 % confidence intervals)

First author (year of publication)	Study name	Country	Sex	Age at baseline	No. of participants/ cases	Follow-up (years)	Study quality	Measure of intake	Quantity (highest *v.* lowest intake)	RR	95 % CI	Adjustment for covariates
Van Woudenbergh (2009)[23]	The Rotterdam Study	Netherlands	F/M	≥55	4472/463	15	7	Validated FFQ, 170 food items	35·6 g/d (quartile 4) *v.* 0 g/d (quartile 1)	1·32	1·02, 1·7	Age, sex, smoking, education level, intake of energy, alcohol, *trans*-fatty acids and fibre
Kaushik (2009)[6]	The Nurses' Health Study	USA	F	30–55	61031/4159	15	7	Validated SFFQ, 120 items	>5 times/week (Q5) *v.* < 1 time/month (Q1)	1·29	1·05, 1·57	Smoking, alcohol consumption, physical activity, family history of diabetes, BMI, intake of saturated fat, *trans*-fats, linolenic acid, caffeine, and cereal fibre; glycaemic index, energy intake, menopausal status and postmenopausal hormone use
Kaushik (2009)[6]	The Nurses' Health Study 2	USA	F	26–46	91669/2728	15	7	Validated SFFQ, 120 items	>5 times/week (Q5) *v.* < 1 time/month (Q1)	1·32	0·99, 1·74	Smoking, alcohol consumption, physical activity, family history of diabetes, BMI, intake of saturated fat, linolenic acid, caffeine, and cereal fibre; glycaemic index, hormone replacement therapy and oral contraceptive use
Kaushik (2009)[6]	The Health Professionals Follow-up Study	USA	M	39–78	42504/2493	15	7	Validated SFFQ, 120 items	>5 times/week (Q5) *v.* < 1 time/month (Q1)	1·16	0·96, 1·41	Smoking, alcohol consumption physical activity family history, BMI, intake of saturated fat, linoleic acid, caffeine, cereal fibre
Krishnan (2010)[38]	The Black Women's Health Study	USA	F	30–69	12303/2777	10	6	Validated FFQ	Two times/week (Q5) *v.* 0 times/week (Q1)	0·89	0·64, 1·24	Age, time period, education, family history of diabetes, television watching, vigorous activity, smoking, coffee consumption, sugar-sweetened soda, Ca, vitamin D, energy and BMI, menopausal status and postmenopausal hormone use, *trans*-fats, linolenic acid, linoleic acid, caffeine, and cereal fibre; glycaemic index, energy intake
Villegas (2011)[41]	The Shanghai Women's Health Study	China	F/M	40–70F	64193/2262F	8·9 F	6	Validated FFQ	80·2 g/d (Q5) *v.* 9·5 g/d (Q1) F	0·89 F	0·78, 1·01 F	Age, energy intake (kcal/d), waist:hip ratio, BMI, smoking, alcohol consumption, physical activity, income level, educational level, occupation, family history of diabetes, hypertension, and dietary pattern
	The Shanghai Men's Health Study			40–74M	51963/833M	4·1 M			79·0 g/d (Q5) *v.* 9·7 g/d (Q1) M	0·94 M	0·74, 1·17 M	
Djousse (2011)[40]	The Cardiovascular Health Study	USA	F/M	75	2831/177	10·6	7	Validated FFQ	<1 time/month (Q1) *v.* > 5 times/week (Q5)	1·07	0·35, 3·30	Age, race, sex, clinic site, BMI, alcohol consumption, physical activity, current smoking, total energy intake, and LDL-cholesterol
Djousse (2011)[19]	The Women's Health Study	USA	F	≧ 45	36328/2370	12·4	6	Validated FFQ	3·93 servings/week (Q5) *v.* 0·47 servings/week (Q1)	1·49	1·30, 1·70	Age, BMI, parental history of diabetes, smoking, exercise, alcohol intake, menopausal status, red-meat intake, quintiles of energy intake, linoleic acid, α-linolenic acid, dietary Mg, *trans*-fat, saturated fat, cereal fibre, glycaemic index

F, female; M, male; Q, quintile; SFFQ, semiquantitative FFQ.

