Mediterranean Diet, Retinopathy, Nephropathy, and Microvascular Diabetes Complications: A Post Hoc Analysis of a Randomized Trial

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OBJECTIVE

To date no clinical trials have evaluated the role of dietary patterns on the incidence of microvascular diabetes complications. We hypothesized that a nutritional intervention based on the Mediterranean diet (MedDiet) would have greater protective effect on diabetic retinopathy and nephropathy than a low-fat control diet.

RESEARCH DESIGN AND METHODS

This was a post hoc analysis of a cohort of patients with type 2 diabetes participating in the PREvención con DIeta MEDiterránea (PREDIMED) study, a multicenter randomized nutritional intervention trial conducted in a population at high cardiovascular risk. Individuals with type 2 diabetes who were free of microvascular complications at enrollment (*n* = 3,614, aged 55–80 years) were randomly assigned to one of three dietary interventions: MedDiet supplemented with extra-virgin olive oil (MedDiet+EVOO), MedDiet supplemented with mixed nuts (MedDiet+Nuts), or a low-fat control diet. Two independent outcomes were considered: new onset of diabetic retinopathy and nephropathy. Hazard ratios (HRs) were calculated using multivariable-adjusted Cox regression.

RESULTS

During a median follow-up of 6.0 years, we identified 74 new cases of retinopathy and 168 of nephropathy. Compared with the control diet, multivariable-adjusted HRs for diabetic retinopathy were 0.56 (95% CI 0.32–0.97) for the MedDiet+EVOO and 0.63 (0.35–1.11) for the MedDiet+Nuts. No between-group differences were found for nephropathy. When the yearly updated information on adherence to the MedDiet was considered, the HR for retinopathy in the highest versus the lowest quintile was 0.34 (0.13–0.89; *P* = 0.001 for trend). No significant associations were found for nephropathy.

CONCLUSIONS

A MedDiet enriched with EVOO may protect against diabetic retinopathy but not diabetic nephropathy.

PATHOPHYSIOLOGY/COMPLICATIONS

Type 2 diabetes is a growing public health problem with an increased risk of developing cardiovascular diseases (CVD) and microvascular complications, including retinopathy and nephropathy, which decrease the quality of life and may cause premature death (1,2). The etiology of type 2 diabetes complications is poorly understood. Diet is one of the lifestyle factors that may play an important role in preventing and managing these conditions (3,4), particularly diabetic retinopathy and nephropathy (5–10). However, few studies have explored the relationship between dietary habits and diabetes complications. Most studies have examined the associations between individual foods or food groups and nutrients and diabetes complications (7,8,11–17) instead of focusing on dietary patterns, which is the most sensible approach to test the role of the overall diet on nutrition-related diseases.

To the best of our knowledge, only one prospective study (10) has evaluated the relationship between diet and nephropathy in individuals with diabetes, showing an increased risk of microalbuminuria and rapid estimated glomerular filtration rate (eGFR) decline in those who adhered to a Western-type diet. In contrast, no studies to date have examined the effect of diet on diabetic retinopathy, a frequent and severe complication of diabetes and an important cause of blindness.

The Mediterranean diet (MedDiet) is recognized as one of the healthiest dietary patterns, and has proven to be beneficial for CVD and other health outcomes (18,19). In fact, previous reports on the PREvención con DIeta MEDiterránea (PREDIMED) study have shown that a traditional MedDiet intervention had more beneficial effects on several cardiovascular risk factors (20), including hypertension (21), diabetes (22), and metabolic syndrome (23), than a low-fat diet and also reduced cardiovascular events (24).

No randomized trial to date has assessed the long-term effect of a MedDiet on diabetes complications. We hypothesized that two MedDiets, one enriched with extravirgin olive oil (EVOO) and another enriched with mixed nuts, would be associated with a lower risk of diabetic retinopathy and nephropathy compared with a low-fat control diet in an elderly Mediterranean population with type 2 diabetes.

RESEARCH DESIGN AND METHODS

Design Overview

This post hoc analysis was conducted within the frame of the PREDIMED study (25), a parallel-group, randomized, primary cardiovascular prevention trial in persons at high risk of CVD (www.predimed.es). The main results of the trial at the primary cardiovascular end point have been published elsewhere (24).

