Mediterranean diet, retinopathy and nephropathy microvascular diabetes complications: A *post hoc* analysis of a randomized trial

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ABSTRACT

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 would have greater protective effect on diabetic retinopathy and nephropathy than a low-fat control diet .

Research design and methods: *Post hoc* analysis of a cohort of patients with type 2 diabetes participating in the PREvención con DIeta MEDiterránea (PREDIMED) study, a multi-center randomized nutritional intervention trial conducted in a population at high cardiovascular risk. Individuals with type 2 diabetes who were free of microvascular complications at enrolment (3614 participants, aged 55-80 years) were randomly assigned to one of three dietary interventions: MedDiet supplemented with extra virgin olive oil (MedDiet+EVOO), 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 and 168 new cases of retinopathy and nephropathy, respectively. In comparison 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 vs the lowest quintile was 0.34 (0.13-0.89); P-trend=0.001. No significant associations were found for nephropathy.

Conclusions: A Mediterranean diet enriched with EVOO may protect against diabetic retinopathy but not diabetic nephropathy.

Type 2 diabetes mellitus is a growing public health problem with an increased risk of developing both 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 (18) has evaluated the relationship between diet and nephropathy in diabetic individuals, showing an increased risk of microalbuminuria and rapid 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 (19,20). 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 (21) (i.e. hypertension (22), diabetes (23) and metabolic syndrome (24)) than a low-fat diet, and also reduced cardiovascular events (25).

To date, no randomized trial has assessed the long-term effect of a MedDiet on diabetes complications. Therefore, we hypothesized that two MedDiets, one enriched with extra-virgin olive oil and another enriched with mixed nuts, would be associated with a lower risk of diabetic retinopathy and nephropathy, in comparison 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 (26), a parallel-group, randomized, primary cardiovascular prevention trial in persons at high risk CVD. The main results of the trial at the primary cardiovascular endpoint have been published elsewhere (25).

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 either type 2 diabetes or at least three of the following cardiovascular risk factors: current smoking, hypertension, dyslipidemia, overweight/obesity, or family history of early-onset CVD. Exclusion criteria have been reported previously (25,26).

Randomization and intervention

Participants were recruited in primary care centers affiliated with 11 Spanish teaching hospitals between October 2003 and January 2009. In total, 7447 participants were enrolled in the PREDIMED study, and randomly assigned in a 1:1:1 ratio, to one of the following three intervention groups: MedDiet supplemented with extra-virgin olive oil (MedDiet+EVOO), MedDiet supplemented with mixed nuts (MedDiet+Nuts), or control diet or control diet (advice on a low fat-diet following the American Heart Association guidelines). Dietary interventions (25,26) are detailed in the Supplementary Appendix. Randomization was performed centrally by means of a computer-generated random-number sequence. Four strata for stratified randomization were built by sex and age (cut-off point: 70 years). Investigators and members of all committees were blinded to the treatments assigned to individual participants.

In the present analysis, our main objective was to determine the effect of the three dietary

interventions on the incidence of diabetes complications. Therefore, we analysed a subset of 3614 participants of the PREDIMED trial who had type 2 diabetes at baseline. All participants (n=3614) were included to assess 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 based on two consecutive visits: albuminuria (urinary ACR ≥30 mg/g) or impaired renal function (eGFR<60 ml/min/1.73m^2), two widely used measures for assessing kidney dysfunction. The effective sample size for statistical analyses of diabetic nephropathy incidence was 2129 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 semi-quantitative food-frequency questionnaire (25); and a validated Spanish version of the Minnesota Leisure-Time Physical Activity Questionnaire (27). In addition, electrocardiography and anthropometric variables and blood pressure were 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 GFR was estimated based on creatinine using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (28). Urinary creatinine and albumin concentrations were also measured by the Jaffé method and bromcresol green albumin method, respectively, and 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 (25). Laboratory technicians were blinded to intervention group.

Ascertainment of diabetes complications

Diabetes complications (externally confirmed by an Adjudication Committee) were not a 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 either 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 charts. These reports and all relevant documentation, including medical records made by ophthalmologists, were sent to the PREDIMED 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 charts 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 non-mydriatic fundus camera to all diabetic patients, 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/1.73m^2 based on serum creatinine, and the latter as the transition from normo- to micro- or macroalbuminuria (urinary ACR ≥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 the SPSS software version 19.0 (SPSS Inc, Chicago, IL) and Stata 12.0 (StataCorp, College Station, TX, USA).

The assumptions for power calculations were based on expected rates of complications >=3% in the control group and >=1.5% in the two intervention groups considered together, with sample sizes of 1200 and 2400 subjects, respectively and two-tailed alpha error=0.05. Under these assumptions the statistical power to find a relative risk <=0.5 is 80 percent. Baseline differences between the three dietary intervention groups were tested using analysis of variance (ANOVA-test) or chi-square, and results were expressed as means ±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 hazard models to assess the effect of the two MedDiet interventions on diabetes complications

(retinopathy and nephropathy) in comparison with the control group. Hazard ratios (HRs) and 95% confidence intervals (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 group of intervention during follow-up. Pre-specified 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 evaluating the diagnosis of diabetic nephropathy according to incident hyperalbuminuria or incident GFR impairment (<60mL/min/1.73m^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 hazard 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 modelled as a continuous variable. All statistical tests were two-tailed and the significance level was set at P≤ 0.05.

RESULTS

Of the 3614 PREDIMED participants with type 2 diabetes assessed in the present report 1282, 1142, and 1190 were allocated to MedDiet+EVOO, MedDiet+Nuts, and control diet groups, respectively (Supplemental figure S1). The mean age of the participants was 67 years – 47 % of whom were men –and they had a sizeable burden of cardiovascular risk factors (90% were overweight/obese, 77% had hypertension and 61% dyslipidemia). The baseline characteristics of study participants by dietary intervention group are listed in Table 1. Although there were small differences in BMI and the proportion of men between the three intervention groups, these are irrelevant in magnitude or from a clinical point of view, and 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 difference between them from a clinical point of view; i.e. 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 over 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) (Supplemental figure S2). Also, the percentage of participants with a MedDiet score of 10 or greater was higher in the two MedDiet groups. There were significant differences between both MedDiet groups and the control group in 10 of the 14 items after 3 and 5 years of follow-up (Supplementary table S1 and table S2). Changes in objective biomarkers (measured in a small random sample of diabetics) 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 (Supplemental table S3). We found no significant differences in changes in body weight, waist circumference, or physical activity among the three groups during follow-up (Supplemental table S4).

During follow-up, 74 participants developed new-onset retinopathy (22 in MedDiet+EVOO; 20 in MedDiet+Nuts; 32 in the control group). Among the 2129 participants (among 3614 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 displays the HRs and 95% CIs of the effects of the two MedDiet interventions on diabetes complications in comparison with the control group. Compared with the control group, the unadjusted HRs for diabetic retinopathy were 0.57 (95%CI, 0.32 to 0.98) for the MedDiet+EVOO and 0.62 (0.35 to 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 [0.32 to 0.97]), and a non-significant risk reduction (37% lower risk, HR 0.63 [0.35 to 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 to 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 as compared with the control group or when both MedDiet groups were merged (Table 2). The unadjusted Kaplan–Meier curves illustrating the survival free of diabetic retinopathy and nephropathy by group of intervention during follow-up are shown in Supplemental figures S3 and S4, 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 (Supplemental table S5).

