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Dietary Marine ω-3 Fatty Acids and Incident Sight-Threatening Retinopathy in Middle-Aged and Older Individuals With Type 2 Diabetes

Prospective Investigation From the PREDIMED Trial

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IMPORTANCE Diabetic retinopathy (DR) is a devastating complication of individuals with type 2 diabetes mellitus. The retina is rich in long-chain ω-3 polyunsaturated fatty acids (LCω3PUFAs), which are substrate for oxylipins with anti-inflammatory and antiangiogenic properties. Experimental models support dietary LCω3PUFA protection against DR, but clinical data are lacking.

OBJECTIVE To determine whether LCω3PUFA intake relates to a decreased incidence of sight-threatening DR in individuals with type 2 diabetes older than 55 years.

DESIGN, SETTING, AND PARTICIPANTS In late 2015, we conceived a prospective study within the randomized clinical trial Prevención con Dieta Mediterránea (PREDIMED), testing Mediterranean diets supplemented with extra virgin olive oil or nuts vs a control diet for primary cardiovascular prevention. The trial was conducted in primary health care centers in Spain. From 2003 to 2009, 3614 individuals aged 55 to 80 years with a previous diagnosis of type 2 diabetes were recruited. Full data were available for 3482 participants (48% men; mean age 67 years).

EXPOSURES Meeting the dietary LCω3PUFA recommendation of at least 500 mg/d for primary cardiovascular prevention, as assessed by a validated food-frequency questionnaire.

MAIN OUTCOMES AND MEASURES The main outcome was incident DR requiring laser photocoagulation, vitrectomy, and/or antiangiogenic therapy confirmed by an external adjudication committee.

RESULTS Of the 3482 participants, 48% were men and the mean age was 67 years. A total of 2611 participants (75%) met target LCω3PUFA recommendation. During a median follow-up of 6 years, we documented 69 new events. After adjusting for age, sex, intervention group, and lifestyle and clinical variables, participants meeting the LCω3PUFA recommendation at baseline (≥500 mg/d) compared with those not fulfilling this recommendation (<500 mg/d) showed a 48% relatively reduced risk of incident sight-threatening DR, with a hazard ratio of 0.52 (95% CI, 0.31-0.88; P = .001). This association was slightly stronger for yearly updated LCω3PUFA intake (relative risk, 0.48; 95% CI, 0.28-0.82; P = .007).

CONCLUSIONS AND RELEVANCE In middle-aged and older individuals with type 2 diabetes, intake of at least 500 mg/d of dietary LCω3PUFA, easily achievable with 2 weekly servings of oily fish, is associated with a decreased risk of sight-threatening DR. Our results concur with findings from experimental models and the current model of DR pathogenesis.

TRIAL REGISTRATION clinicaltrials.gov Identifier: http://www.controlled-trials.com/ISRCTN35739639

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The increasing prevalence of type 2 diabetes mellitus, coupled with an increased lifespan, has resulted in a steady rise of disability in older diabetic individuals.[1] A major concern for this population group is diabetic retinopathy (DR), a leading global cause of vision loss.[2] Given the economic and societal burden of DR, developing effective strategies to prevent or at least delay its onset is a major public health issue.[3]

The pathogenesis of DR is not yet fully understood, but inflammation, oxidative stress, and hipoxia-driven microvascular alterations play a pivotal role in the worsening of retinal function in individuals with diabetes.[4] The retina is rich in long-chain ω-3 polyunsaturated fatty acids (LCω3PUFAs), particularly docosahexaenoic acid (DHA; C22:6n-3).[5] Once released from cell membranes, these fatty acids are transformed to oxylipins with anti-inflammatory and antiangiogenic properties.[6,7] Because cell membrane DHA status is modifiable and dependent on intake, dietary DHA or consumption of its parent food oily fish has been suggested to protect against DR.[5] Experimental studies have consistently reported a protective role of supplemental DHA or LCω3PUFA against DR[8,9] or neovascularization of the retina,[10,11] a hallmark of proliferative DR. However, to our knowledge, human data are lacking.