The study was conducted in accordance with the Declaration of Helsinki. The institutional review board of the respective recruitment centers approved the study protocol, and all participants gave their informed consent.

Participants

Eligible participants were men and women (55 to 80 years) initially free of CVD but who had type 2 diabetes or at least three of the following cardiovascular risk factors: current smoking, hypertension, dyslipidemia, overweight/obesity, or a family history of early-onset CVD. Exclusion criteria have been reported previously (24,25).

Randomization and Intervention

Participants were recruited in primary care centers affiliated with 11 teaching hospitals in Spain between October 2003 and January 2009. The PREDIMED study enrolled 7,447 participants who were randomly assigned in a 1:1:1 ratio to one of the following three intervention groups: MedDiet supplemented with EVOO (MedDiet+EVOO), MedDiet supplemented with mixed nuts (MedDiet+Nuts), or control diet (advice on a low fat-diet following the American Heart Association guidelines). Dietary interventions (24,25) are detailed in Supplementary Data. Randomization was performed centrally by means of a computer-generated random-number sequence. Four strata for stratified randomization were built by sex and age (cutoff point: 70 years). Investigators and members of all committees were blinded to the treatments assigned to individual participants.

Our main objective in the present analysis was to determine the effect of the three dietary interventions on the incidence of diabetes complications. We therefore analyzed a subset of 3,614 participants of the PREDIMED trial who had type 2 diabetes at baseline and were included to assess the incidence of retinopathy because they did not have the condition at baseline. For the analysis of diabetic nephropathy, participants who lacked measurements at baseline or who did not have at least two consecutive urinary albumin/creatinine ratio (ACR) or serum creatinine measurements for whom we could ascertain the diabetic nephropathy during the follow-up (n = 986) were excluded. Participants were also excluded (n = 499) if they had any of the following conditions at baseline at two consecutive visits: albuminuria (urinary ACR $\geq$30 mg/g) or impaired renal function (eGFR $<$60 mL/min/1.73 m^2), two widely used measures for assessing kidney dysfunction. The effective sample size for statistical analyses of diabetic nephropathy incidence was 2,129 participants.

At baseline and yearly during follow-up, all participants completed a 47-item questionnaire about lifestyle variables, educational achievement, history of illnesses, and medication use; a 137-item validated semiquantitative food-frequency questionnaire (24); and a validated Spanish version of the Minnesota Leisure-Time Physical Activity Questionnaire (26). Electrocardiography and anthropometric variables and blood pressure were also determined by trained staff.

Fasting blood and spot urine were sampled at baseline and yearly during the follow-up, and laboratory biochemical analyses were performed. Plasma glucose, total cholesterol, HDL-cholesterol, and triglycerides were measured by routine laboratory tests using standard enzymatic methods. Serum creatinine was measured by enzymatic reaction using the Jaffé method, and eGFR was calculated based on creatinine using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (27). Urinary creatinine and albumin concentrations

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were also measured by the Jaffé method and bromcresol green albumin method, respectively, and the urinary ACR was calculated (mg/g). Biomarkers of adherence to the MedDiet interventions were measured in a random sample of PREDIMED participants during the first 5 years of follow-up, including urine hydroxytyrosol levels and plasma α-linolenic acid proportions, which are reliable biomarkers of EVOO and walnut intake, respectively (24). Laboratory technicians were blinded to intervention group.

Ascertainment of Diabetes Complications

Diabetes complications (externally confirmed by an adjudication committee) were not an explicitly prespecified secondary outcome of the PREDIMED trial; therefore, this study must be considered a post hoc analysis. However, given that 50% of participants in the trial had type 2 diabetes, these two complications of diabetes were always included as relevant outcomes in all interim analyses and in all reports prepared every year for the data and safety monitoring board of the PREDIMED trial. Type 2 diabetes was considered to be present at baseline by clinical diagnosis or antidiabetic medication use.