Table 2. Characteristics of prospective studies on *n*-3 fatty acid intake and type 2 diabetes
(Relative risks (RR) and 95 % confidence intervals)

First author (year of publication)	Study name	Country	Sex	Age at baseline	No. of participants/cases	Follow-up (years)	Study quality	Measure of intake	Quantity (highest *v.* lowest intake)	RR	95 % CI	Adjustment for covariates
Meyer (2001)[37]*	The Iowa Women's Health Study	USA	F	55–69	35988/1890	11	6	Validated FFQ, 127 items	0·39 g/d (Q5) *v.* 0·03 g/d (Q1)	1·20	1·03, 1·39	Age, total energy, waist:hip ratio, BMI, physical activity, cigarette smoking, alcohol consumption, education, marital status, residential area, hormone replacement, therapy and dietary Mg
Song (2004)[36]†	Women's Health Study	USA	F	≧ 45	37309/1558	8·8	6	SFFQ, 131 items	1·88 g/d (Q5) *v.* 0·95 g/d (Q1)	1·10	0·93, 1·30	Age, BMI, total energy intake, smoking, exercise, alcohol use family history of diabetes, dietary intake of fibre intake, glycaemic load, Mg and total fat
van Dam (2002)[35]*	The Health Professionals Follow-up Study*	USA	M	40–75	42504/1321	12	7	Validated FFQ, 131 items	0·57 g/d (Q5) *v.* 0·08 g/d (Q1)	0·90	0·75, 1·07	Age, total energy intake, time period, physical activity, cigarette smoking, alcohol consumption, hypercholesterolaemia, family history of type 2 diabetes, hypertension, intake of cereal fibre and Mg and BMI
Kaushik (2009)[6]*	The Nurses' Health Study	USA	F	30–55	61031/4159	15	7	Validated SFFQ, 120 items	0·49 g/d (Q5) *v.* 0·06 g/d (Q1)	1·23	1·11, 1·37	Smoking, alcohol consumption, physical activity, family history of diabetes, BMI, intake of saturated fat, *trans*-fats, linolenic acid, caffeine, and cereal fibre; glycaemic index, energy intake, menopausal status and postmenopausal hormone use
Kaushik (2009)[6]*	The Nurses' Health Study 2	USA	F	26–46	91669/2728	15	7	Validated SFFQ, 120 items	0·36 g/d (Q5) *v.* 0·06 g/d (Q1)	1·25	1·10, 1·42	Smoking, alcohol consumption, physical activity, family history of diabetes, BMI, intake of saturated fat, linolenic acid, caffeine, and cereal fibre, glycaemic index, hormone replacement therapy and oral contraceptive use
Kaushik (2009)[6]*	The Health Professionals Follow-up Study	USA	M	39–78	42504/2493	15	7	Validated SFFQ, 120 items	0·62 g/d (Q5) *v.* 0·06 g/d (Q1)	1·12	0·98, 1·28	Smoking, alcohol consumption physical activity family history, BMI, intakes of saturated fat, linoleic acid, caffeine, cereal fibre

Table 2. *Continued*

First author (year of publication)	Study name	Country	Sex	Age at baseline	No. of participants/cases	Follow-up (years)	Study quality	Measure of intake	Quantity (highest *v.* lowest intake)	RR	95 % CI	Adjustment for covariates
Villegas (2011)[41]*	The Shanghai Women's Health Study	China	F/M	40–70 F	64193/2262 F	8·9 F	6	Validated FFQ	0·2 g/d (Q5) *v.* 0·02 g/d (Q1) F	0·84 F	0·74, 0·95 F	Age, energy intakes (kcal/d), waist:hip ratio, BMI, smoking, alcohol consumption, physical activity, income level, educational level, occupation, family history of diabetes, hypertension, and dietary pattern
	The Shanghai Men's Health Study			40–74 M	51963/833 M	4·1 M			0·2 g/d (Q5) *v.* 0·02 g/d (Q1) M	0·89 M	0·70, 1·12 M	
Brostow (2011)[39]†	The Singapore Chinese Health Study	Singapore	F/M	45–74	43176/2252	5·7	7	Validated SFFQ, 165 items	1·54 g/d (Q5) *v.* 0·45 g/d (Q1)	0·78	0·65, 0·94	Age, sex, dialect, year of interview, educational level, BMI, physical activity, smoking status, alcohol use, and hypertension, monounsaturated fat and saturated fat, dietary fibre, protein, and total energy
Djousse (2011)[19]†	The Women's Health Study	USA	F	≥45	36328/2370	12·4	6	Validated FFQ	0·43 g/d (Q5) *v.* 0·07 g/d (Q1)	1·44	1·25, 1·65	Age, BMI, parental history of diabetes, smoking, exercise, alcohol intakes, menopausal status, red-meat intakes, and quintiles of energy intakes, linoleic acid, α-linolenic acid, dietary Mg, *trans*-fat, saturated fat, cereal fibre, and glycaemic index

