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Association between Omega-3 fatty acids consumption and the risk of Type 2 Diabetes: a meta-analysis of cohort studies

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Running title: Omega 3 and T2D risk

Abstract:

Aims/Introduction: Epidemiologic evidences for the effect of omega 3 fatty acids on the risk of type 2 diabetes (T2D) are controversial. A meta-analysis based on prospective cohorts was conducted to evaluate this issue.

Materials and Methods: Pooled diabetic risk was calculated using fixed or random effects model. Dose-response relationship was assessed by meta-regression analysis.

Results: Our study revealed that consumption of single omega-3 was associated with an increased risk of T2D (Relative Risk, RR=1.45, $p<0.001$); while the RR for mixed omega-3 was statistically insignificant. Dose-response curve presented an inverted U-shape of diabetes risk corresponding to the dose of omega-3 consumption. Sub-analysis showed that omega-3

was inversely associated with T2D risk in Asians (RR=0.82, p<0.001); while the risk was increased in Westerners (RR=1.30, p<0.001). Studies with follow-up duration≥16 years and baseline age≥54 years demonstrated positive association between T2D risk and omega-3 intake.

Conclusions: Our findings suggest that dosage and composition of omega-3, ethnicity, trial duration and recruited age may influence the effect of omega-3 on T2D progression.

Keywords: meta-analysis; omega-3 fatty acids; type 2 diabetes

Introduction

Type 2 diabetes (T2D) is a complex metabolic disorder featured with chronic hyperglycemia, the prevalence of which is estimated to raise from 171 million in 2000 to 366 million in 2030 worldwide [1-3]. There are various factors contributing to the growth of diabetic incidence and daily diet is standing out as a quite important factor [4, 5].

Since the year 1966, it has been reported that the incidence of T2D was significantly reduced with high fish and seafood consumption [6-8] in north western Greenland, which may be attributed to the effect of omega-3 fatty acids, the predominant fatty acid composition of seafood. In the past decades, notwithstanding plenty of reports have been published about the effects of omega-3 fatty acids on diabetes prevention, discrepancies still remain. Several cohort studies demonstrated that high intake of n-3 fatty acids was in relation to a lower prevalence of T2D [5, 9-11]; while some other studies showed positive [12, 13] or null associations [14, 15]. Inconsistent results were also reported in clinical trials which investigate the effect of fish oil supplementation on glucose homeostasis [16-20].

Recent published systematic reviews reported that omega-3 fatty acids supplementation was either positive or insignificant associated with T2D development [21-25]. These conclusions suggest an unfavourable effect of omega 3 supplementation on people who are prone to develop to diabetes, for example people with obesity, insulin resistance and hyperlipidemia. However, some other systematic reviews reported that omega 3 presents beneficial effects on metabolic related diseases; it exerts cardio-protective effect, reduces ischemic stroke risk, corrects high TG level, and increases insulin sensitivity [21, 26]. These contrary notions may confound physicians and nutritionists on dietary guidance. Additionally, these meta-analysis papers failed to dissect the source that results in this contradiction. We therefore, conducted a meta-analysis with dose-response model and subgroup analysis in prospective cohort studies to evaluate the potential factors which influence the effect of omega-3 fatty acids consumption on T2D incidence.

Methods

Data Sources and Searches

A comprehensive literature search was conducted from Pubmed, Cochrane Library, Medline, SIGLE, EMBASE databases, and National Research Register with last date of inclusion to be the end of May 2016. The Medical Subject Heading (MeSH) terms and keywords for database searching include 1) omega-3 or n-3 or ω-3 fatty acids; 2) docosapentaenoic acid or DPA; 3) eicosapentaenoic or EPA; 4) docosahexaenoic or DHA; 5) fish oil(s). We combined these terms with diabetes mellitus, type 2 diabetes, or T2D, which was detailedly described in our previous work [26]. Cross references of studies or reviews were also examined manually.

Two investigators (Shao and Chen) worked independently to determine the eligible studies by reviewing the titles, abstracts and keywords. The manuscripts were obtained in full-text version for further assessment if the study 1) was a cohort design investigating the association

between omega-3 supplementation and the incidence of T2D; 2) analyzed relative risk (RR), hazard ratios (HR) or odds ratios (OR) with 95% confidence interval (CI); 3) reported at least 3 quantitative exposure levels of omega-3 for dose-response analysis. 4) showed the method of dietary assessment; and 5) included the participants at baseline who were not diagnosed as T2D.