For this report, two independent outcomes were considered during follow-up. Our first outcome—new-onset diabetic retinopathy—was defined by the medical diagnosis made by an ophthalmologist of any nonproliferative or proliferative diabetic retinopathy, or laser photocoagulation treatment for diabetic retinopathy, as reported in the medical records. These reports and all relevant documentation, including medical records made by ophthalmologists, were sent to the members of the clinical adjudication events committee, who were blinded to the intervention. Even though retinopathy was not a primary end point in the trial, the adjudication events committee reviewed the medical records for potential retinopathy, and only definitively confirmed cases were included in this analysis. Because the public health system in Spain recommends early diabetic retinopathy detection by yearly examination of the fundus by an ophthalmologist or assessment of diabetic retinopathy by nonmydriatic fundus camera to all patients with diabetes, in the present report we assume that participants were free of diabetic retinopathy at baseline.

Our second outcome considered was new-onset diagnosis of diabetic nephropathy ascertained by the adjudication events committee based on assessments recorded in clinical records. For this study, an incident case of diabetic nephropathy was also defined by chronic kidney disease progressing from moderate to severe (stage 3 or greater) or albuminuria progressing during follow-up; the former was defined as a sustained eGFR value <60 mL/min/1.73 m^2 based on serum creatinine and the latter as the transition from normo- to micro- or macroalbuminuria (urinary ACR $\geq$30 mg/g). Serum creatinine and ACR were measured regularly, at least once yearly, in 67% and 43% of participants, respectively. Both transitions needed to be confirmed by at least two consecutive measurements during follow-up. The end point for diabetic nephropathy was the time to first occurrence.

Statistical Analyses

Analyses were performed using SPSS 19.0 software (IBM Corp., Armonk, NY) and Stata 12.0 (StataCorp LP, College Station, TX).

The assumptions for power calculations were based on expected rates of complications $\geq$3% in the control group and $\geq$1.5% in the two intervention groups considered together, with sample sizes of 1,200 and 2,400 subjects, respectively, and a two-tailed α error = 0.05. Under these assumptions, the statistical power to find a relative risk $\leq$0.5 is 80%. Baseline differences among the three dietary intervention groups were tested using ANOVA or χ^2, and results are expressed as mean ± SD, median and interquartile range (IQR), or numbers (percentages), respectively. The normality of variables was examined by using the Kolmogorov-Smirnov test. All analyses were performed on an intention-to-treat principle.

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 diabetes complication was diagnosed, death from any cause, or date of the last contact visit, whichever came first.

We used unadjusted, age- and sex-adjusted, and multivariable time-dependent Cox proportional hazards models to assess the effect of the two MedDiet interventions on diabetes complications (retinopathy and nephropathy) compared with the control group. Hazard ratios (HRs) and 95% CIs were calculated using the control group as the reference. A fully adjusted multivariable analysis was repeated after both MedDiet groups had been merged into a single category for comparison with the control group. The assumption of proportional hazards was tested by analysis of the scaled Schoenfeld residuals, and it was not violated ($P >$ 0.50). The test for time-varying covariates also suggested that the assumption of proportional hazards was met. We also used the Kaplan-Meier method to graphically estimate the cumulative diabetes complications-free survival by intervention group during follow-up.

Prespecified subgroup analyses were conducted within strata of sex, baseline age, BMI, prevalence of dyslipidemia, and adherence to the MedDiet. We also conducted sensitivity analyses stratified by follow-up periods and evaluated the diagnosis of diabetic nephropathy according to incident hyperalbuminuria or incident GFR impairment (<60 mL/min/1.73 m^2) separately. Finally, taking advantage of the yearly repeated measurements of adherence to the MedDiet, systolic blood pressure, and HDL-cholesterol levels, we used time-dependent Cox proportional hazards models to assess the risk of diabetic retinopathy and nephropathy during follow-up. We calculated the *P* for linear trend by taking the median of each category of adherence to the MedDiet. This new variable was modeled as a continuous variable. All statistical tests were two-tailed, and the significance level was set at $P \leq 0.05$.

RESULTS

Of the 3,614 PREDIMED participants with type 2 diabetes assessed in the present report, 1,282 were allocated to the MedDiet+EVOO group, 1,142 to the MedDiet+Nuts group, and 1,190 to the control diet group (Supplementary Fig. 1). Mean age of the participants was 67 years, 47% were men, and they had a sizeable burden of cardiovascular risk factors: 90% were overweight/obese, 77% had hypertension, and 61% had dyslipidemia. The baseline characteristics of study participants by dietary intervention group are listed in Table 1.