F, female; Q, quintile; M, male; SFFQ, semiquantitative FFQ.
* Long-chain *n*-3 fatty acids.
† *n*-3 Fatty acids.

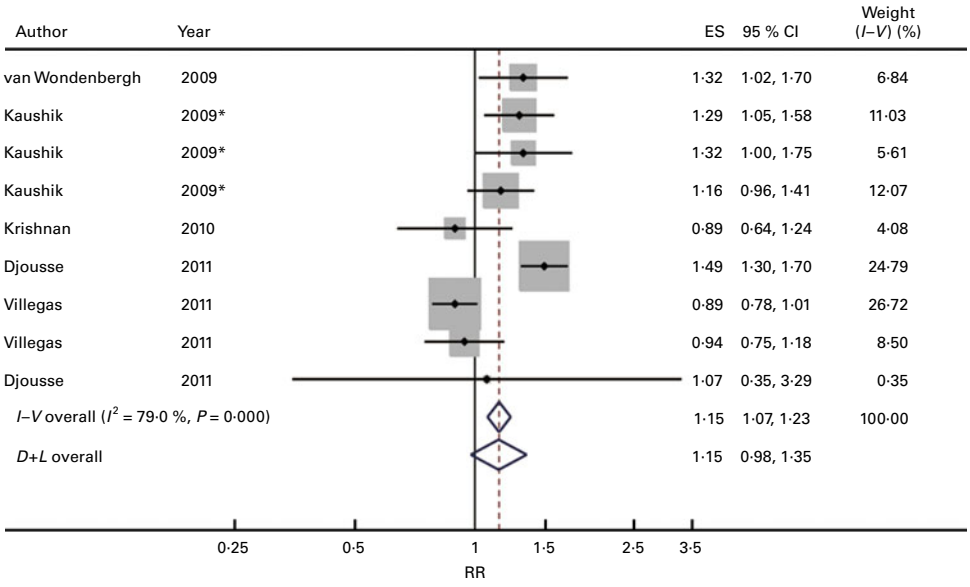

Fig. 2. Forest plot of relative risk (RR) of high *v.* low analysis for fish intake with type 2 diabetes mellitus risk. ◇ Denotes the pooled RR. ◆ Indicates the RR in each study, with the square sizes inversely proportional to the standard error of the RR. Horizontal lines represent the 95 % CI. * One study with different cohorts. ES, effect size; $I - V$, fixed effects model; $D + L$, random effects model. (A colour version of this figure can be found online at http://www.journals.cambridge.org/bjn).

1·026, 1·058), with no between-study heterogeneity ($P_{\text{heterogeneity}} = 0{\cdot}421$, $I^2 = 0{\cdot}00\,\%$). For the non-linear trend analysis, the overall association was significant ($P_{\text{overall association}} < 0{\cdot}001$), with an increase of fish intake generally associated with higher T2DM risk, but the non-linearity was not significant ($P_{\text{non-linearity}} = 0{\cdot}150$) (Fig. 3).

n-3 Fatty acids

High v. low analysis

Overall, seven publications with ten cohort studies[6,19,35–37,39,41] involving 506 665 subjects were included in the analysis on the association of *n*-3 fatty acid intake with T2DM risk. The pooled RR was 1·076 (95 % CI 0·955, 1·213), with substantial between-study heterogeneity ($P_{\text{heterogeneity}} < 0{\cdot}001$, $I^2 = 84{\cdot}8\,\%$) (Fig. 4).