We hypothesized that LCω3PUFA intake with the usual diet relates to lower vision-threatening DR. To test this hypothesis, we longitudinally investigated the association of dietary LCω3PUFA intake with incident DR requiring laser photocoagulation, vitrectomy, and/or antiangiogenic therapy in a cohort of middle-aged and older individuals with type 2 diabetes enrolled into the Prevención con Dieta Mediterránea (PREDIMED) study, a nutrition intervention trial for the primary prevention of cardiovascular disease conducted in Spain.[12] Because of the lack of available recommendations for LCω3PUFA intake regarding DR and given the vascular nature of DR pathophysiology, we tentatively set the exposure target to meeting the recommendation for dietary LCω3PUFA for primary cardiovascular protection (500 mg/d),[13] a goal that can be achieved by following the advice of the American Heart Association to consume 2 weekly servings of fish, preferably oily fish.[14]

Key Points

Question Do dietary long-chain ω-3 fatty acids protect against diabetic retinopathy?

Findings A substudy of the PREDIMED randomized clinical trial analyzed as an observational longitudinal cohort, considering only participants with type 2 diabetes at baseline, showed after median follow-up of 6 years that those reporting intake of at least 500 mg/d of long-chain ω-3 fatty acids at baseline had a 46% decreased risk of sight-threatening diabetic retinopathy compared to those not meeting this target.

Meaning Results suggest fish-derived long-chain ω-3 fatty acids are a healthy fat in this cohort.

Methods

Setting

This substudy, which was conceived in late 2015, was conducted within the frame of the PREDIMED trial (http://www.predimed.es), the design of which has been described in detail[15] (http://www.controlled-trials.com/ISRCTN35739639). From October 2003 to June 2009, a total of 8713 candidates were screened for eligibility, and 7447 were randomly assigned to 1 of 3 interventions. Participants were men aged 55 to 80 years and women aged 60 to 80 years at high cardiovascular risk but with no cardiovascular disease at enrollment. Criteria for eligibility were the presence of either type 2 diabetes or at least 3 cardiovascular risk factors: current smoking, hypertension, dyslipidemia, overweight or obesity, and family history of early-onset coronary heart disease. The study protocol was conducted according to the guidelines laid down in the Declaration of Helsinki and approved by the institutional review boards of Hospital Clínic, Barcelona, Spain; Rovira i Virgili University, Reus, Spain; Hospital del Mar, Barcelona, Spain; University of Valencia, Valencia, Spain; University of Navarra, Navarra, Spain; University of Málaga, Málaga, Spain; Instituto de Investigación Sanitaria de Palma, Palma de Mallorca, Spain; San Pablo Health Center, Sevilla, Spain; University Hospital of Alava, Vitoria, Spain; University of Las Palmas de Gran Canaria, Las Palmas, Spain; and Hospital Universitari de Bellvitge, L'Hospitalet de Llobregat, Spain. Written informed consent was obtained from all study participants. The formal trial protocol can be found in Supplement 1.

Assessment of Risk Factors

The diagnosis of type 2 diabetes was based on at least 1 of the following criteria: current treatment with insulin or oral hypoglycemic drugs; fasting (no caloric intake at least for 8 hours) glucose at least 126 mg/dL in 2 determinations (to convert to millimoles per liter, multiply by 0.0555); casual glucose at least 200 mg/dL with polyuria, polydipsia, or unexplained weight loss; or glucose at least 200 mg/dL in 2 measurements after an oral glucose tolerance test. Participants were considered to have hyperlipidemia or hypertension if they had a previous diagnosis of these conditions and/or they were treated with cholesterol-lowering or antihypertensive agents, respectively. Smoking status was categorized into never, current, or past smoking according to self-reports. Physical activity was determined with a validated Spanish version of the Minnesota Leisure-Time Physical Activity questionnaire and expressed in minutes at a given metabolic equivalent per day.[16] Anthropometric variables (height, weight, and waist circumference) and blood pressure were measured by standard methods.