Although small differences were found in BMI and the proportion of men between the three intervention groups, these were irrelevant in magnitude or clinically. These variables are used as covariates in our analysis, and therefore, they were controlled for in all analyses. The three groups were well balanced without any important clinical difference between them, such as CVD-related risk factors, including overweight/obesity, hypertension, diabetes, dyslipidemia, smoking, and medication use, as well as biochemical parameters such as HDL-cholesterol, triglycerides, and plasma fasting glucose levels.

During follow-up (a median of >6.0 years), mean scores on the 14-item MedDiet screener increased for the participants allocated to the two MedDiet groups and were higher than in the control group ($P < 0.001$ for all yearly comparisons) (Supplementary Fig. 2). The percentage of participants with a MedDiet score of 10 or greater was also higher in the two MedDiet groups. There were significant differences between the MedDiet groups and the control group in 10 of the 14 items after 3 and 5 years of follow-up (Supplementary Tables 1 and 2). Changes in objective biomarkers (measured in a small random sample of patients with diabetes) of the supplemental foods also indicated good compliance with the dietary assignments in the two MedDiet groups, but these biomarkers did not change in the control group (Supplementary Table 3). We found no significant differences in changes in body weight, waist circumference, or physical activity among the three groups during follow-up (Supplementary Table 4).

During follow-up, new-onset retinopathy developed in 74 participants (22 in MedDiet+EVOO, 20 in MedDiet+Nuts, 32 in the control group). Among the 2,129 participants (among 3,614 initially selected participants with type 2 diabetes) in the analysis of diabetic nephropathy, there were a total of 168 incident cases of nephropathy (64 in MedDiet+EVOO, 51 in MedDiet+Nuts, 53 in the control group). Table 2 summarizes the HRs and 95% CIs of the effects of the two MedDiet interventions on diabetes complications compared with the control group. Compared with the control group, the unadjusted HRs for diabetic retinopathy were 0.57 (95% CI 0.32–0.98) for the MedDiet+EVOO and 0.62 (0.35–1.07) for the MedDiet+Nuts. Further adjustment for potential confounders gave similar results. We found a significantly lower risk of diabetic retinopathy in the MedDiet+EVOO group (44% lower risk; HR 0.56 [95% CI 0.32–0.97]) and a nonsignificant risk reduction (37% lower risk; HR 0.63 [0.35–1.11]) for retinopathy in the MedDiet+Nuts group versus the control group. As expected, the risk of diabetic retinopathy was significantly lower than in the control group (multivariable-adjusted HR 0.60 [0.37–0.96]) when the two MedDiet groups were merged (Table 2). No differences in the incidence of diabetic nephropathy were found in the two MedDiet interventions compared with the control group or when both MedDiet groups were merged (Table 2). The unadjusted Kaplan-Meier curves illustrating the survival free of

Table 1—Baseline characteristics of the study population (participants with type 2 diabetes from the PREDIMED trial) by intervention group

	MedDiet+EVOO (n = 1,282)	MedDiet+Nuts (n = 1,142)	Control group (n = 1,190)	P values†
Age, years	67.5 ± 6.2	67.1 ± 6.1	67.5 ± 6.4	0.15
Men	574 (45)	593 (52)	540 (45)	0.001
BMI, kg/m^2	29.8 ± 3.8	29.5 ± 3.9	30.2 ± 4.3	<0.001
Weight, kg	76.4 ± 11.7	76.9 ± 11.9	77.2 ± 12.8	0.25
Waist circumference, cm	101.0 ± 10.0	100.9 ± 10.7	101.2 ± 10.2	0.05
Tobacco use				
Never smoker	796 (62)	662 (58)	742 (62)	
Current smoker	154 (12)	139 (12)	139 (12)	0.14
Former smoker	332 (26)	341 (29)	309 (26)	
Educational level				
Primary/secondary education	1,034 (81)	880 (77)	982 (82)	0.004
University/some college	248 (19)	262 (23)	208 (18)	
Overweight/obesity	1,157 (90)	1,009 (88)	1,085 (91)	0.07
Hypertension	974 (76)	850 (74)	922 (77)	0.22
Dyslipidemia	764 (60)	673 (59)	705 (59)	0.94
Medication use				
Antihypertensive agents*	629 (49)	588 (51)	596 (50)	0.49
Statins	509 (39)	406 (36)	451 (37)	0.10
HDL-cholesterol, mg/dL	50.0 (43.0, 59.0)	49.6 (42.2, 58.2)	50.0 (42.0, 59.1)	0.59
Triglyceride, mg/dL	125.5 (92.0, 172.0)	124.0 (91.0, 166.0)	125.0 (91.0, 170.0)	0.30
Plasma fasting glucose, mg/dL	136.0 (116.8, 163.0)	134.0 (115.0, 162.0)	134.0 (115.0, 163.0)	0.34
Family history of premature CHD	278 (22)	263 (23)	242 (20)	0.28
Leisure-time physical activity, MET min/day	177 (70, 325)	202 (75, 350)	152 (48, 295)	0.002
MedDiet adherence (14-point score)	8.7 ± 1.8	8.7 ± 1.9	8.3 ± 1.8	<0.001