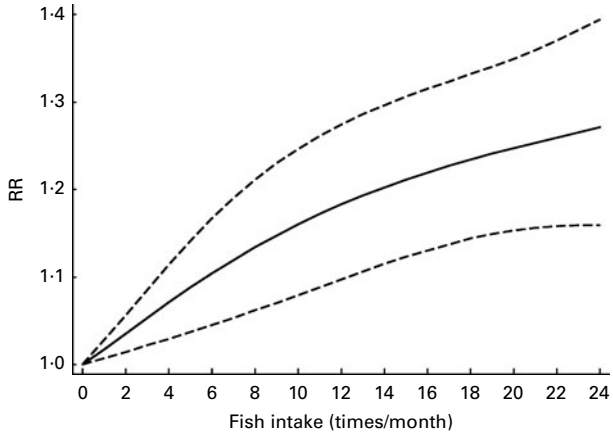

Fig. 3. Non-linear dose–response relationship between fish intake and type 2 diabetes mellitus risk assessed by restricted cubic spline model with three knots. Relative risk (RR, ——). ····, 95 % CI.

Sources of heterogeneity and sensitivity analysis

To explore the heterogeneity, we performed meta-regression for covariate, and sensitivity analysis for individual results. However, the univariate meta-regression analysis, with the covariates publication year, sex (male, female, both sexes), sample size, methods of dietary assessment (FFQ, SFFQ), duration of follow-up, type of *n*-3 fatty acid (*n*-3 fatty acids, long-chain *n*-3 fatty acids), and study quality, showed that no covariate had a significant impact on between-study heterogeneity. In the sensitivity analysis, three studies conducted by Djousse *et al.*[19], Brostow *et al.*[39] and Villegas *et al.*[41] for the Shanghai Women's Health Study were found to be the key contributors to the between-study heterogeneity. After excluding these three studies, no substantial between-study heterogeneity was observed among the seven cohorts left ($P_{\text{heterogeneity}} = 0{\cdot}108$, $I^2 = 42{\cdot}5\,\%$) and the pooled RR was 1·155 (95 % CI 1·094, 1·220).

No significant influence and publication bias were observed before and after the sensitivity analysis.

Dose–response meta-analysis

Overall, four studies with six cohorts[6,19,36,39] were available to evaluate the dose–response association of *n*-3 fatty acid intake with T2DM risk. For the linear trend analysis, the pooled RR for an increment of 0·1 g/d of *n*-3 fatty acid intake was 1·030 (95 % CI 1·002, 1·058), with substantial between-study heterogeneity ($P_{\text{heterogeneity}} < 0{\cdot}001$, $I^2 = 92{\cdot}1\,\%$). There was not much evidence for an overall association ($P_{\text{overall association}} = 0{\cdot}076$) with an increase of *n*-3 fatty acid intake with an almost slight increase of T2DM risk, and the non-linearity was also not significant ($P_{\text{non-linearity}} = 0{\cdot}084$).