Dietary Intake

Dietary intake was assessed at baseline and yearly during follow-up by using a 137-item semiquantitative food-frequency questionnaire validated for the PREDIMED study.[17] In face-to-face interviews, participants were asked about the frequency of consumption of each food item during the past year, specifying usual portion sizes. Nine possibilities of frequency were offered, ranging from never to more than 6 times/d. Information on seafood products was collected in 8 items of the questionnaire (uncanned oily fish; lean fish; smoked/salted fish;

mollusks; shrimp, prawn, and crayfish; octopus, baby squid and squid; oily fish canned in oil; and oily fish canned in salted water). Nutrient intakes were computed using Spanish food composition tables. The validation of the food-frequency questionnaire against 4 3-day food records showed energy-adjusted intraclass correlation coefficients of 0.51 for LCω3PUFA ($P < .001$).

After the screening visit, suitable candidates were randomly assigned to 1 of 3 interventions: Mediterranean diet (MeDiet) supplemented with extra virgin olive oil, MeDiet supplemented with nuts, or control diet (advice to reduce all dietary fat). Quarterly individual and group sessions were scheduled for the 2 MeDiet groups; in them, participants were educated on how to follow the MeDiet and received supplemental foods at no cost. Extra virgin olive oil (1 L/wk) was provided to 1 group and 30 g/d of mixed nuts (15 g walnuts, 7.5 g hazelnuts, and 7.5 g almonds) to the other group. Those allocated to the control group were educated on how to follow a low-fat diet and received small nonfood gifts. In each session, a dietary screener of adherence to the MeDiet was used to track diet changes. The score was determined by 12 questions on food consumption frequency and 2 questions on food consumption habits considered characteristic of the MeDiet (each question scored 0 or 1).[18] For this particular substudy, because a higher adherence to the MeDiet among frequent consumers of seafood could introduce confounding, the question about seafood consumption was omitted from the brief screener; therefore, a 13-point score was used as a covariate (minimum, 0; maximum, 13).

End point Ascertainment

Diabetic retinopathy was not an explicitly prespecified secondary outcome of the PREDIMED trial. However, given that nearly one-half of participants had type 2 diabetes, this complication was always included as a relevant outcome in all interim analyses supervised by the Data and Safety Monitoring Board.

New events were identified prospectively through yearly review of the medical records of each participant. The PREDIMED dietitians of the different teams were responsible for the accurate filling of the follow-up questionnaires, which included 3 items about noncardiovascular complications of diabetes. Suspected DR was investigated by clinical records made by ophthalmologists and hospital discharge reports. All the information was sent to the adjudication committee, which determined the validity of diagnoses based on the information received. Participants were considered to have sight-threatening DR if they had undergone laser photocoagulation, intravitreal antivascular endothelial growth factor injections, and/or vitreoretinal surgery. Only cases that were confirmed by the adjudication committee and that occurred between October 1, 2003, and December 1, 2012 (date of the last update in the extended follow-up of the PREDIMED cohort, 2 years after the end of the trial), were included in the analyses.

Statistical Analyses

Person-time of follow-up was calculated as the interval between the randomization date and the earliest date of the follow-up contact at which a new event was identified, death from any cause, or date of the last contact visit, whichever came first.

After excluding participants free of type 2 diabetes at baseline (n = 3833) with missing data on year after diagnosis of type 2 diabetes (n = 25), food-frequency questionnaires (n = 36), 13-point score of adherence to MeDiet (n = 5), and those who reported total energy intakes outside predefined limits (>4000 or <800 kcal/d in men, and >3500 or <500 kcal/d in women; n = 64) or with implausible intakes of LCω3PUFA (>4 g/d; n = 2), 3482 study participants (n = 1151 control diet, n = 1236 MeDiet plus extra virgin olive oil, and n = 1095 MeDiet plus nuts) remained for inclusion in the analysis (eFigure in Supplement 2).

The exposure of interest was meeting the International Society for the Study of Fatty Acids and Lipids recommendation to consume at least 500 mg/d of LCω3PUFA (DHA + eicosapentaenoic acid [C20:5ω-3]) for primary cardiovascular prevention (yes/no), which was released in June 2004.[13] Baseline differences in demographic, clinical, and selected dietary variables between groups of exposure were assessed by analysis of variance or χ^2 tests, as appropriate.