Data Extraction

Data extraction was independently performed by Shao and Chen. The extracted information included composition and intake amounts of omega-3, number of cases and person-years of follow-up in each exposure category, follow-up years, study setting, diabetes diagnosis, baseline characteristics of included subjects (number of participants, age at recruitment, gender, and ethnicity), and the adjusted RR with 95% CI. For studies with OR or HR data, we converted OR and HR into RR using a previously published formula [27]. The corresponding CI Values were also converted. When studies reported results with different models for variable adjustment, data were extracted from models including the most potential confounders.

Quality Assessment

Quality assessment of cohort studies was conducted based on "Newcastle-Ottawa Scale (NOS)" Criteria [28], which were performed by Shao and Chen independently with discrepancies resolved by Yang. The maximum score that can be assigned by NOS is nine points in three broad items: 1) selection of study groups (up to four points); 2) comparability of groups (up to two points); and 3) assessment of exposure and outcomes (up to three points). The overall evaluation of included trials is presented in Table S1.

Statistical analysis

The meta-analysis was conducted by STATA11.0. Extracted data from cohort studies were analyzed with fixed effects model to calculate the pooled RR comparing the highest versus the lowest intake of EPA, DHA or mixed omega-3 with 95%CI. $p<0.05$ was considered to be statistically significant. Heterogeneity was assessed using the chi-square method [29]. Random effects model was used when heterogeneity was statistically significant ($p<0.1$) [30].

The methods proposed by Greenland and Orsini [31, 32] were used in our dose-response analysis. To assess the possible non-linear trends between ω-3 fatty acid intake and T2D risk, we employed a restricted cubic spline model, in which 4 fixed knots at the 5th, 35th, 65th and 95th percentile of the exposure distribution were set up [33, 34]. The regression coefficients of the second and third splines were assumed equal to zero to test the nonlinearity using the Wald test [31]. Linear analysis was chosen in the subsequent calculating steps if $p\geq0.05$; otherwise a nonlinear model was employed.

The generalized least-square model was used to estimate the RR of T2D for daily dose increment of ω-3 fatty acid consumption; and a random-effect model was applied to synthesis the study-specific regression coefficients [35, 36]. The mean or median value of each exposure level was allocated to the corresponding RR [36].The lowest exposure category was defined as a referent; while other categories were centralized to the referent dose. Incomplete data was calculated using an improved method of Bekkering et al [37].

Subgroup analysis was performed according to 1) duration of studies (< 16 years versus ≥ 16 years); 2) ethnicity (Asian versus US/European); 3) age at the initial stage of studies (<54 years versus ≥54 years).

The potential source of heterogeneity between included studies was investigated through meta-regression analysis with p-value<0.1 as statistical significance. The items including type of omega-3 fatty acids, study duration, ethnicity and age at recruitment were analyzed in the regression model. Funnel plot with Egger's linear regression analysis was used to determine the risk of publication bias by assessing the asymmetry of funnel plot. p-value <0.1 was considered to be of significant bias [38, 39].

Results

Description of studies

The initial search obtained 1450 publications in 2016, of which 30 studies were investigated in full-text articles (Figure S1). Finally, 5 papers with 10 cohort trials were potentially eligible [5, 9, 10, 12, 13].

The scores of included studies, according to NOS Criteria, ranged from 5 to 8 (Table S1). A total of 426,852 participants were included. The individual sample size varied from 35,988 to 91,669 participants and the follow-up duration was 4.1~18 years. These participants were non-diabetic at the beginning of each study, aged from 26 to 78 years. Validated Food Frequency Questionnaire (VFFQ) was used to assess the dietary method. EPA, DHA, and mixed omega-3 fatty acids were examined as dietary factors, the amounts of which were organized in five quintiles. The pooled RR for T2D was calculated by comparing the RR corresponding to the highest exposure category of omega-3 with that of the lowest one.