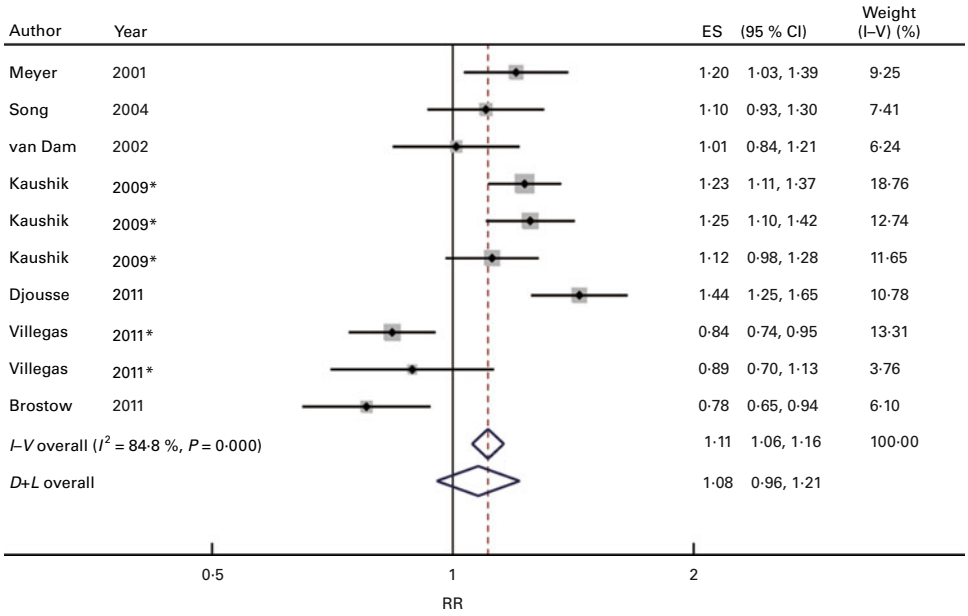

Fig. 4. Forest plot of relative risk (RR) of high *v.* low analysis for *n*-3 fatty acid intake with type 2 diabetes mellitus risk. ◇ Denotes the pooled RR. ◆ Indicates the RR in each study, with the square sizes inversely proportional to the standard error of the RR. Horizontal lines represent the 95 % CI. * One study with different cohorts. ES, effect size; $I - V$, fixed effects model; $D + L$, random effects model. (A colour version of this figure can be found online at http://www.journals.cambridge.org/bjn).

Overall, three studies conducted by Kaushik *et al.*[6] for the Health Professionals Follow-up Study, Song *et al.*[36] and Brostow *et al.*[39] were the key contributors to the between-study heterogeneity assessed by the 'leave one out' sensitivity analysis[28]. After excluding these three studies, no between-study heterogeneity was observed among the three cohorts left ($P_{\text{heterogeneity}} = 0{\cdot}46$, $I^2 = 0{\cdot}0\,\%$), and the linear trend of pooled RR for an increment of 0·1 g/d of *n*-3 fatty acid intake was 1·057 (95 % CI 1·042, 1·073). Moreover, after excluding these three studies, the overall association in the non-linear dose–response model was significant ($P_{\text{non-linear model}} < 0{\cdot}001$), with an increase of *n*-3 fatty acid intake generally associated with higher T2DM risk, but the non-linearity was not significant ($P_{\text{non-linearity}} = 0{\cdot}105$) (Fig. 5).

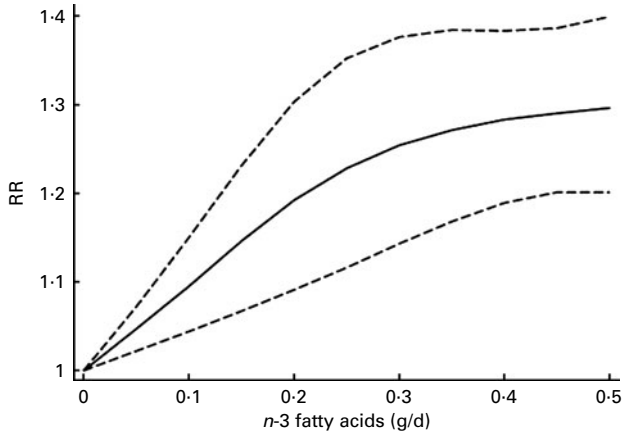

Fig. 5. Non-linear dose–response relationship between *n*-3 fatty acid intake and type 2 diabetes mellitus risk assessed by restricted cubic spline model with three knots. Relative risk (RR, ——). ····, 95 % CI.

Discussion

In this meta-analysis, a weakly positive association of fish and *n*-3 fatty acid intake with T2DM risk was found. For high *v.* low intake analysis, an increased but not significant T2DM risk was found before sensitivity analysis, and the increased T2DM risk was significant after sensitivity analysis. For dose–response analyses, the linear dose–response analyses reported a significantly positive association before and after sensitivity analysis. Considering the fact that categories of fish and *n*-3 fatty acid intake differed between studies, which might complicate the interpretation of the pooled results across study populations with different categories, a dose–response meta-analysis could provide a more robust method to combine results from individual studies and would better quantify the relationship between fish and *n*-3 fatty acid and T2DM risk than does the 'high *v.* low intake' analysis.