To assess the associations between meeting the recommendation for LCω3PUFA intake (yes/no) at baseline and the risk of incident sight-threatening DR, we used unadjusted, age- and sex-adjusted, and multivariable time-dependent Cox proportional hazard models including age, sex, body mass index, intervention group, year after diagnosis of diabetes (≤5 or >5), use of insulin (yes/no), use of oral hypoglycemic agents (yes/no), smoking status (never, former, or current smoker), systolic blood pressure, history of hypertension (yes/no), use of angiotensin-converting-enzyme inhibitor and/or angiotensin-II receptor blockers (yes/no), physical activity, and adherence to the MeDiet (13-point score) as potential confounders. All analyses were stratified by recruitment center. Additional analyses were done after stratifying for history of hypertension, year after diagnosis of diabetes, and use of insulin and oral hypoglycemic agents at baseline. We also used Cox regression models to assess the risk of the prespecified endpoint according to the joint categories of meeting target intake recommendations for LCω3PUFA intake (yes/no) and intervention group (3 groups, 2 dummy variables).

Several sensitivity analyses were conducted refitting Cox regression analyses for LCω3PUFA after (1) excluding early cases of sight-threatening DR that occurred in the 2 first years of follow-up; (2) including only events occurring after at least 3 years of follow-up; and (3) excluding cases occurring after 5 years of follow-up. Effect modification by sex, intervention group, and LCω3PUFA intake on incident DR was evaluated by calculating the likelihood ratio test between the fully adjusted model and the same model adding the interaction product term.

As a secondary analysis, we repeated the analyses using generalized estimating equations to assess the association for yearly updated LCω3PUFA intake. We assumed a binomial distribution with logit models and the unstructured matrix as the working correlation structure. For each 1-year follow-up period, we used as exposure the average of total LCω3PUFA intake of all repeated measurements from baseline to the beginning of that yearly period. We defined the cohort risk as participants who remained free of DR at the beginning of each 1-year follow-up period. Participants who had been classified as incident cases were excluded from subsequent follow-up analyses.

Finally, we explored the association for meeting the American Heart Association recommendation to consume at least 2 weekly servings of fish (particularly oily fish) at baseline. Analyses were done using SPSS software, version 19.0 (IBM Corp) and STATA software, version 14.0 (StataCorp).

Results

The mean age of participants at inclusion was 67 years, and 48% of them were men. At baseline, from the whole cohort (n = 3482), 2611 participants (75%) met the target recommendation of LCω3PUFA intake. **Table 1** summarizes the baseline clinical characteristics and treatment regimens of participants. Those not meeting the recommendation of LCω3PUFA intake were older, smoked less, had a higher prevalence of hypertension (and treatment with antihypertensive drugs), and were treated with insulin more frequently than those meeting the LCω3PUFA recommendation. Intake of energy and nutrients and consumption of key foods are shown in **Table 2**. In brief, compared with participants meeting the LCω3PUFA recommendation, those who did not also adhered less to the MeDiet, although they consumed less red meat.

During a mean follow-up of 6 years, we documented 69 new events. **Table 3** shows the hazard ratios (HRs) and 95% CIs associated with the exposure of interest. After adjusting for intervention group and classic risk factors, participants reporting intake of at least 500 mg/d of eicosapentaenoic acid + DHA at baseline showed a 46% decreased risk of incident sight-threatening DR (HR, 0.52; 95% CI, 0.31-0.88; P = .001) in comparison with participants not meeting the recommendation of LCω3PUFA intake. Higher risk reductions were observed in participants with hypertension, those with diabetes of greater than 5 years duration, and those treated with insulin at baseline (eTable in Supplement 2). The **Figure** shows the multivariate-adjusted HRs by meeting the recommendation for LCω3PUFA at baseline by intervention group. When compared with the reference category (participants not meeting target intake and allocated to the control diet group, n = 326), those meeting the recommendation had a significant decreased risk regardless of the intervention group to which they were allocated. Notably, compared with the reference category, only participants not meeting the recommendation but allocated to the MeDiet with extra virgin olive oil intervention group (n = 280) had a borderline 63% (95% CI, 87-2) decreased risk of sight-threatening DR.