Omega-3 fatty acids intake and Risk of T2D

10 cohort studies with 3 dietary factors (EPA, DHA and mixed omega-3) were included in this meta-analysis. As shown in Figure 1, the overall effect of total omega-3 fatty acids on the risk of T2D was insignificant (RR=1.14, 95% CI 0.99 to 1.31, p=0.062). Furthermore, we analyzed the association of mixed omega-3 fatty acids supplementation with T2D, which was found to

be insignificant (RR=1.07, 95% CI 0.92 to 1.25, p=0.35) as well. Interestingly, despite the limited included studies, the consumption of single omega-3 subtype (either EPA or DHA) was related to an increased risk of T2D (Figure 1) with the pooled effect size 1.45 (95%CI, 1.31 to 1.60, p<0.001).

Dose-response analysis

Among the 10 included studies, a significant non-linear association was identified (p＜0.001 for non-linear test). The dose-response curve showed an inverted U-shape with 0.43 g/d as the peak point. Specifically, within people consuming 0.10-0.43 g n-3 fatty acids per day, an increment dose of fatty acids intake was generally associated with a significant higher T2D risk as compared to the referent dosage (0 g/d). However, supplementation of n-3 fatty acids with 0.43-0.75g/d showed a decreased tendency in the T2D risk as compared to the 0.10-0.43g/d dose range. RR in more than 0.75g/d category (0.75-1.08 g/d) was further decreased with no statistic significance (Figure 2A).

Two studies investigated the association of T2D risk and single omega-3 fatty acids intake. In accordance with results we obtained in Figure 1, a significant positive association between EPA/DHA with T2D risk was observed in the non-linear model (Figure 2B). To exclude the possible interference of single n-3 fatty acids, we conducted a non-linear analysis in 8 studies with mixed omega-3 as dietary factor (Figure 2C) and a similar curve was obtained with that in Figure 2A (p＜0.001 for non-linear test, p＜0.001 for overall association).

Subgroup analysis and Heterogeneity evaluation

High degree of heterogeneity was observed in overall analysis (I-squared=89.8%, p<0.001). Thus, we further performed sub-analysis according to ethnicity, study duration, and age of subjects at recruitment to explore the source of heterogeneity. As shown in Figure 3A, studies based on Asian population indicated a protective effect of omega-3 fatty acids intake against

the development of T2D (pooled RR=0.82, p<0.001). Conversely, studies on western populations demonstrated increased risk of T2D (pooled RR =1.30, p<0.001).

We chose the median values as the cut-off points of study duration (16 years) and subject age (54-year old). There was no significant association between omega-3 fatty acids intake and T2D incidence in studies with less than 16 years of follow-up (pooled RR =0.97, p=0.782); while studies with more than 16 years of follow-up (including 16 years) indicated an increased risk of T2D (pooled RR =1.33, p<0.001) (Figure 3B). In subgroup analysis according to age at recruitment (Figure 3C), individuals who were recruited at the age of more than 54-year old presented increased risk of T2D (pooled RR=1.24, p=0.04). No significant association was observed in subgroup which including individuals with initial age less than 54-year old (pooled RR =1.05, p=0.574).

According to the above data, high degree of heterogeneity was still observed in all subgroup analysis except in studies with Asian subjects. Subsequently, we performed a meta-regression analysis according to the following covariates: ethnicity, age of subjects at baseline, omega-3 composition, and follow-up duration (Table S2). Univariate analysis showed that no significant association was observed in the covariate of age (p=0.783); while ethnicity (p<0.01), omega-3 composition (p=0.10) and study duration (p=0.01) were possible factors that caused the heterogeneity. Thus, we selected these factors for multivariate analysis and identified ethnicity (p=0.007) and omega-3 composition (p=0.064) as the major factors that contribute to the heterogeneity between studies involved (Table 1).

Furthermore, we performed subgroup analysis in US/European population according to omega-3 composition. As shown in Figure 4, no significant heterogeneity was observed in single (I^2=0.0%, p=0.328) or mixed (I^2=42.1%, p=0.141) omega-3 subgroup, which further confirmed the source of heterogeneity identified in this study.

Publication bias evaluation

As shown in Figure 5, the risk of publication bias was evaluated using the method of funnel plot with Egger's linear regression line. Although the graph of funnel plot displayed asymmetric shape, no publication bias was observed in Egger's test (p=0.411).