Data are mean ± SD, median (interquartile range), or n (%). CHD, coronary heart disease. †P value for comparisons between groups calculated with χ^2 tests for categorical variables or ANOVA test for quantitative variables. *Angiotensin-type 2 receptor blocker and ACE inhibitors.

Table 2—Incidence of diabetic retinopathy and diabetic nephropathy according to intervention group in the PREDIMED trial after a median 6.0 years of follow-up

Outcomes	MedDiet+EVOO	MedDiet+Nuts	Control group
Diabetic retinopathy, *n*	1,282	1,142	1,190
Cases, *n*/person-years of follow-up	22/7,830	20/6,622	32/6,856
Diabetic retinopathy by intervention group, HR (95% CI)			
Crude model	0.57 (0.32–0.98)	0.62 (0.35–1.07)	1 (Ref.)
Age- and sex-adjusted model	0.56 (0.33–0.98)	0.64 (0.36–1.12)	1 (Ref.)
Multivariable-adjusted model 1†	0.56 (0.32–0.97)	0.63 (0.35–1.11)	1 (Ref.)
MedDiets combined vs. control, HR (95% CI)			
Multivariable-adjusted model 1†	0.60 (0.37–0.96)		1 (Ref.)
Diabetic nephropathy, *n*	740	672	717
Cases, *n*/person-years of follow-up	64/4,419	51/3,985	53/4,180
Diabetic nephropathy by intervention group, HR (95% CI)			
Crude model	1.12 (0.77–1.62)	0.99 (0.97–1.46)	1 (Ref.)
Age- and sex-adjusted model	1.10 (0.76–1.59)	1.05 (0.71–1.54)	1 (Ref.)
Multivariable-adjusted model 1†	1.15 (0.79–1.67)	1.06 (0.72–1.58)	1 (Ref.)
MedDiets combined vs. control, HR (95% CI)			
Multivariable-adjusted model 1†	1.11 (0.79–1.55)		1 (Ref.)

Cox regression models with outcome of diabetic retinopathy and diabetic nephropathy and exposure to MedDiet intervention group vs. control group. †Model 1 was additionally adjusted for baseline BMI (continuous variable), waist circumference (continuous variable), smoking (never, current, or former smoker), physical activity in MET min/day (continuous variable), educational level (primary/secondary education or academic/graduate), hypertension (yes or no), dyslipidemia (yes or no), family history of premature coronary heart disease (yes or no), and baseline adherence to the MedDiet (low, <10 points; high, ≥10 points). All models were stratified by recruitment center.

diabetic retinopathy and nephropathy by group of intervention during follow-up are shown in Supplementary Figs. 3 and 4, respectively.

The observed reduction in the risk of diabetic retinopathy in the MedDiet+EVOO group was similar between subgroups of sex, age, baseline BMI, dyslipidemia, and adherence to the MedDiet, and there was no evidence of statistical interaction (Table 3). Results for diabetic nephropathy were not meaningfully different across the assessed subgroups (Supplementary Table 5).