Sensitivity analyses were consistent with the findings of the primary analysis (Table 4 and Supplemental Table S6). When the early cases of diabetic retinopathy which 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 [0.26 to 0.91]) in comparison to the control diet. Similarly, a significant relative risk reduction was found, when both MedDiet groups were merged together (HR, 0.57 [0.34 to 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 to 0.99) in the MedDiet+EVOO group and 0.51 (0.26 to 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 diastolic blood pressure or HDL-cholesterol levels, regardless of the allocated intervention group, to evaluate associations with the incidence of diabetes complications. A 66% reduction in the risk of diabetic retinopathy (multivariable-adjusted HR, 0.34 (95% CI: 0.13 to 0.89); P for trend=0.001) was found for those individuals in the highest quintile of adherence to the MedDiet as compared to the lowest (reference) quintile. In contrast, no association was observed between adherence to the MedDiet and the development of diabetic nephropathy (Supplemental figure S5 and figure S6). An increased risk of diabetic nephropathy (multivariable-adjusted HR, 1.84 (1.10 to 3.07); *P* for trend=0.03) was found for those individuals in the highest quintile of average levels of diastolic blood pressure during follow-up as compared to the lowest quintile (Supplemental figure S7). However, no differences between quintiles of HDL-cholesterol levels were shown.

CONCLUSIONS

This *post hoc* analysis of the PREDIMED randomised 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 6.0 years of median follow-up, a statistically significant relative reduction in the risk of diabetic retinopathy of 43% and a non-significant 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 in comparison 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 macronutrients or micronutrients. Given that our study did not specifically restrict energy intake or promote physical activity, and 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 novel risk factors such as markers of oxidation, inflammation and endothelial dysfunction (21). Moreover, we have also previously reported that in comparison

with a low-fat control diet, the MedDiet protects against cardiovascular events (25) and related conditions, such as hypertension (22), metabolic syndrome (24) and diabetes (23). In fact, we have 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, in comparison to a low-fat control diet (23). Therefore, our results add new knowledge from first-class 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 both EVOO, the major fat component of the diet, and nuts 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 (29,30). Most of these 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 decreasing insulin resistance and secretion, which are pathogenic factors in diabetes (31) and diabetic microvascular complications (32). 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 (33,34). 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 present study is not in agreement with some observational studies that have noted favourable effects of the MedDiet on kidney function in apparently healthy young or middle-aged individuals from different populations (35–37). Contrary to our hypothesis, in the present *post hoc* analysis we could not show a statistically protective effect of either theMedDiet+EVOO nor the MedDiet+Nuts group 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 carried out at the Reus PREDIMED centre with 785 participants in which we assessed the 1-year effects of three interventions on kidney function. In this 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 (38). 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 confirm 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 present 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 meaningful from a clinical point of view. The most relevant imbalance was a higher proportion of males in one of the intervention groups (MeDiet+nuts). As male sex was strongly related with a higher risk of complications, this imbalance may act against our hypothesis. Nevertheless we took these imbalances into account by controlling always for all these factors in multivariable-adjusted analyses. Other, more relevant, limitations of our study should be acknowledged. First, it was carried out in elderly individuals with diabetes at high risk for CVD. Consequently

our findings cannot be extrapolated to other populations. Second, that the assessment of diabetes complications was not the primary end-point, since the PREDIMED trial was designed to assess the effect of 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 in order to ensure a high degree of specificity in the diagnosis of retinopathy. In the case of diabetic nephropathy, only 13% of the cases diagnosed were confirmed by the Adjudication Committee. Serum creatinine or urinary ACR were regularly measured and used for new case ascertainment of nephropathy , although a second test was used to confirm the diagnose. Third, unfortunately we do not have repeated measures of glycated haemoglobin as a marker of diabetes control during the follow-up to test the hypothesis that both MedDiets interventions have been superior to the low-fat diet in terms of diabetes control. Fourth, the CKD-EPI equation used for the ascertainment of diabetic nephropathy was not validated in overweight or obese diabetic people at high cardiovascular risk. Therefore, it may not be the most appropriate for our population. However, GFR-estimating equations, such as CKD-EPI equation, which includes age, sex and race, have been shown to be a more accurate assessment of the level of kidney function than serum creatinine alone (39). Finally, other potential limitations include that the observed number of events was relatively small and 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 both diabetic retinopathy and nephropathy, and the results did not significantly changed. 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 MeDiet; and, finally, the control for several potential confounders, which together with

the randomisation 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 type 2 diabetes participants at high cardiovascular risk. The possible beneficial effect of a low-fat diet compared to a MedDiet on diabetic nephropathy remains to be elucidated.

Authors' contributions: MA.M-G, D.C, M.F, R.E, F.A, E.G-G, M.Fiol, J.L, LS-M, and J.S-S designed the research. A.D-L, N.B, MA.M-G, D.C, AJ.A, M.F, R.E, F.A, E.G-G, M.Fiol, J.L, LS-M, J.B, FJ. B-G, V.Z-M, MA.M and J.S-S conducted the research. A.D-L and J.S-S analyzed the data. A.D-L and J.S-S wrote the paper. MA.M-G, D.C, AJ.A, M.F, R.E, F.A, E.G-G, M.Fiol, J.L, L.S-M and J.S-S were the coordinators of participants recruitment at the outpatient clinics. A.D-L and J.S-S are the guarantors of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors revised the manuscript for important intellectual content, read and approved the final manuscript.

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[14]**LIST OF PREDIMED INVESTIGATORS** (Supplementary Appendix).

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

	MedDiet+EVOO (n=1282)	MedDiet+Nuts (n=1142)	Control group (n=1190)	P values†
Age, years	67.5 ± 6.2	67.1 ± 6.1	67.5 ± 6.4	0.15
Men, n (%)	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, n (%)	796 (62)	662 (58)	742 (62)	
Current smoker, n (%)	154 (12)	139 (12)	139 (12)	0.14
Former smoker, n (%)	332 (26)	341 (29)	309 (26)	
Educational level, n (%)				
Primary/Secondary education	1034 (81)	880 (77)	982 (82)	0.004
University/Some college	248 (19)	262 (23)	208 (18)	
Overweight/obesity, n (%)	1157 (90)	1009 (88)	1085 (91)	0.07
Hypertension, n (%)	974 (76)	850 (74)	922 (77)	0.22
Dyslipidemia, n (%)	764 (60)	673 (59)	705 (59)	0.94
Medication use, n (%)				
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, n (%)	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 means±SD, median and interquartile range [IQR] or numbers (%). Abbreviations: MedDiet+EVOO, Mediterranean diet supplemented with extra-virgin olive oil; MedDiet+Nuts, Mediterranean diet supplemented with nuts; BMI, Body mass index; HDL, High-density lipoprotein; CHD, coronary heart disease. *Angiotensin-type 2 receptor blocker and angiotensin converting enzyme inhibitors. †P value for comparisons between groups calculated with chi-square tests for categorical variables or analysis of variance (ANOVA-test) for quantitative variables.

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 (DR)	n=1282	n=1142	n=1190
Cases, n/ person-years of follow-up	22/7830	20/6622	32/6856
Hazard ratios of DR by intervention group (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.)
Hazard ratio for Mediterranean diets combined *vs*. control (95% CI)			
Multivariable-adjusted model 1†	0.60 (0.37–0.96)		1 (Ref.)
Diabetic nephropathy (DN)	n=740	n=672	n=717
Cases, n/ person-years of follow-up	64/4419	51/3985	53/4180
Hazard ratios of DN by intervention group (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.)
Hazard ratio for Mediterranean diets combined *vs*. control (95% CI)			
Multivariale-adjusted model 1†	1.11 (0.79–1.55)		1 (Ref.)