We conducted several sensitivity analyses to investigate possible sources of bias in the estimation of the relationship between meeting the recommendation for LCω3PUFA intake at baseline and sight-threatening DR (Table 3). The results of these analyses were consistent with the findings of the primary analysis. Additionally, the magnitude of the association was strengthened when using as exposure the yearly updated LCω3PUFA intake (HR, 0.48; 95% CI, 0.28-0.82; P = .007). Finally, a lower risk (HR, 0.41; 95% CI, 0.23- 0.72; P = .002) was also observed when grouping the participants by reporting to meet the advice of the American Heart Association to consume at least 2 weekly servings of oily fish at baseline (**Table 4**).

Table 1. Participants' Clinical Characteristics and Treatment Regimens at Baseline by Meeting the ISSFAL and Lipids Recommendation for LCω3PUFA

Variable	All Participants (n = 3482)	Difference Between Meeting/Not Meeting the ISSFAL Recommendation for LCω3PUFA[a]	
Categorical variables, No.		**Yes/No**	***P* Value[b]**
Men	1656	1315/360	<.001
Family history of early-onset CHD	541	402/139	.69
Smoking status			<.001
Current	419	315/104	
Former	948	751/197	
Never	2115	1545/570	
Hypertension	2549	1882/667	.009
Use of antihypertensive drugs[c]	1753	1280/473	.007
Dyslipidemia	2058	1537/521	.62
Use of statins	1605	1183/422	.11
>5 y After diagnosis	1740	1315/425	.42
Use of oral hypoglycemic agents	2301	1714/587	.35
Use of insulin	493	345/148	.006
Continuous variables, mean (95% CI)		**"Yes" minus "no"**	***P* Value[d]**
Age, y	67.5 (67.3-67.7)	−1.2 (−1.6 to −0.7)	<.001
Weight, kg	76.8 (76.4-77.2)	0.6 (−0.3 to 1.5)	.20
BMI	29.8 (29.7-30.0)	−0.3 (−0.6 to 0.1)	.06
Waist circumference, cm[e]	101 (101-102)	0 (0 to 1)	.53
Energy expenditure in physical activity, MET-min/d	239 (231-248)	16 (−3 to 36)	.11
Systolic blood pressure, mm Hg	149 (148-150)	1 (0-1)	.90
Diastolic blood pressure, mm Hg	82 (81-82)	0 (0-1)	.76

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); CHD, coronary heart disease; ISSFAL, International Society for the Study of Fatty Acids; LCω3PUFA, long-chain ω-3 polyunsaturated fatty acid; MET-min, minutes at a given metabolic equivalent level (units of energy expenditure in physical activity, 1 MET-min is roughly equivalent to 1 kcal).

[a] A minimum intake of 500 mg/d of combined eicosapentaenoic and docosahexaenoic acids, for primary cardiovascular protection.

[b] P between groups obtained by the χ^2 test.

[c] Angiotensin-converting enzyme inhibitor and/or angiotensin-II receptor blockers.

[d] P value between groups obtained by analysis of variance.

[e] Data from 3348 participants (2515 meeting the ISSFAL recommendation and 833 not meeting the ISSFAL recommendation).

Discussion

In this substudy of the PREDIMED trial analyzed as an observational longitudinal cohort and considering only participants with type 2 diabetes, we found that those reporting

Table 2. Baseline Intake of Energy, Nutrients, and Key Foods by Meeting the ISSFAL and Lipids Recommendation for LCω3PUFA