Between-study heterogeneity is common in meta-analysis, and our meta-analysis also showed significant between-study heterogeneity in the analyses of both fish and *n*-3 fatty acid intake. Although most studies in this meta-analysis used multivariate regression to adjusted confounders, other indeterminate characteristics that vary among studies, such as design quality, characteristics of the sample, non-comparable measures of fish and *n*-3 fatty acid intake, variation of the unmeasured covariate, diagnosis criteria of diabetes, etc. could be the causes of between-study heterogeneity. Hence, we used meta-regression and 'leave one out' sensitivity analysis[28], which aims to reduce between-study heterogeneity and explore the potential important causes of between-study heterogeneity for both covariate and studies. However, our meta-analysis did not identify any of the aforementioned covariates as being an important contributor to between-study

heterogeneity. Moreover, T2DM has a complex aetiology and pathophysiology generated by the combined effects of genes and environmental factors. Although the aforementioned covariates were not found to be important sources of disease–effect heterogeneity across the studies in this meta-analysis, other genetic background and other environmental variables as well as their possible interaction also deserve to be considered as potential contributors to this disease–effect unconformity. In this respect, the lack of relevant study-level covariate in the reported articles precluded our more robust assessment of sources of this heterogeneity. Whatever the reason, disease–effect inhomogeneity will finally influence the pooled-effect estimate. Thus, we performed the 'leave one out' sensitivity analysis[28] using $I^2 > 50\,\%$ as the criterion to exclude the key studies that had substantial impact on between-study heterogeneity; and the results suggested that higher intake of fish and *n*-3 fatty acids might weakly increase the T2DM risk.

In the explanation of our present results, the limitations in our meta-analysis should be taken into consideration. First, measurement errors in the assessment of dietary intake are known to bias effect estimates, particularly when using FFQ to assess *n*-3 fatty acid consumption, although most of the studies included in our meta-analysis used a validated FFQ. Random measurement error in dietary exposures most frequently attenuates risk estimates[42]. We cannot exclude the possibility that measurement errors and lack of accurate data on categories of exposure might have resulted in attenuated associations and that such attenuation might explain, in part, why the associations we observed are weak. Second, most of the included studies did not assess extensive details about the specific subcategories of fish and *n*-3 fatty acid consumed. EPA and DHA are present mainly in fatty fish, which may indicate that it is also important to pay attention to the type of fish consumption instead of total fish intake alone. In our present study, only two publications by van Woudenbergh *et al.*[23] and Villegas *et al.*[41] reported results for both total fish and the type of fish (lean and fatty fish or freshwater and saltwater fish). As for *n*-3 fatty acids, only one study conducted by Djousse *et al.*[19] reported results for both total marine *n*-3 fatty acids and three types of *n*-3 fatty acids (α-linolenic acid, EPA and DHA). Therefore, we cannot perform our meta-analysis for the subtype of fish or *n*-3 fatty acid to assess the potential effects. Third, considering the small number of studies included in our meta-analysis for both high *v.* low intake and linear and non-linear dose–response analyses, the validity of our publication bias test might be questioned.

Several suggestions should be considered in further studies. First, the data on *n*-3 fatty acids from these studies are derived from FFQ, which is very useful for ranking within populations, but have provided narrow ranges of estimated intake that may be questionable as biologically relevant[39]. Thus, in contrast to estimates from FFQ, the measurement of plasma phospholipid or cholesteryl ester fatty acids may provide an objective measure of exposure. Second, further cohort studies are warranted to estimate the specific type of fish and *n*-3 fatty acids, because only three studies[19,23,41] in our meta-analysis assessed the subtype of fish and *n*-3 fatty acid consumed. Third, most of the studies included were conducted in Americans, only one in Dutch and two in Chinese; considering the underlying disease–effect unconformity across different geographical locations, more studies deserve to be conducted in other populations. Fourth, the meta-analysis of observational studies presented particular challenges because of inherent biases and variations in study design; and hence, more research and different approaches such as randomised feeding or supplementation studies are warranted to investigate which, if any, specific type of *n*-3 fatty acid is involved in T2DM aetiology.

In summary, this meta-analysis suggested that higher fish and *n*-3 fatty acid consumption might be associated with a weak increase of T2DM risk. Since the potential biases and confounders could not be ruled out completely in this meta-analysis, further studies are warranted to confirm these results.