Cox regression models with outcome of DR and DN, and exposure to MedDiet intervention group vs. control group. Abbreviations: MedDiet+EVOO, Mediterranean diet supplemented with extra-virgin olive oil; MedDiet+Nuts, Mediterranean diet supplemented with nuts; CI, confidence interval. † Model 1 was additionally adjusted for baseline body-mass index (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 adherence to the Mediterranean diet (< 10- point, low or ≥ 10-point, high). All models were stratified by recruitment center.

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			Hazard Ratios (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. Abbreviations: MedDiet+EVOO, Mediterranean diet supplemented with extra-virgin olive oil; MedDiet+Nuts, Mediterranean diet supplemented with nuts; CI, confidence interval; BMI, body mass index. †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.

Table 4. Sensitivity analyses. Hazard ratios (95% confidence intervals) of diabetic retinopathy by intervention group

	Hazard Ratios (95% confidence intervals) [1]		
	MedDiet+EVOO versus control group	MedDiet+Nuts versus control group	Both MedDiets versus control group
Early cases excluded (< 1 yr) (62 events included)[2]	0.49 (0.26-0.91)	0.67 (0.36-1.22)	0.57 (0.34-0.95)
Late cases excluded (> 6 yr) (67 events included)[3]	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[4] (42 events included)	0.48 (0.23-0.99)	0.54 (0.26-1.11)	0.51 (0.26-0.95)

Abbreviations: MedDiet+EVOO, Mediterranean diet supplemented with extra-virgin olive oil; MedDiet+Nuts, Mediterranean diet supplemented with nuts.

[1]All models are fully adjusted for the confounders shown in model 1 in Table 2 and stratified by center.

[2]Of the 74 incident diabetic retinopathy cases, 12 were excluded.

[3]Of the 74 incident diabetic retinopathy cases, 7 were excluded.

[4]Of the 74 incident diabetic retinopathy cases, 32 were excluded.

SUPPLEMENTAL APPENDIX

The set of details, tables and figure included in this file are non-essential files that may clarify or add additional information that Diabetes care's readers may found important to take into consideration. For this reason, we would like this file of supplemental material to be included with our paper, in case of acceptance, as online-only supplemental material.

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DESCRIPTION OF THE DIETARY INTERVENTIONS

The main focus of the PREDIMED Study was to change the dietary pattern instead of focusing on changes in macronutrients. As opposed to recommendations to participants allocated the Control diet, total fat intake for the two Mediterranean diet groups was ad *libitum* [a high fat intake was allowed, as long as most fat was derived from fatty fish and vegetable sources, particularly extra-virgin olive oil (EVOO) and nuts]. Registered dietitians were directly responsible for all aspects of the dietary intervention at each site. All PREDIMED dietitians were trained and certified to deliver the intervention protocol.

Mediterranean diet groups

For participants in both MedDiet groups, a behavioural intervention promoting the MedDiet was implemented, as described previously (1). Dietitians gave personalized advice to participants about how to use olive oil for cooking and dressing and how much; weekly intake of nuts; increased consumption of vegetables, fruits, legumes and fish; recommended intake of white meat instead of red or processed meat; avoidance of butter, fast food, sweets, pastries, or sugar-sweetened beverages; and the dressing of dishes with "sofrito" sauce (using tomato, garlic, onion, and spices simmered in olive oil). Participants were advised to reduce their intake of all alcohol except wine. Moderate wine consumption with meals was recommended only to habitual drinkers.

At baseline and quarterly thereafter, dietitians conducted individual and group dietary training sessions with no more than 20 participants to provide information on typical Mediterranean foods, seasonal shopping lists, meal plans, and recipes. In each session, a 14-item questionnaire was used to assess adherence to the MedDiet (1), and to provide personalized advice to upgrade participants' adherence to this healthy dietary pattern. The same questionnaire was used yearly in the control group.

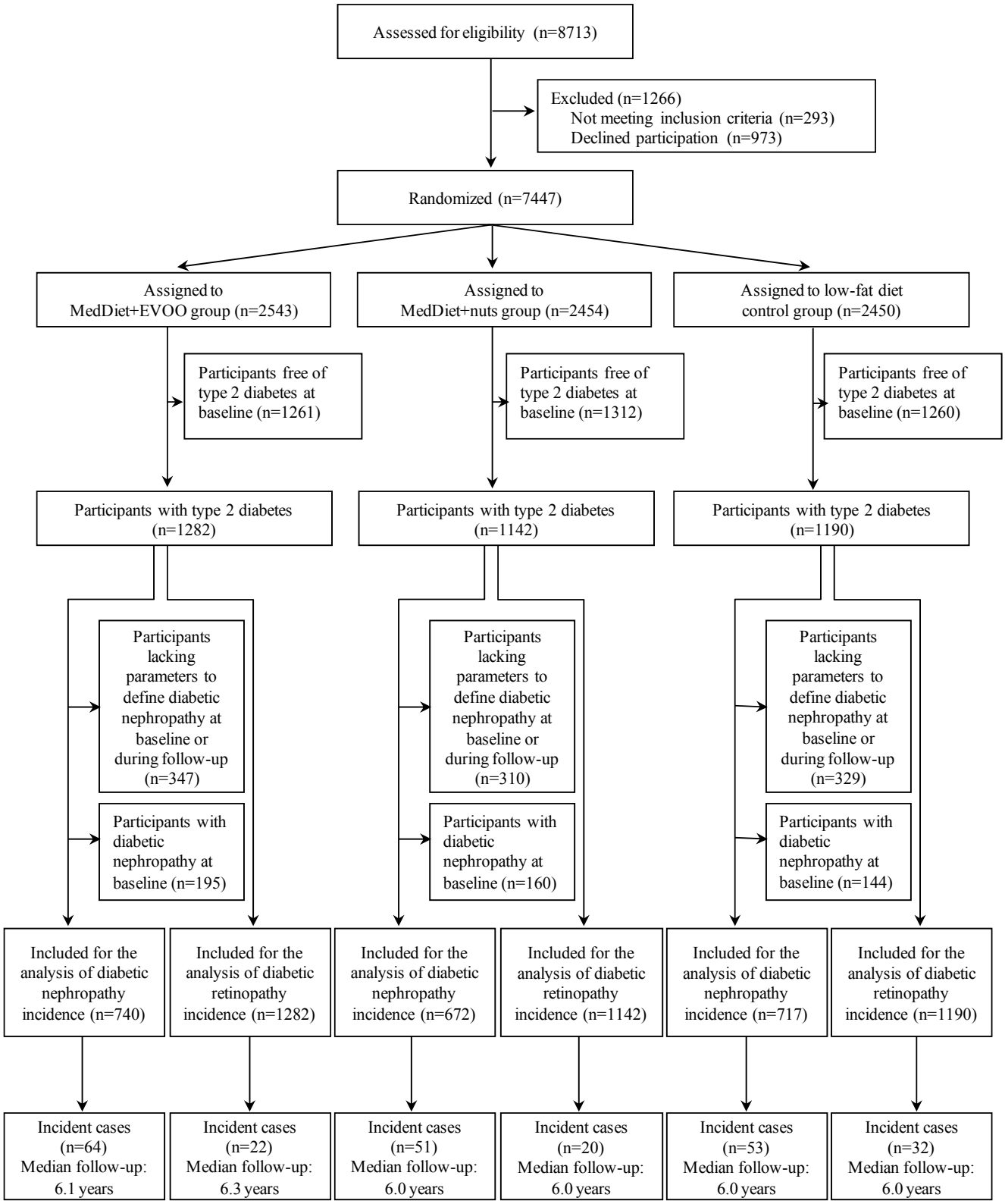
Participants assigned to the two MedDiet intervention groups were given packages of typical MedDiet foods at no cost during the intervention. EVOO (1 l/week for the participant and his/her family) was provided to the MedDiet+EVOO group, and 30 g/day of mixed nuts (15 g of walnuts, 7.5 g of almonds, and 7.5 g of hazelnuts) to the MedDiet+Nuts group. These foods, key elements in the traditional MedDiet, were provided to ensure high consumption, and to promote better overall adherence to the target dietary pattern.