Discussion

This meta-analysis pooled 10 cohort studies with 426,852 participants to explore the association between omega-3 consumption and T2D risk. It is known that omega-3 fatty acids include EPA, DPA, DHA and α-linolenic acid (ALA), etc [40]. Dietary factors included in these cohort studies were EPA, DHA and mixed omega-3. Although the overall effect of total omega-3 fatty acids was insignificant on T2D development, the supplementation of single omega-3 subtype was correlated to an increased risk of T2D. The dose-response analysis presented an inverted U-shaped curve of T2D risk with the peak point at 0.43 g/d of omega-3 supplementation. Subgroup analysis identified that omega-3 consumption only showed beneficial effect in Asian subjects.

It was identified that subjects with single omega-3 supplementation presented a more obvious tend on T2D progression when compared with mixed omega-3 intake. Although the limited included trials, this finding still impelled us to assume whether synergic action in vivo of different omega-3 individuals could alleviate the detrimental effects of single omega-3 subtype intake on T2D development. Mixed omega-3 fatty acids used in WHS3 study include EPA, DHA and DPA [12]. In SWHS study, subjects were supplemented with omega-3 mixtures of EPA and DHA [5]. The composition of omega-3 fatty acids in SCHS study was EPA, DHA, and ALA [9]. However, in NHS, NHS2, HPFS, SMHS and IWHS studies, detailed information of mixed omega-3 was unavailable [10, 13]. Additionally, it is not clear about the percentage of the individual fatty acid in mixed omega-3 supplementation. Our previous study found that high

ratio of EPA/DHA could improve insulin resistance [26]. It is known that insulin resistance is the major pathophysiological feature of T2D [27]. Therefore, we assumed that the varied compositions and proportions of omega-3 subtypes might explain, at least in part, the divergences of their effects on T2D prevention and development. Our heterogeneity analysis also identified that the composition of omega 3 contributed a lot to the high degree of heterogeneity between involved trails. More investigations focusing on this assumption remain to be performed, which may bring a new concept on omega 3 supplementation.

According to the inverted U-shaped dose-response curve with ~1g/d dose range (Figure 2), we presume that daily intake of around 0.43 g omega-3 fatty acids may impose the most significant detrimental effect on T2D development. Either lower or higher supplementation dose showed decreasing tendency. On the other hand, several studies with higher dose of omega-3 fatty acids intake have shown beneficial effects on some diseases that share similar pathological processes and/or risk factors with T2D, such as hyperlipidemia [41], insulin sensitivity [42-44], obesity [45, 46], nonalcoholic fatty liver disease (NAFLD) [47], inflammatory reaction [48] and cardiovascular diseases [49]. Pirillo et al suggested that the optimal dosage should be 3-4g/d to achieve a significant lipid-lowering effect [41]. Daily consumption of 0.85-1.8g/d omega-3 was advised to provide a protective efficacy in people with documented CVD [50, 51]. Additionally, 0.7-5.1g/d of n-3 fatty acids supplementation was associated with a greater reduction on urine protein excretion [52]. These findings together with the dose-response curve in this study led us to get the concept that higher omega-3 intake amount (more than 0.43 g/d at least) may provide a protective effect, or at least a risk-lowering tendency in T2D. An appropriate dose range to achieve a beneficial effect on T2D is waiting for identification.

Furthermore, our subgroup analysis showed that omega-3 fatty acids exerted beneficial effects in Asian populations but were detrimental in Western populations. However, there were only two studies that included Asian populations (Chinese people in particular). More

investigations across different Asian groups were required. Nevertheless, the distinct lifestyle between these two populations, especially the cooking manner, may result in the contrary observations. It is speculated that cooking manner (e.g., fried in Asian style or raw in Western style) can influence the generation of omega-3 derived lipid mediators and resultantly change the physiological effects of omega-3 fatty acids in vivo. Additionally, Eastern and Western populations share totally different dietary patterns. It would be significant to identify the biochemical interactions between omega-3 fatty acids and other multifarious dietary factors. Furthermore, fish oil related gene polymorphisms in different ethnicity may also contribute to the opposite effects observed in US/European and Asian populations. A recent study reported that carriers of ELOVL2 SNP minor alleles with a daily intake of 1.8 g omega-3 showed a more obvious increase of plasma EPA and DHA when compared to non-carriers. Thus these carriers may benefit from high levels of plasma omega-3 [53]. It is known that adiponectin has a beneficial effect on the improvement of insulin sensitization [54]. Alsaleh reported that ADIPOQ gene polymorphism interacted with fish oil to affect plasma adiponectin levels [55]. More studies that assess the relationship between omega-3 fatty acids and Single Nucleotide polymorphisms (SNPs) in different ethnicities may shed light on the complicated effects of omega-3 on different ethnicities.