Variable	All Participants, Mean (95% CI) (n = 3482)	Difference Between Meeting/Not Meeting the ISSFAL Recommendation for LCω3PUFA[a]	
		Mean (95% CI)	*P* Value[b]
Energy intake, kcal/d	2121 (2100-2142)	296 (255-338)	<.001
Carbohydrate, g/d	230 (228-231)	−20 (−24 to −17)	<.001
Fiber, g/d	25.0 (24.8-25.3)	−0.2 (−0.78 to 0.38)	.50
Protein, g/d	90 (89-90)	9 (7-10)	<.001
Fat, g/d	96 (95-97)	5 (3-6)	<.001
Saturated fatty acids	24.9 (24.6-25.1)	0.8 (0.4-1.3)	<.001
Monounsaturated fatty acids	47.3 (46.8-47.7)	2.9 (2.0-3.7)	<.001
ω-6 Polyunsaturated fatty acids	13.1 (12.9-13.3)	−0.3 (−0.7 to 0.1)	.14
α-Linolenic acid	1.37 (1.34-1.40)	0.12 (0.05 to 0.19)	.001
LCω3PUFA	0.68 (0.66-0.69)	0.62 (0.59-0.65)	<.001
Cholesterol, mg/d	349 (345-353)	62 (55-70)	<.001
Cereals, g/d	142 (139-145)	6 (1-13)	.048
Vegetables, g/d	339 (334-344)	57 (45-68)	<.001
Fruits, g/d	366 (359-372)	15 (0-30)	.05
Total nuts, g/d	10.0 (9.5-10.5)	2.9 (1.8-4.0)	<.001
Dairy products, g/d	395 (387-402)	−15 (−32 to 2)	.08
Red meat, g/d	78 (77-80)	22 (19-26)	<.001
Seafood, g/d[c]	99 (97-101)	59 (55-62)	<.001
Olive oil, g/d	33.7 (32.6-34.9)	6.2 (4.8-7.6)	<.001
Alcohol, g/d	7.5 (7.0-8.0)	0.8 (−0.2 to 1.8)	.12
13-Point score of adherence to Mediterranean Diet[d]	8.05 (7.99-8.10)	0.51 (0.37-0.64)	<.001

Abbreviations: ISSFAL, International Society for the Study of Fatty Acids; LCω3PUFA, long-chain ω-3 polyunsaturated fatty acid.

SI conversion factor: To convert cholesterol to millimoles per liter, multiply by 0.0259.

[a] A minimum intake of 500 mg/d of combined eicosapentaenoic and docosahexaenoic acids for primary cardiovascular protection. For nutrients, values are estimated means (95% confidence interval) after adjusting for energy intake; for foods, values are means (95% confidence interval).

[b] *P* value for comparison between groups (analysis of variance).

[c] Sum of uncanned oily fish; lean fish; smoked/salted fish; mollusks; shrimp, prawn, and crayfish; octopus, baby squid, and squid; oily fish canned in oil; and oily fish canned in salted water.

[d] Determined by 11 questions on food consumption frequency and 2 questions on food intake habits characteristic of the Mediterranean diet (each question scored 0 or 1).

Table 3. Hazard Ratios (95% Confidence Intervals) for Incidence of DR by Meeting the ISSFAL Recommendation for LCω3PUFA Intake at Baseline

Variable	Meeting the ISSFAL Recommendation for LCω3PUFA,[a] HR (95% CI)		
	No	Yes	*P* Value
Cases/person-years	27/4916	42/15491	NA
Crude model	1 [Reference]	0.50 (0.30-0.81)	.006
Age- and sex-adjusted model	1 [Reference]	0.50 (0.30-0.82)	.006
Multivariate-adjusted model[b]	1 [Reference]	0.52 (0.31-0.88)	.001
Sensitivity analyses			
Early cases excluded (<2 y)			
Cases/person-years	18/4902	28/15478	NA
Age and sex-adjusted model	1 [Reference]	0.46 (0.26-0.82)	.009
Multivariate-adjusted model[b]	1 [Reference]	0.43 (0.24-0.78)	.006
Late cases excluded (>3 y)			
Cases/person-years	16/4899	23/15463	NA
Age and sex-adjusted model	1 [Reference]	0.42 (0.22-0.77)	.006
Multivariate-adjusted model[b]	1 [Reference]	0.40 (0.21-0.77)	.006
Very late cases excluded (>5 y)			
Cases/person-years	22/4882	33/15437	NA
Age and sex-adjusted model	1 [Reference]	0.49 (0.28-0.83)	.009
Multivariate-adjusted model[b]	1 [Reference]	0.52 (0.29-0.92)	.02

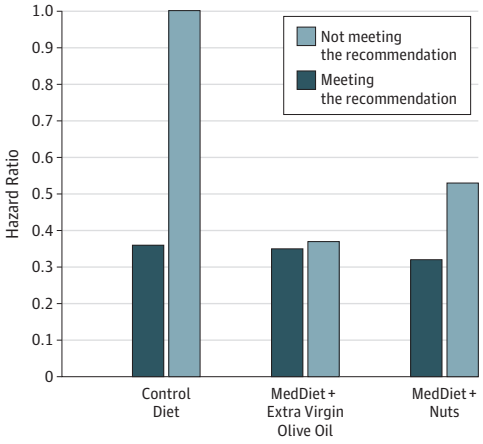
Abbreviations: DR, diabetic retinopathy; HR, hazard ratio; ISSFAL, International Society for the Study of Fatty Acids; LCω3PUFA, long-chain ω-3 polyunsaturated fatty acid; NA, not applicable.