Control diet group

Participants assigned to the control group did not receive education on the MedDiet. Instead they were given advice on following a low-fat diet, including recommendations for reducing intake of all types of fat, with particular emphasis in recommending the consumption of lean meats, low-fat dairy products, cereals, potatoes, pasta, rice, fruits and vegetables, in accordance with American Heart Association guidelines (2). A 9-item dietary questionnaire (3) was used to assess adherence to the low-fat diet. The last assessment of the 9-item score helped dietitians to give personalized advice in order to upgrade it in a similar way than the 14-item Mediterranean diet score.

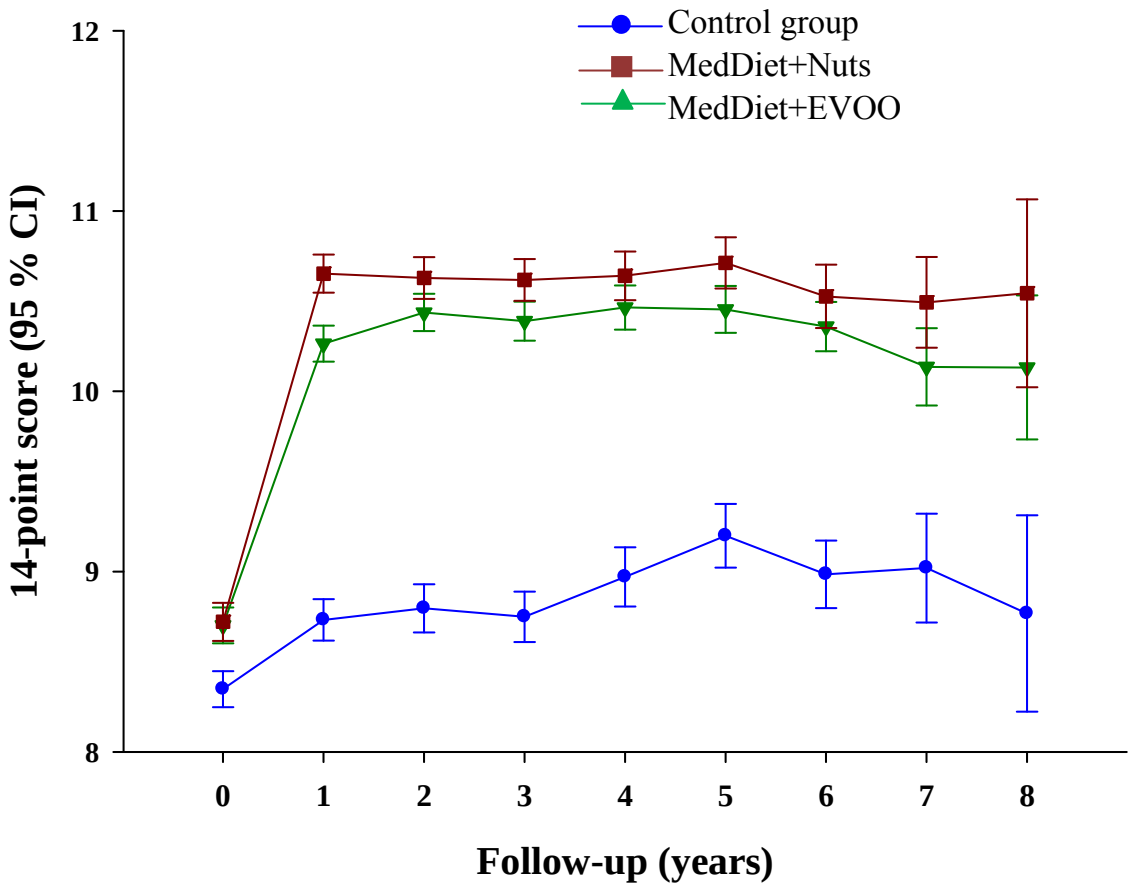
To encourage adherence, participants were given small non-food gifts, such as kitchenware, tableware, aprons or shopping bags. In October 2006, the participants in the control group received only a leaflet describing the low-fat diet. Thereafter, participants assigned to the control diet also received personalized advice and were invited to group sessions with the same frequency and intensity as those in the Mediterranean diet groups. Neither energy restriction nor increased physical activity was advised for any intervention group.

Supplemental figure S1. Flow-chart of study participants



Abbreviations: MedDiet, Mediterranean diet; EVOO, extra-virgin olive oil.

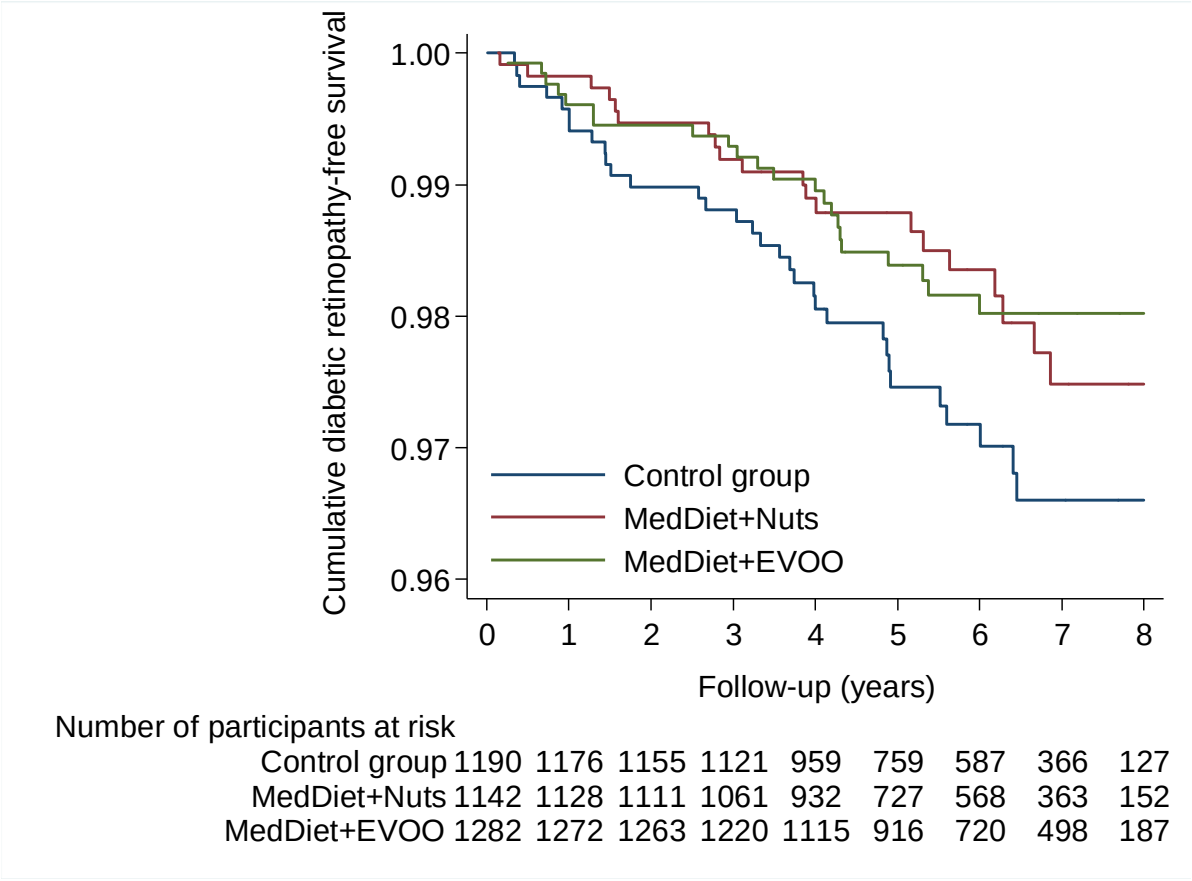
Supplemental figure S2. Adherence to the Mediterranean diet of the study population (diabetic participants of the PREDIMED trial) by intervention group, as assessed by the repeated 14-item questionnaires collected at baseline and during follow-up (means and 95% confidence intervals)