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References

1. Carter P, Gray LJ, Troughton J, *et al.* (2010) Fruit and vegetable intake and incidence of type 2 diabetes mellitus: systematic review and meta-analysis. *BMJ* **341**, c4229.
2. Knowler WC, Barrett-Connor E, Fowler SE, *et al.* (2002) Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med* **346**, 393–403.
3. Mostad IL, Bjerve KS, Bjorgaas MR, *et al.* (2006) Effects of *n*-3 fatty acids in subjects with type 2 diabetes: reduction of insulin sensitivity and time-dependent alteration from carbohydrate to fat oxidation. *Am J Clin Nutr* **84**, 540–550.
4. Woodman RJ, Mori TA, Burke V, *et al.* (2002) Effects of purified eicosapentaenoic and docosahexaenoic acids on glycemic control, blood pressure, and serum lipids in type 2 diabetic patients with treated hypertension. *Am J Clin Nutr* **76**, 1007–1015.
5. Puhakainen I, Ahola I & Yki-Jarvinen H (1995) Dietary supplementation with *n*-3 fatty acids increases gluconeogenesis from glycerol but not hepatic glucose production in patients with non-insulin-dependent diabetes mellitus. *Am J Clin Nutr* **61**, 121–126.
6. Kaushik M, Mozaffarian D, Spiegelman D, *et al.* (2009) Long-chain omega-3 fatty acids, fish intake, and the risk of type 2 diabetes mellitus. *Am J Clin Nutr* **90**, 613–620.
7. Vessby B, Karlstrom B, Boberg M, *et al.* (1992) Polyunsaturated fatty acids may impair blood glucose control in type 2 diabetic patients. *Diabet Med* **9**, 126–133.
8. Osterud B & Elvevoll EO (2011) Dietary omega-3 fatty acids and risk of type 2 diabetes: lack of antioxidants? *Am J Clin Nutr* **94**, 617–618.
9. Lee DH, Lee IK, Song K, *et al.* (2006) A strong dose–response relation between serum concentrations of persistent organic pollutants and diabetes: results from the National