Sensitivity analyses were consistent with the findings of the primary analysis (Table 4 and Supplementary Table 6). When the early cases of diabetic retinopathy that occurred in the first year were excluded (*n* = 12), the fully adjusted HR in the MedDiet+EVOO group showed a relative risk reduction of 51% (HR 0.49 [95% CI 0.26–0.91]) compared with the control diet. Similarly, a significant relative risk reduction was found when the MedDiet groups were merged (HR 0.57 [0.34–0.95]). When only the events that occurred after at least after 3 years of follow-up were included (*n* = 42), the HRs were 0.48 (0.23–0.99) in the MedDiet+EVOO group and 0.51 (0.26–0.95) in both MedDiet groups versus the control, respectively (Table 4).

Finally, we considered yearly updated information on actually observed adherence to the MedDiet and on diastolic blood pressure or HDL-cholesterol levels, regardless of the allocated intervention group, to evaluate associations with the incidence of diabetes complications. A 66% relative reduction in the risk of diabetic retinopathy (multivariable-adjusted HR 0.34 [95% CI 0.13–0.89]; *P* = 0.001 for trend) was found for those individuals in the highest quintile of adherence to the MedDiet compared with the lowest (reference) quintile. In contrast, no association was observed between adherence to the MedDiet and the development of diabetic nephropathy (Supplementary Figs. 5 and 6). An increased risk of diabetic nephropathy (multivariable-adjusted HR 1.84 [1.10–3.07]; *P* = 0.03 for trend) was found for those individuals in the highest quintile of average levels of diastolic blood pressure during follow-up compared with the lowest quintile (Supplementary Fig. 7). However, no differences between quintiles of HDL-cholesterol levels were shown.

CONCLUSIONS

This post hoc analysis of the PREDIMED randomized trial suggests that a nutritional intervention based on a MedDiet supplemented with EVOO reduces the incidence of diabetic retinopathy in an elderly Mediterranean population with type 2 diabetes. After a median follow-up of 6.0 years, a statistically significant relative reduction in the risk of diabetic retinopathy of 43% and a nonsignificant reduction of 38% were apparent in the MedDiet group supplemented with EVOO and the MedDiet group supplemented with mixed nuts, respectively. Our results also suggest that the two MedDiet interventions had no beneficial effect on diabetic nephropathy. Indeed, the MedDiets were associated with a nonsignificant increased risk of diabetic nephropathy compared with the control diet, and we cannot exclude that our intervention may even increase the rates of diabetic nephropathy.

The main focus of the intervention in the PREDIMED trial was to change the overall dietary pattern instead of focusing on changes in single macro- or micronutrients. Given that our study did not specifically restrict energy intake or promote physical activity and that between-group changes in body weight were negligible, the observed benefit is likely attributable to the MedDiet plus the supplementary foods given for free. This reported benefit can be explained because participants in the two MedDiet groups, unlike those in the control group, increased their adherence to the MedDiet during the trial. We also observed that participants who best adhered to the MedDiet during the follow-up period showed the strongest reductions in the incidence of diabetic retinopathy. Moreover, changes in objective biomarkers in the MedDiet groups, but not in the control group, also indicated good compliance with the dietary assignments.

Our results are consistent with previous PREDIMED reports showing that the MedDiet had protective effects on traditional cardiovascular risk factors, such as blood pressure, lipid profile, and glucose metabolism, and on novel risk

Table 3—Subgroup analyses of the incidence of diabetic retinopathy by intervention group in the PREDIMED trial after a median 6.1 years of follow-up