	Baseline	1 year	2 years	3 years	4 years	5 years	6 years	7 years	8 years
Control group	1182	818	674	623	427	380	302	136	37
MedDiet+Nuts	1137	898	806	762	580	504	394	182	51
MedDiet+EVOO	1274	1099	1042	1026	751	709	611	271	71

*Number of participants

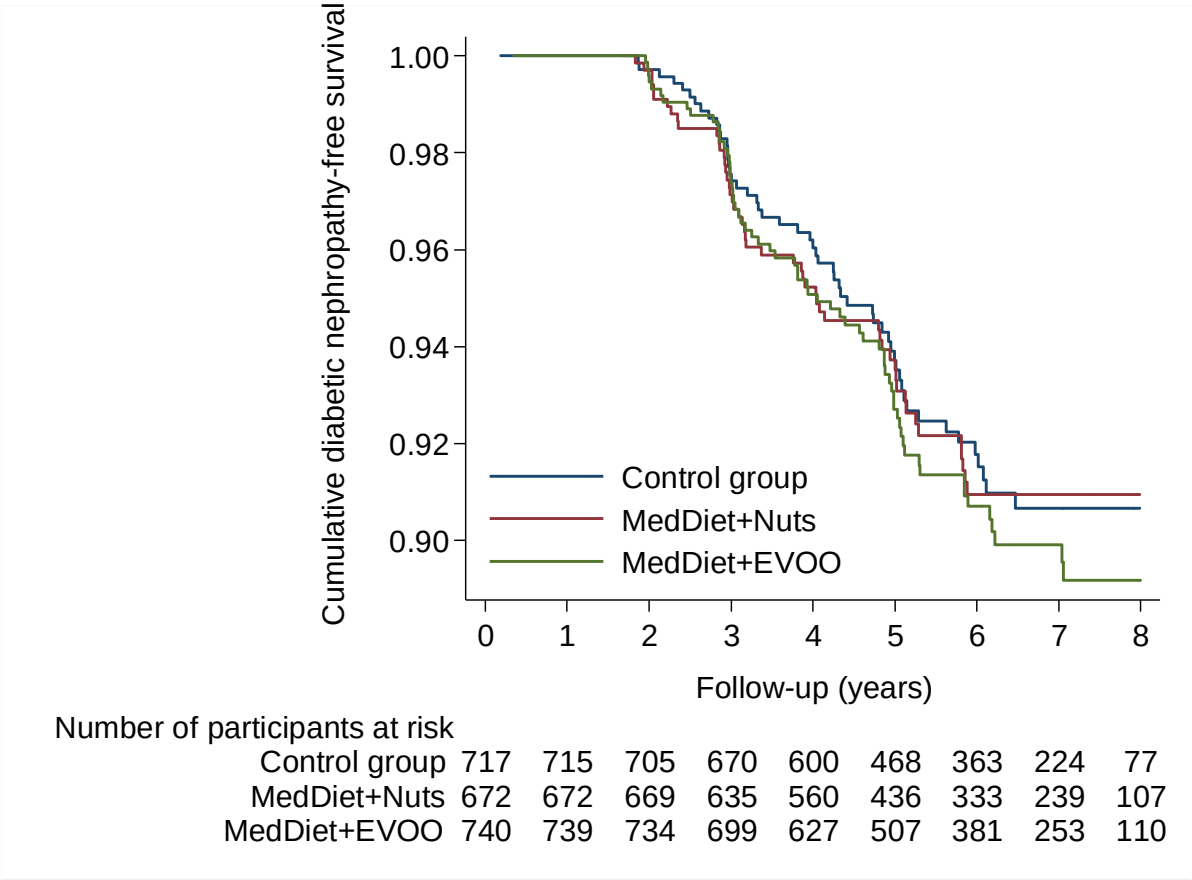
Supplemental figure S3. Kaplan-Meier survival curves were plotted to estimate the probability of remaining free of diabetic retinopathy during follow-up.



Number of participants at risk

Control group	1190	1176	1155	1121	959	759	587	366	127
MedDiet+Nuts	1142	1128	1111	1061	932	727	568	363	152
MedDiet+EVOO	1282	1272	1263	1220	1115	916	720	498	187

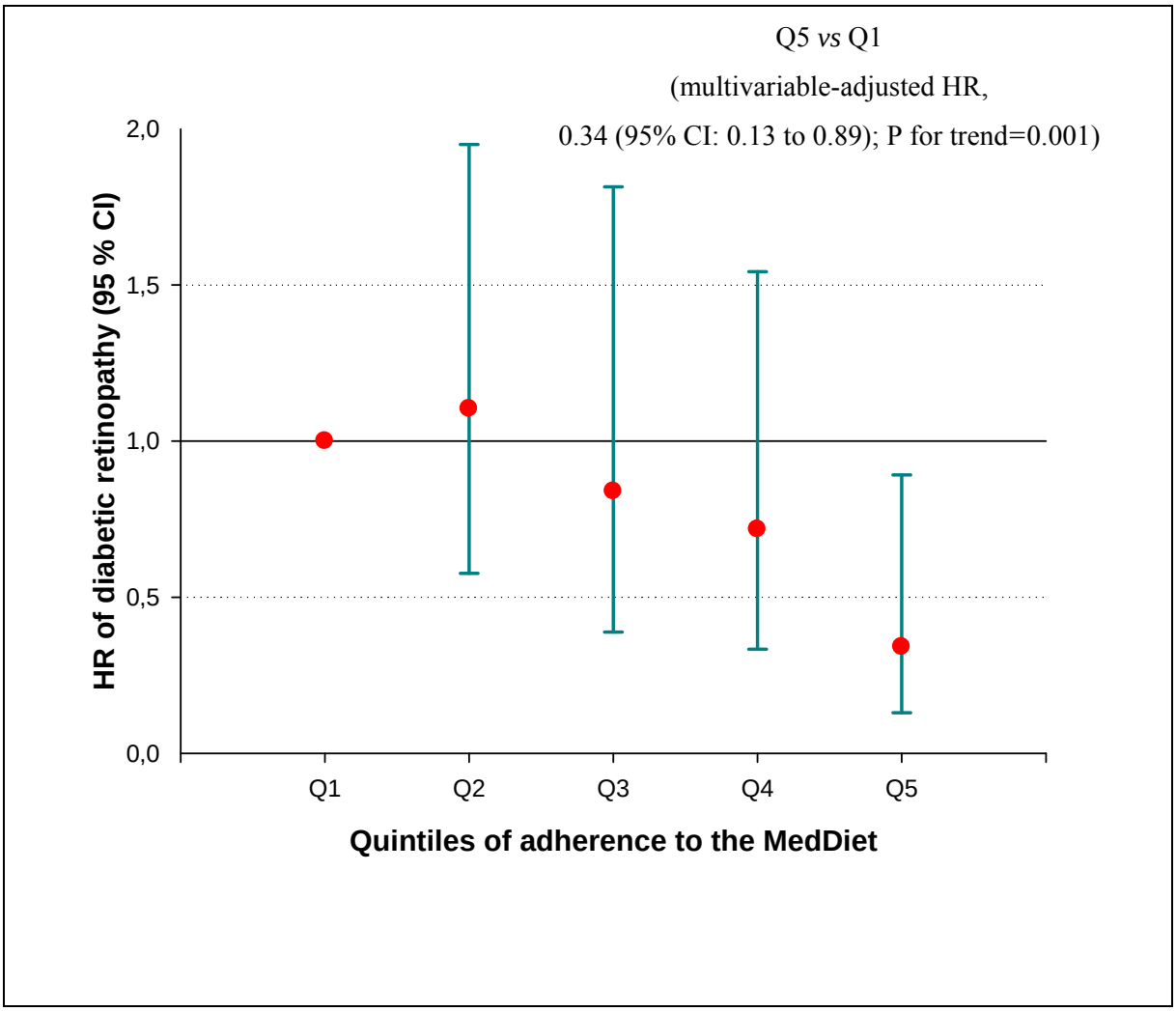
Supplemental figure S4. Kaplan-Meier survival curves were plotted to estimate the probability of remaining free of diabetic nephropathy during follow-up.