[a] A minimum intake of 500 mg/d of combined eicosapentaenoic and docosahexaenoic acids for primary cardiovascular protection.

[b] Adjusted for age, sex, body mass index, intervention group, years after diagnosis of diabetes (≤5 or >5), use of insulin (yes/no), use of oral hypoglycemic agents (yes/no), smoking status (never, former, or current smoker), systolic blood pressure, history of hypertension (yes/no), use of angiotensin-converting enzyme inhibitor and/or angiotensin-II receptor blockers (yes/no), physical activity, and adherence to the Mediterranean diet (13-point score). All models were stratified by recruitment center. Cox regression models according to categories of reporting to meet the recommendation for LCω3PUFA intake at baseline.

intake of at least 500 mg/d of LCω3PUFA (or at least 2 weekly servings of oily fish) at baseline had a significantly decreased risk of incident sight-threatening DR compared with those not meeting this target. The reduction remained significant when using as exposure the yearly updated LCω3PUFA intake. To the best of our knowledge, this is the first study in humans on LCω3PUFA and DR, and the results reinforce a notion heretofore only explored in experimental models. Our findings support the view that regular consumption of oily fish might be beneficial to delay the onset or progression

Figure. Adjusted Hazard Ratios of Sight-Threatening Diabetic Retinopathy by Meeting the International Society for the Study of Fatty Acids and Lipids Recommendation to Consume at Least 500 mg/d of Eicosapentaenoic Acid + Docosahexaenoic Acid at Baseline and PREDIMED Intervention Group



Multivariable Cox regression model stratified by recruitment center. Further information about the adjustment can be found in the text. Values for the "not meeting the recommendation" category are 1 [reference] for the low-fat control diet group; hazard ratio (HR), 0.37 (95% CI, 0.13-1.02) for the Mediterranean diet (MedDiet) supplemented with extra virgin olive oil group; and HR, 0.53 (95% CI, 0.20-1.36) for the MedDiet supplemented with nuts group. Values for the "meeting the recommendation" category are HR, 0.36 (95% CI, 0.17-0.76) for the control diet group; HR, 0.35 (95% CI, 0.17-0.73) for the MedDiet supplemented with extra virgin olive oil group; and HR, 0.32 (95% CI 0.14-0.71) for the MedDiet supplemented with nuts group.

Table 4. Hazard Ratios (95% Confidence Intervals) for Incidence of Diabetic Retinopathy by Meeting the American Heart Association Recommendation to Consume at Least 2 Weekly Servings of Oily Fish

	Reporting Consumption of ≥2 Weekly Servings of Oily Fish, HR (95% CI)[a]		
Variable	No	Yes	*P* Value
Cases/person-years	52/10 587	17/9817	
Crude model	1 [Reference]	0.38 (0.22-0.67)	.001
Age- and sex-adjusted model	1 [Reference]	0.39 (0.22-0.68)	.001
Multivariate-adjusted model[b]	1 [Reference]	0.41 (0.23-0.72)	.002

Abbreviation: HR, hazard ratio.

[a] Including uncanned oily fish (130 g per serving) and oily fish canned either in salted water or in oil (50 g per serving).

[b] Adjusted for age, sex, body mass index, intervention group, years after diagnosis of diabetes (≤5 years or >5 years), use of insulin (yes/no), use of oral hypoglycemic agents (yes/no), smoking status (never, former, or current smoker), systolic blood pressure, history of hypertension (yes/no), use of angiotensin-converting-enzyme inhibitor and/or angiotensin-II receptor blockers (yes/no), physical activity, and adherence to the Mediterranean diet (13-point score). All models were stratified by recruitment center. Cox regression models according to categories of reporting to meet the recommendation of consumption of ≥2 servings of oily fish per week at baseline.

of vascular diseases in arterial beds other than the coronary and cerebrovascular ones.