Sub-analysis according to follow-up duration recognized that studies with more than 16 years of follow-up showed positive effect of omega-3 intake on T2D development; while insignificant finding was identified in studies with less than 16 years of follow-up. Due to aging as one of the primary risk factors for T2D [56, 57], there should be no question on this conclusion since longer follow-up duration implies more aged subjects. The result from sub-analysis by age with cut-off point 54 years old get the consistent result. These data indicate that early supplementation of omega-3 fatty acids may get beneficial outcome for T2D prevention.

Moreover, we evaluated the risk of publication bias with the method of funnel plot. Although there was some suggestion of asymmetry from visual inspection, no statistical significance was identified. Thus, it is assumed that such asymmetric shape should not be a marked factor that affects our conclusions. An asymmetric funnel plot usually indicates a possible publication bias; nevertheless there are other factors that can cause the asymmetry of a funnel plot, such as the involvement of small-size studies and marked heterogeneity between included studies [58]. As shown in Figure 5, dots in the funnel plot, which represent included studies, spread widely, suggesting a big heterogeneity between these studies. Thus, it is estimated that the asymmetry of the funnel plot might result from the study heterogeneity but not publication bias.

In this study, high degree of heterogeneity can be observed either in overall or in subgroup analysis, which may overestimate or underestimate the effect of omega-3 fatty acids on T2D risk. Such heterogeneity may be attributed to several limitations of included studies. First, included studies contained people from different ethnicities. Only 3 trials showed protective effect of omega-3 fatty acids on T2D, all of which chose Asian populations as recruited subjects. Second, various dietary factors (single or mixed omega-3) were used in these studies; however, the detailed information about the composition and percentage of supplemented omega-3 was not available. Thirdly, included studies ranged from 1990 to 2011; hence the progression of technique and the update of testing device may affect the obtained data. Thus, we performed meta-regression analysis to trace the source of heterogeneity. Ethnicity and omega-3 composition were identified as the major factors.

In conclusion, our data are relevant to clinicians and nutritionists on adopting optimized dietary guidance for diabetes-prone populations. We assumed that dietary supplementation with different subtypes of omega-3 results in varied effects on T2D prevention. However, this study does not provide evidence to discourage the use of omega-3 fatty acids because the

analyzed studies have showed the decreased incidence of T2D with mixed omega-3 supplementation in Asian population. The appropriate dosage and compositions of omega-3, the optimized cooking method, and early omega-3 supplementation may be beneficial for T2D prevention.

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Author Contributions: S.S., S.H., and X.Y. conceived and designed the experiments; C.C., Y.Y. and S.S. performed the experiments; C.C., Y.Y., and S.S. analyzed the data; S.S., S.H., and C.C. wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

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Legend

Figure 1. Forest plot of meta-analysis for T2D risk and omega-3 fatty acids supplementation.

Figure 2. Non-linear dose-response relationship between omega-3 fatty acids consumption and T2D risk based on total omega-3 (A), single omega-3 (B), and mixed omega-3 (C).

Figure3. Forest plot of meta-analysis for T2D risk and omega-3 fatty acids supplementation according to Asian/Western populations (A), duration of follow-up (B), and age of subjects at baseline (C).

Figure 4. Forest plot of meta-analysis for T2D risk and omega-3 fatty acids supplementation based on omega-3 composition in US/European population.

Figure 5. Funnel plot with Egger´s regression line for risk ratio of T2D.

Table 1. Results of Source Meta-regression analysis to explore heterogeneity (Multivariate analysis).

Covariates	exp (b)	SE	P
Ethnicity	0.63	0.07	0.01
Omega-3 composition	0.86	0.06	0.06
Follow-up duration	0.99	0.01	0.65

Note: The dependent variable is the lnRR for T2D incidence from each study. Weights were assigned according to the estimated variance of lnRR. RR, relative risk; exp (b), relative risk of estimates; SE, standard error of exp (b).

Supplementary Materials:

Figure S1: Flow chart of article selection process

Table S1: Characteristics and quality assessment of included cohort studies

Table S2: Results of Source Meta-regression analysis to explore heterogeneity (Univariate analysis).