Number of participants at risk

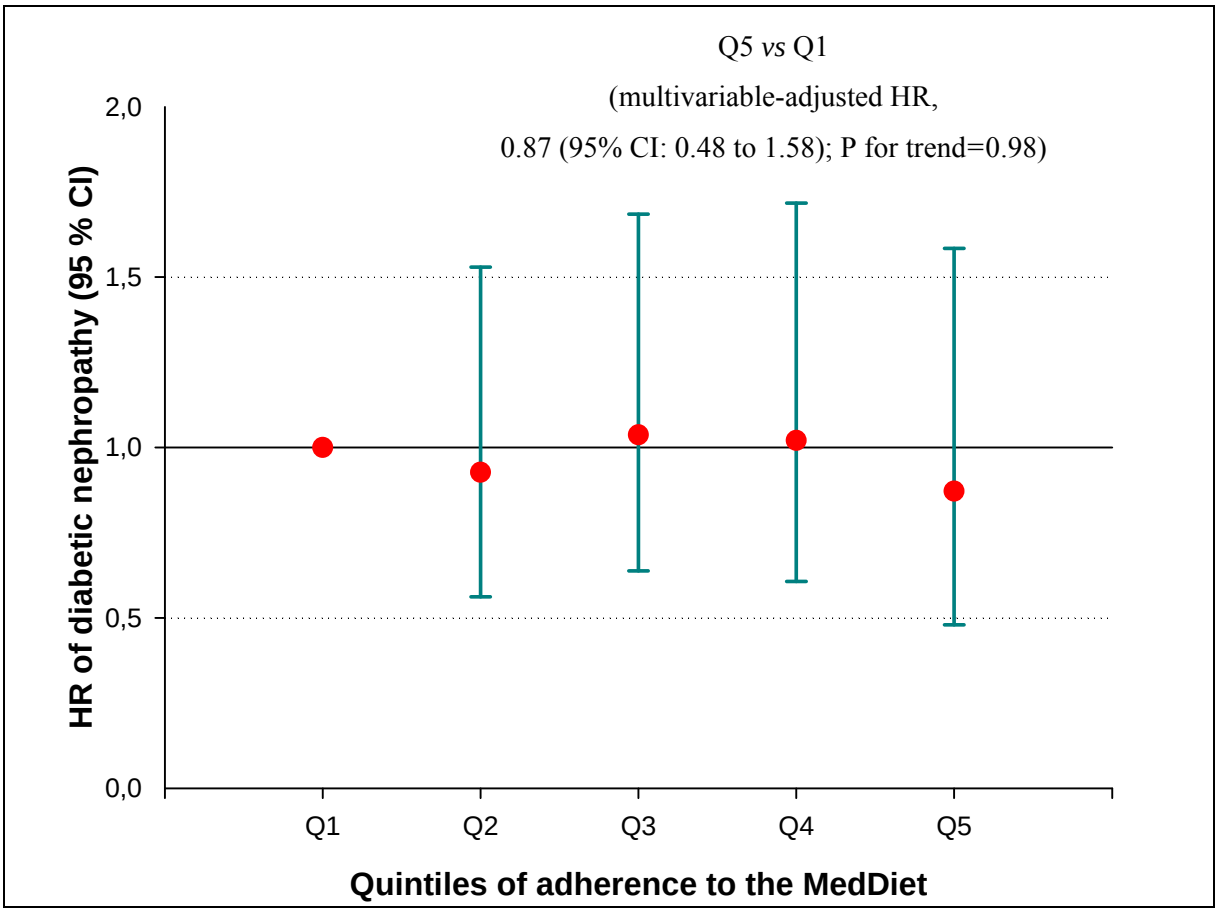
Control group	717	715	705	670	600	468	363	224	77
MedDiet+Nuts	672	672	669	635	560	436	333	239	107
MedDiet+EVOO	740	739	734	699	627	507	381	253	110

Supplemental figure S5. Hazard ratios (HRs) of diabetic retinopathy by quintiles of average adherence to the Mediterranean diet during follow-up



Time-dependent Cox regression models with outcome of diabetic retinopathy and yearly updated information on adherence to the MedDiet (assessed as the average of all available repeated measurements at baseline and during follow-up) as exposure. Abbreviations: MedDiet, Mediterranean diet; Q, quintile. Number of events/person-years of follow-up of each quintile of adherence to the Mediterranean diet: Q1, 21/5094; Q2, 19/4329; Q3, 14/4148; Q4, 12/3919; Q5, 7/3705.The model was adjusted for age, sex, baseline body-mass index (continuous variable), waist circumference (continuous variable), smoking (never, current or former smoker), physical activity in MET-min/day (continuous variable), education 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 dietary intervention group. All models were stratified by recruitment center.

Supplemental figure S6. Hazard ratios (HRs) of diabetic nephropathy by quintiles of average adherence to the Mediterranean diet during follow-up



Time-dependent Cox regression models with outcome of diabetic nephropathy and yearly updated information on adherence to the MedDiet (assessed as the average of all available repeated measurements at baseline and during follow-up) as exposure. Abbreviations: MedDiet, Mediterranean diet; Q, quintile. Number of events/person-years of follow-up of each quintile of adherence to the Mediterranean diet: Q1, 36/2989; Q2, 31/2532; Q3, 35/2356; Q4, 37/2524; Q5, 29/2170. The model was adjusted for age, sex, baseline body-mass index (continuous variable), waist circumference (continuous variable), smoking (never, current or former smoker), physical activity in MET-min/day (continuous variable), education 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 dietary intervention group. All models were stratified by recruitment center.