Epidemiologic studies assessing exposure to LCω3PUFA for the primary prevention of cardiovascular disease (in particular fatal coronary heart disease) prompted the debate of whether increasing LCω3PUFA intake is beneficial for patients with diabetes. The effectiveness of this approach has been questioned in recent years given the null reduction of the rate of cardiovascular events reported in the Outcome Reduction With an Initial Glargine Intervention (ORIGIN) trial of LCω3PUFA intake in patients with diabetes with a much lower background LCω3PUFA intake[19,20] coupled with a suggested increased risk of DR secondary to peroxidation of accumulated PUFA in the retina.[21] However, no clincal data support a deleterious effect of LCω3PUFA on the retina. Actually, a small cross-sectional study reported that increased PUFA intake (although not distinguishing between ω-3 or ω-6 fatty acids) was associated with reduced odds of prevalent DR and lesser DR severity in patients with well-controlled diabetes, while no associations were found for those poorly controlled.[22] Suprisingly, neither epidemiologic studies nor clinical trials focused on dietary LCω3PUFA and DR, although the link has repeatedly been proven in experimental models.[8-11] By reporting that regular LCω3PUFA intake seems to reduce the risk of the most severe form of DR, particularly in patients with hypertension or advanced diabetes, we reinforce the benefits of these particular fatty acid species in a critical population group.

Our results are mechanistically supported by experimental studies showing that anti-inflammatory LCω3PUFA-derived compounds, mostly lipoxins, resolvins, and protectins, delay progression of DR, particularly neovascularization, the hallmark of proliferative DR, and improve diabetic macular edema.[11] Nevertheless, we must underline that this study was conducted in a Mediterranean population with high adherence to a plant-based diet, as attested by relatively high mean MeDiet scores (Table 2). Therefore, it seems plausible that a high intake of antioxidants, a main feature of this dietary pattern, might contribute to counteracting the presumed membrane peroxidation of PUFA. Indeed, a prior analysis of the PREDIMED trial concerning diabetic complications by intervention arm described a significant reduction of incident DR in participants allocated to the MeDiet supplemented with extra virgin olive oil.[23] In our study, when considering the 3 intervention groups, a marginal additional reduction in DR was observed by meeting the LCω3PUFA target precisely in the MeDiet with extra virgin olive oil group (Figure 1). This suggests that phytochemicals with antioxidant and anti-inflammatory properties contained in extra virgin olive oil could overrun the protection afforded by LCω3PUFA.

Our study has limitations. First, this is a prospective analysis of a subsample in a larger clinical trial that was not specifically designed to examine incidence of sight-threatening DR. Second, the assessment of diabetes complications was not the primary end point for the PREDIMED trial, and no data on prevalent DR were available at baseline. Nonetheless, we took care to ensure that all cases of incident sight-threatening DR were diagnosed by experienced ophthalmologists and confirmed by an external adjudication committee. Additionally, individuals with DR at baseline would have been more likely to be diagnosed with

complicated DR in the first 2 years of follow-up, and results after excluding events reported during this period were similar to those of the primary analysis. Third, we had a relatively low number of events with rates slightly lower than those observed in a Spanish general population,[24] resulting in imprecise estimates. Finally, because the study participants were middle-aged and older individuals with type 2 diabetes at high risk for cardiovascular disease living in a Mediterranean country, our findings cannot be easily extrapolated to other populations. There are also strengths to our study, such as a prospective design with a long duration of follow-up, validation of the food frequency questionnaire, adjustment for relevant confounders, and the confirmation of the primary results by both sensitivity analyses and cumulative average estimates of the exposure of interest, that provide a more robust measure than a single baseline dietary assessment.

Conclusions

In summary, we report that meeting the target of at least 500 mg/d of dietary LCω3PUFA is associated with a reduced incidence of severe DR in individuals older than 55 years with type 2 diabetes. Our findings, which are consistent with the current model of the pathogenesis of DR and data from experimental models, add to the notion of fish-derived LCω3PUFA as a healthy fat.

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