	Events/total			HR (95% CI)		*P* for interaction†	
	MedDiet+EVOO	MedDiet+Nuts	Control group	MedDiet+EVOO	MedDiet+Nuts	EVOO	EVOO+Nuts
Sex							
Male	9/574	10/593	8/540	0.76 (0.28–2.04)	0.82 (0.31–2.16)	0.38	0.33
Female	13/708	10/549	24/650	0.46 (0.23–0.92)	0.51 (0.24–1.08)		
Age, years*							
<70	18/790	14/729	17/709	0.84 (0.42–1.66)	0.74 (0.36–1.54)	0.20*	0.38*
≥70	4/492	6/413	15/481	0.24 (0.07–0.73)	0.47 (0.17–1.26)		
BMI, kg/m^2							
<30	15/689	11/630	12/608	1.00 (0.46–2.18)	0.80 (0.34–1.86)	0.59*	0.74*
≥30	7/596	9/512	20/582	0.26 (0.10–0.62)	0.50 (0.23–1.12)		
Hypertension							
No	7/308	5/292	14/268	0.35 (0.14–0.89)	0.31 (0.10–0.90)	0.62	0.28
Yes	15/974	15/850	18/922	0.70 (0.34–1.42)	0.90 (0.45–1.82)		
Dyslipidemia							
No	13/518	14/469	18/485	0.60 (0.28–1.23)	0.78 (0.38–1.62)	0.86	0.47
Yes	9/764	6/673	14/705	0.50 (0.20–1.17)	0.41 (0.16–1.10)		
MedDiet adherence at baseline (0 to 14 score)							
<10	11/841	15/735	24/878	0.47 (0.23–0.97)	0.75 (0.38–1.48)	0.10	0.18
≥10	11/441	5/407	8/312	0.68 (0.26–1.79)	0.31 (0.09–1.09)		

All models are fully adjusted for the confounders shown in model 1 in Table 2 and stratified by center. †Two interactions were assessed: only for the effect of MedDiet+EVOO (1 degree of freedom) and for both groups (2 degrees of freedom). *The interactions with age and BMI were assessed using age and BMI as continuous variables.

factors such as markers of oxidation, inflammation, and endothelial dysfunction (18,20). Moreover, we have also previously reported that the MedDiet protects against cardiovascular events (24) and related conditions, such as hypertension (21), metabolic syndrome (23) and diabetes, compared with a low-fat control diet (22). In fact, we recently reported that after a median 4.1 years of follow-up, a MedDiet supplemented with EVOO or mixed nuts reduces the incidence of type 2 diabetes by 40% and 18%, respectively, compared with a low-fat control diet (22). Therefore, our results add new knowledge from first-level evidence and confirm once again the health benefits of adopting a MedDiet, which may be of help not only in lowering the incidence of diabetes but also in halting the development of microvascular complications in individuals with diabetes.

In our study, we found that the MedDiet supplemented with EVOO had a protective effect on retinopathy but that the MedDiet supplemented with mixed nuts only had a marginal effect. The dissimilar benefit of the two MedDiet interventions may be a chance finding because EVOO, the major fat component of the diet, and nuts both contributed an extra load of nutrients, including mono- and polyunsaturated fatty acids, and other bioactive compounds (including fiber, minerals, tocopherols, phytosterols, and phenolic compounds) with strong anti-inflammatory and antioxidant effects (28,29). Most of these components have been related to decreases in the risk of diabetic retinopathy (5,7,11–13,16). The MedDiet pattern promoted in both MedDiet interventions included several other dietary components reported to be beneficial in alleviating inflammation and oxidative stress and in decreasing insulin resistance and secretion, which are pathogenic factors in diabetes (30) and diabetic microvascular complications (31). In conjunction with the improvement in the aforementioned cardiometabolic risk factors, this adds biological plausibility to the present results. For instance, many vegetables, fruits, and seeds, such as cereals and legumes, contain minerals, polyphenols, and other phytochemicals that combat oxidative stress, inflammation, and insulin resistance (32,33). In fact, high consumption of flavonoid-rich fruits and vegetables (7,8) has been associated to a lower risk of diabetic retinopathy.

Very few studies have evaluated the effect of a Mediterranean-style dietary pattern on kidney function in individuals with type 2 diabetes. The current study is not in agreement with some observational studies that have noted favorable effects of the MedDiet on kidney function in apparently healthy young or

Table 4—Sensitivity analyses

	MedDiet+EVOO vs. control group	MedDiet+Nuts vs. control group	Both MedDiets vs. control group
Early cases excluded (<1 year) (62 events included)*	0.49 (0.26–0.91)	0.67 (0.36–1.22)	0.57 (0.34–0.95)
Late cases excluded (>6 years) (67 events included)†	0.66 (0.37–1.15)	0.60 (0.32–1.10)	0.63 (0.38–1.03)
Only cases observed after the first 3 years (42 events included)‡	0.48 (0.23–0.99)	0.54 (0.26–1.11)	0.51 (0.26–0.95)