Supplemental table S1. Number and percentage (%) of diabetic participants of the PREDIMED trial with a MedDiet score ≥ 10 points at baseline and during follow-up

Years of follow-up	MedDiet+EVOO n (%)	MedDiet+Nuts n (%)	Control group n (%)	P values[†]
Baseline	443 (34.8)	407 (35.8)	312 (26.4)	<0.001
1	733 (66.7)	688 (76.6)	277 (33.9)	<0.001
2	748 (72.0)	611 (75.8)	246 (36.5)	<0.001
3	708 (69.0)	580 (76.1)	216 (34.7)	<0.001
4	532 (70.8)	439 (75.7)	178 (41.7)	<0.001
5	511 (72.1)	390 (77.4)	170 (44.7)	<0.001
6	415 (67.9)	277 (70.3)	112 (37.1)	<0.001
7	171 (63.1)	127 (69.8)	56 (41.2)	<0.001
8	41 (57.7)	36 (70.6)	12 (32.4)	<0.001

Abbreviations: MedDiet, Mediterranean diet; EVOO, extra-virgin olive oil. [†]P values of the difference between group interventions (chi-square test).

Supplemental table S2. Diabetic participants of the PREDIMED trial with a positive answer (%) to each of the 14 items of the MedDiet score by intervention group at baseline and during follow-up

	Baseline			3-year follow-up			5-year follow-up		
	MedDiet+ EVOO (n=1373)	MedDiet+ Nuts (n=1246)	Control group (n=1303)	MedDiet+ EVOO (n=1102)	MedDiet+ Nuts (n=844)	Control group (n=633)	MedDiet+ EVOO (n=775)	MedDiet+ Nuts (n=563)	Control group (n=424)
1. Use olive oil as main culinary fat	89.2	89.6	88.4*	99.0	96.6	90.6	100.0	96.8	95.8
2. Olive oil >4 tablespoons	72.3	69.7	64.9	93.3	73.9	49.3	92.7	78.4	59.8
3. Vegetables ≥ 2 servings/d	43.8	45.9	41.6*	66.8	66.4	55.9	74.0	74.6	67.5
4. Fruits ≥ 3 servings/d	52.3	52.3	49.0*	58.5	60.7	51.6	62.5	64.7	58.0
5. Red or processed meats < 1/d	86.7	87.0	85.1*	94.2	94.0	92.3*	97.9	96.6	95.5*
6. Butter, cream, margarine < 1/d	89.2	89.9	90.0*	97.3	94.8	93.3	98.0	96.0	95.5
7. Soda drinks <1/d	91.0	90.3	87.7	93.5	92.5	91.0*	94.2	93.5	94.8*
8. Wine glasses ≥ 7/wk	28.2	29.2	25.6	26.5	27.8	24.6*	26.7	27.2	23.4
9. Legumes ≥ 3/wk	25.5	28.7	25.3*	46.5	48.0	31.1	42.5	39.1	32.3
10. Fish or seafood ≥ 3/wk	54.0	53.3	55.9*	75.4	73.1	60.5	74.0	72.6	62.7
11. Commercial bakery ≤ 2/wk	71.2	71.2	69.0*	75.8	74.0	70.3*	74.6	76.4	70.6*
12. Nuts ≥ 3/wk	32.0	39.1	28.4	38.0	93.6	23.0	37.5	90.7	16.8
13. Poultry more than red meats	68.7	65.9	65.9*	84.2	82.8	76.1	81.8	81.7	79.0*
14. Use of sofrito sauce ≥ 2/wk	63.9	62.5	57.6	87.1	82.9	64.5	86.0	81.2	65.1

MedDiet denotes Mediterranean diet; EVOO, extra-virgin oil. All comparisons between each of the two MedDiet groups and the control group for each year were statistically significant (chi-square tests), with exception of those with an asterisk* ($p>0.050$).

Supplemental table S3. Levels at baseline and at 1, 3, and 5 years of follow-up of the objective Biomarkers of compliance plasma α-linolenic acid (a marker of walnut intake) and urinary hydroxytyrosol (a marker of extra-virgin olive oil consumption) in diabetic participants of the PREDIMED trial

	Intervention group		
	MedDiet+EVOO	MedDiet+Nuts	Control group
Plasma α-linolenic acid (%)	n=20	n=25	n=17
Baseline	0.36 (0.25- 0.46)	0.31 (0.25-0.38)	0.31 (0.26-0.36)
1 year	0.37 (0.27-0.47)	0.65 (0.40-0.51)*	0.33 (0.26-0.40)
3 years	0.34 (0.26-0.41)	0.44 (0.36-0.51)	0.34 (0.27-0.41)
5 years	0.29 (0.24-0.33)	0.40 (0.33-0.48)	0.28 (0.23-0.32)
Urinary hydroxytyrosol (µg/L)	n=102	n=67	n=70
Baseline	181 (138-225)	173 (125-221)	158 (88-228)
1 year	207 (156-256)	194 (131-258)	168 (128-208)
3 years	294 (223-365)*	231 (148-313)	202 (147-257)
5 years	271 (199-343)*	141 (108-173)	192 (123-262)

Abbreviations: MedDiet, Mediterranean diet; EVOO, extra-virgin olive oil. Data expressed as mean and 95% CI. Repeated-measures generalized linear model; * $p<0.050$ vs baseline.

Supplemental table S4. Baseline values and changes at 1, 3, and 5 years of follow-up of body weight, waist circumference, and physical activity

	Intervention groups			Between-group changes	
	MedDiet+EVOO	MedDiet+Nuts	Control group	MedDiet+EVOO vs control	MedDiet+Nuts vs control
Total body weight (kg)					
Baseline (n=1282/1142/1190)†	76.4 (75.7, 77.0)	76.8 (76.1, 77.5)	77.2 (76.1, 77.9)		
1 year (n=1166/963/899)	-0.24 (-0.43,-0.04)	-0.06 (-0.26,0.12)	-0.21 (-0.43,0.005)	-0.02 (-0.36,0.32) P=1.00	0.15 (-0.20,0.51) P=0.94
3 years (n=1071/842/727)	-0.82 (-1.09,-0.55)	-0.28 (-0.57,-0.001)	-0.57 (-0.99,-0.14)	-0.25 (-0.80,0.29) P=0.80	0.28 (-0.29,0.80) P=0.71
5 years (n=765/598/533)	-1.12 (-1.48,-0.77)	-0.63 (-1.04,-0.22)	-0.78 (-1.25,-0.30)	-0.34 (-1.04,0.35) P=0.71	0.14 (-0.59,0.89) P=1.00
Waist circumference (cm)					
Baseline (n=1227/1099/1134)	101.0 (100.4, 101.6)	100.9 (100.4, 101.5)	101.8 (101.2, 102.4)		
1 year (n=1056/881/804)	-0.65 (-0.99,-0.30)	-0.57 (-0.95,-0.19)	-0.42 (-0.81,0.02)	-0.22 (-0.87,0.41) P=1.00	-0.14 (-0.52,0.82) P=1.00
3 years (n=999/753/625)	-0.35 (-0.77, 0.05)	0.11 (-0.35,0.59)	0.21 (-0.31,0.74)	-0.57 (-1.39,0.24) P=0.27	-0.09 (-0.96,0.76) P=1.00
5 years (n=689/488/368)	0.62 (0.12, 1.13)	0.62 (0.008, 1.23)	1.3 (0.62, 2.08)	-0.72 (-1.79,0.33) P=0.30	-0.73 (-1.872,0.40) P=0.37
Physical activity (METS/min/day)					
Baseline (n=1282/1142/1190)	230.8 (217.6, 244.0)	257.0 (242.2, 271.9)	220.6 (227.5,244.0)		
1 year (n=1057/891/815)	11.14 (-5.64, 27.93)	2.14 (-14.00, 18.28)	3.77 (-14.17, 21.72)	7.36 (-21.99,36.72) P=1.00	-1.63 (-32.16,28.89) P=1.00
3 years (n=1026/761/622)	17.60 (1.26, 34.01)	8.13 (-12.89, 30.00)	-10.1 (-30.71,10.41)	27.79 (-5.68,61.27) P=0.14	18.29 (-17.32,53.90) P=0.65
5 years (n=706/499/370)	18.23 (-2.04, 38.51)	-4.91 (-31.14,21.31)	-6.59 (-33.91,20.73)	24.83 (-18.32,67.98) P=0.50	1.68 (-44.45,47.81) P=1.00

Abbreviations: MedDiet denotes Mediterranean Diet; EVOO, extra-virgin oil. Changes were calculated between baseline and each of the time points. † Number of diabetic participants allocated to each of the three groups (MedDiet+EVOO, MedDiet+Nuts, and control diet respectively). P values for comparisons between two groups was tested by bivariate analysis of variance (ANOVA) followed by post hoc tests with Bonferroni correction.