British Journal of Nutrition

Health and Examination Survey 1999–2002. *Diabetes Care* **29**, 1638–1644.
10. Mozaffarian D & Rimm EB (2006) Fish intake, contaminants, and human health: evaluating the risks and the benefits. *JAMA* **296**, 1885–1899.
11. Chen YW, Huang CF, Tsai KS, *et al.* (2006) The role of phosphoinositide 3-kinase/Akt signaling in low-dose mercury-induced mouse pancreatic beta-cell dysfunction *in vitro* and *in vivo*. *Diabetes* **55**, 1614–1624.
12. Nkondjock A & Receveur O (2003) Fish-seafood consumption, obesity, and risk of type 2 diabetes: an ecological study. *Diabetes Metab* **29**, 635–642.
13. Fedor D & Kelley DS (2009) Prevention of insulin resistance by *n*-3 polyunsaturated fatty acids. *Curr Opin Clin Nutr Metab Care* **12**, 138–146.
14. Panagiotakos DB, Zeimbekis A, Boutziouka V, *et al.* (2007) Long-term fish intake is associated with better lipid profile, arterial blood pressure, and blood glucose levels in elderly people from Mediterranean islands (MEDIS epidemiological study). *Med Sci Monit* **13**, CR307–CR312.
15. Ruidavets JB, Bongard V, Dallongeville J, *et al.* (2007) High consumptions of grain, fish, dairy products and combinations of these are associated with a low prevalence of metabolic syndrome. *J Epidemiol Community Health* **61**, 810–817.
16. Harding AH, Day NE, Khaw KT, *et al.* (2004) Habitual fish consumption and glycated haemoglobin: the EPIC-Norfolk study. *Eur J Clin Nutr* **58**, 277–284.
17. Adler AI, Boyko EJ, Schraer CD, *et al.* (1994) Lower prevalence of impaired glucose tolerance and diabetes associated with daily seal oil or salmon consumption among Alaska Natives. *Diabetes Care* **17**, 1498–1501.
18. Bjerregaard P, Pedersen HS & Mulvad G (2000) The associations of a marine diet with plasma lipids, blood glucose, blood pressure and obesity among the inuit in Greenland. *Eur J Clin Nutr* **54**, 732–737.
19. Djousse L, Gaziano JM, Buring JE, *et al.* (2011) Dietary omega-3 fatty acids and fish consumption and risk of type 2 diabetes. *Am J Clin Nutr* **93**, 143–150.
20. Schulze MB, Manson JE, Willett WC, *et al.* (2003) Processed meat intake and incidence of type 2 diabetes in younger and middle-aged women. *Diabetologia* **46**, 1465–1473.
21. He K, Song Y, Daviglus ML, *et al.* (2004) Accumulated evidence on fish consumption and coronary heart disease mortality: a meta-analysis of cohort studies. *Circulation* **109**, 2705–2711.
22. Berlin JA, Longnecker MP & Greenland S (1993) Meta-analysis of epidemiologic dose–response data. *Epidemiology* **4**, 218–228.
23. van Woudenbergh GJ, van Ballegooijen AJ, Kuijsten A, *et al.* (2009) Eating fish and risk of type 2 diabetes: a population-based, prospective follow-up study. *Diabetes Care* **32**, 2021–2026.
24. Patel PS, Sharp SJ, Luben RN, *et al.* (2009) 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* **32**, 1857–1863.
25. Wells G, Shea B, O'connell D, *et al.* (2000) The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. Ottawa: Department of Epidemiology and Community Medicine. http://www.ohri.ca/programs/clinical_epidemiology/nos_manual.pdf
26. Higgins JP & Thompson SG (2002) Quantifying heterogeneity in a meta-analysis. *Stat Med* **21**, 1539–1558.
27. Higgins JP, Thompson SG, Deeks JJ, *et al.* (2003) Measuring inconsistency in meta-analyses. *BMJ* **327**, 557–560.
28. Patsopoulos NA, Evangelou E & Ioannidis JP (2008) Sensitivity of between-study heterogeneity in meta-analysis: proposed metrics and empirical evaluation. *Int J Epidemiol* **37**, 1148–1157.
29. Egger M, Davey Smith G, Schneider M, *et al.* (1997) Bias in meta-analysis detected by a simple, graphical test. *BMJ* **315**, 629–634.
30. Tobias A (1999) Assessing the influence of a single study in the meta-analysis estimate. *Stata Tech Bull* **47**, 15–17.
31. Greenland S & Longnecker MP (1992) Methods for trend estimation from summarized dose–response data, with applications to meta-analysis. *Am J Epidemiol* **135**, 1301–1309.
32. Orsini N, Bellocco R & Greenland S (2006) Generalized least squares for trend estimation of summarized dose–response data. *Stata J* **6**, 40–57.
33. White IR (2009) Multivariate random-effects meta-analysis. *Stata J* **9**, 40–56.
34. Bagnardi V, Zambon A, Quatto P, *et al.* (2004) Flexible meta-regression functions for modeling aggregate dose–response data, with an application to alcohol and mortality. *Am J Epidemiol* **159**, 1077–1086.
35. van Dam RM, Willett WC, Rimm EB, *et al.* (2002) Dietary fat and meat intake in relation to risk of type 2 diabetes in men. *Diabetes Care* **25**, 417–424.
36. Song Y, Manson JE, Buring JE, *et al.* (2004) A prospective study of red meat consumption and type 2 diabetes in middle-aged and elderly women: the Women's Health Study. *Diabetes Care* **27**, 2108–2115.
37. Meyer KA, Kushi LH, Jacobs DR Jr, *et al.* (2001) Dietary fat and incidence of type 2 diabetes in older Iowa women. *Diabetes Care* **24**, 1528–1535.
38. Krishnan S, Coogan PF, Boggs DA, *et al.* (2010) Consumption of restaurant foods and incidence of type 2 diabetes in African American women. *Am J Clin Nutr* **91**, 465–471.
39. Brostow DP, Odegaard AO, Koh WP, *et al.* (2011) Omega-3 fatty acids and incident type 2 diabetes: the Singapore Chinese Health Study. *Am J Clin Nutr* **94**, 520–526.
40. Djousse L, Biggs ML, Lemaitre RN, *et al.* (2011) Plasma omega-3 fatty acids and incident diabetes in older adults. *Am J Clin Nutr* **94**, 527–533.
41. Villegas R, Xiang YB, Elasy T, *et al.* (2011) 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* **94**, 543–551.
42. Beaton GH (1994) Approaches to analysis of dietary data: relationship between planned analyses and choice of methodology. *Am J Clin Nutr* **59**, 253S–261S.

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