HRs (95% CI) of diabetic retinopathy by intervention group. All models are fully adjusted for the confounders shown in model 1 in Table 2 and stratified by center. *Of the 74 incident diabetic retinopathy cases, 12 were excluded. †Of the 74 incident diabetic retinopathy cases, 7 were excluded. ‡Of the 74 incident diabetic retinopathy cases, 32 were excluded.

middle-aged individuals from different populations (34–36). Contrary to our hypothesis, in the present post hoc analysis, we could not show a significant protective effect of the MedDiet+ EVOO or the MedDiet+Nuts on diabetic nephropathy, even after performing sensitivity analysis evaluating nephropathy diagnosis according to incident impaired GFR and incident albuminuria. These results are consistent with a previous study conducted at the Reus PREDIMED center with 785 participants in which we assessed the 1-year effects of three interventions on kidney function (37).

In that pilot report, although the three dietary interventions were associated with improved kidney function, as assessed by eGFR, the between-group differences were negligible, and the results did not vary with diabetes status (37). This could be partly explained by the reduction in the fat intake in the control diet group that could have improved kidney function, because it has been reported that a high intake of fat is negatively associated with kidney function measurements (14). Further randomized trials with longer follow-up are needed to assess the hypothesis that the MedDiet is better than other dietary interventions at preventing the development of diabetic nephropathy in adults with type 2 diabetes.

The current study has some limitations and strengths that should be considered. Some statistically significant imbalances (albeit of small magnitude) in baseline characteristics were present in our trial. These imbalances were minor and cannot be considered as clinically meaningful. The most relevant imbalance was a higher proportion of men in the MedDiet+Nuts intervention group. Because male sex was strongly related with a higher risk of complications, this imbalance may act against our hypothesis. Nevertheless, we accounted for these imbalances by always controlling for all of these factors in multivariable-adjusted analyses. Other, more relevant, limitations of our study should be acknowledged.

First, the study was performed in elderly individuals with diabetes at high risk for CVD. Consequently, our findings cannot be extrapolated to other populations.

Second, the assessment of diabetes complications was not the primary end point, because the PREDIMED trial was designed to assess the effect of the MedDiet on primary cardiovascular prevention. However, we took care to ensure that all cases of diabetic retinopathy were medically diagnosed by experienced ophthalmologists. Furthermore, only those cases definitively confirmed by the adjudication committee were included in this post hoc analysis to ensure a high degree of specificity in the diagnosis of retinopathy. Only 13% of the probable cases of diabetic nephropathy diagnosed were confirmed by the adjudication committee. Serum creatinine or urinary ACR was regularly measured and used for new case ascertainment of nephropathy, although a second test was used to confirm the diagnosis.

Third, unfortunately, we do not have repeated measures of glycated hemoglobin as a marker of diabetes control during the follow-up to test the hypothesis that both MedDiets interventions were superior to the low-fat diet in diabetes control.

Fourth, the CKD-EPI equation used for the ascertainment of diabetic nephropathy was not validated in overweight or obese people with diabetes at high cardiovascular risk and, therefore, might not be the most appropriate for our population. However, equations to estimate the GFR, such as CKD-EPI equation, which include age, sex, and race, have been shown to be a more accurate assessment of the level of kidney function than serum creatinine alone (38).

Finally, among other potential limitations are that the observed number of events was relatively small and that our study may lack enough statistical power to detect small effects.

A considerable strength of our study was that to test the robustness of our findings, we conducted additional sensitivity analyses for diabetic retinopathy and nephropathy, and the results did not significantly change. Other major advantages of our study are, first, its randomized design; second, its long-term intervention and good compliance; third, the large study size, which may eventually provide stronger evidence of diabetic retinopathy prevention by the MedDiet; and, finally, the control for several potential confounders, which together with the randomization, allows us to rule out residual confounding.

In summary, the results of our post hoc analysis suggest that a MedDiet intervention supplemented with EVOO could play a beneficial role in the prevention of diabetic retinopathy but not on diabetic nephropathy in participants at high cardiovascular risk with type 2 diabetes. The effect of a low-fat diet compared with a MedDiet on diabetic nephropathy remains to be elucidated.

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