Supplemental table S5. Subgroup analyses of the incidence of diabetic nephropathy by intervention group in the PREDIMED trial after a median 6.1 years follow-up

	Events/Total			Hazard Ratios (95% CI)		*P* for Interaction†	
	MedDiet+EVOO	MedDiet+Nuts	Control group	MedDiet+EVOO	MedDiet+Nuts	EVOO	EVOO+Nuts
Sex							
Male	33/335	18/355	21/328	1.59 (0.90–2.79)	0.83 (0.91–2.79)	0.23	0.50
Female	31/405	33/317	32/389	0.86 (0.52–1.47)	1.17 (0.71–1.95)		
Age, years*							
<70	28/470	26/472	26/459	1.26 (0.72–2.21)	1.10 (0.62–1.94)	0.88*	0.54*
≥70	36/270	25/200	27/258	1.03 (0.61–1.72)	1.02 (0.58–1.78)		
BMI, kg/m^2							
<30	30/407	25/375	27/369	0.97 (0.57–1.64)	0.92 (0.52–1.61)	0.26*	0.33*
≥30	34/333	26/297	26/348	1.38 (0.80–2.39)	1.23 (0.69–2.20)		
Hypertension							
No	13/208	5/185	7/179	1.31 (0.49–3.49)	0.58 (0.17–1.89)	0.40	0.51
Yes	51/532	46/487	46/538	1.12 (0.75–1.69)	1.08 (0.71–1.65)		
Dyslipidemia							
No	30/302	27/271	25/296	1.12 (0.65–1.95)	1.16 (0.66–2.04)	0.66	0.83
Yes	34/438	24/401	28/421	1.21 (0.72–2.02)	1.03 (0.58–1.81)		
MedDiet adherence at baseline (0 to 14 score)							
<10	44/477	26/435	39/530	1.44 (0.92–2.25)	0.97 (0.58–1.61)	0.06	0.15
≥10	20/263	25/237	14/187	0.75 (0.37–1.54)	1.10 (0.55–2.19)		

All models are fully adjusted for the confounders shown in model 1 in Table 2 and stratified by center. Abbreviations: MedDiet+EVOO, Mediterranean diet supplemented with extra-virgin olive oil; MedDiet+Nuts, Mediterranean diet supplemented with nuts; CI, confidence interval; BMI, body mass index. †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.

Supplemental table S6. Incidence of diabetic nephropathy during the follow-up according to the assessment criteria used and the intervention group in the PREDIMED trial

Outcomes	MedDiet+EVOO	MedDiet+Nuts	Control group
Diabetic nephropathy ascertained by incident chronic kidney disease (DN-CKD)	n=735	n=666	n=711
Cases, n/person-years of follow-up	46/4523	39/4059	37/4250
Hazard ratios of DN-CKD by intervention group (95% CI)			
Crude model	0.72 (0.42–1.23)	1.16 (0.70–1.94)	1 (Ref.)
Age- and sex-adjusted model	0.67 (0.38–1.17)	1.19 (0.71–1.99)	1 (Ref.)
Multivariable-adjusted model 1†	0.73 (0.41–1.32)	1.38 (0.81–2.35)	1 (Ref.)
Hazard ratio for Mediterranean diets combined *vs*. control (95% CI)			
Multivariable-adjusted model 1†	1.04 (0.64–1.67)		1 (Ref.)
Diabetic nephropathy ascertained by incident hyperalbuminuria (DN-A)	n=386	n=365	n=363
Cases, n/person-years of follow-up	21/2419	13/2296	19/2223
Hazard ratios of DN-A by intervention group (95% CI)			
Crude model	1.35 (0.68–2.68)	1.63 (0.66–4.02)	1 (Ref.)
Age- and sex-adjusted model	1.49 (0.80–5.33)	2.06 (0.80–5.33)	1 (Ref.)
Multivariable-adjusted model 1†	1.30 (0.56–3.04)	2.59 (0.85–7.88)	1 (Ref.)
Hazard ratio for Mediterranean diets combined *vs*. control (95% CI)			
Multivariable-adjusted model 1†	1.54 (0.69–3.44)		1 (Ref.)

Cox regression models with outcome of DN-CKD and DN-A, and exposure to MedDiet intervention group vs. control group. Abbreviations: MedDiet+EVOO, Mediterranean diet supplemented with extra-virgin olive oil; MedDiet+Nuts, Mediterranean diet supplemented with nuts; CI, confidence interval. Incident CKD was considered by CKD progressing from moderate to severe (stage 3 or greater), defined as a sustained eGFR value <60 ml/min/1.73m^2 based on serum creatinine. Incident hyperalbuminuria was considered by albuminuria progressing during follow-up, defined as the transition from normo- to micro- or

macroalbuminuria (urinary ACR ≥30 mg/g). † Model 1 was additionally adjusted for baseline body-mass index (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 adherence to the Mediterranean diet (<10- point, low or ≥10-point, high). All models were stratified by recruitment center.

Supplemental table S7. Hazard ratios (HRs) of diabetes complications by quintiles of average levels of diastolic blood pressure and high-density lipoprotein cholesterol during follow-up

	Hazard Ratios (95% CI)					
	Q1	Q2	Q3	Q4	Q5	P for trend
Diabetic retinopathy						
Systolic blood pressure						
Cases, n/person-years of follow-up	10/4255	11/4281	11/4216	19/4287	21/4223	
Multivariable-adjusted model 1†	1 (Ref.)	1.23 (0.52–2.91)	1.22 (0.52–2.88)	2.06 (0.90–4.67)	2.04 (090.–4.62)	0.05
HDL-cholesterol, mg/dL						
Cases, n/person-years of follow-up	13/4153	11/4096	20/3998	16/4051	8/4042	
Multivariable-adjusted model 1†	1 (Ref.)	0.79 (0.33–1.86)	1.42 (0.64–3.12)	0.97 (0.42–2.22)	0.55 (0.20–1.47)	0.25
Diabetic nephropathy						
Systolic blood pressure						
Cases, n/person-years of follow-up	20/2549	32/2508	25/2486	33/2534	58/2506	
Multivariable-adjusted model 1†	1 (Ref.)	1.41 (0.80–2.47)	1.05 (0.58–1.91)	1.16 (0.66–2.02)	1.84 (1.10–3.07)	0.03
HDL-cholesterol, mg/dL						
Cases, n/person-years of follow-up	30/2541	40/2488	29/2517	27/2525	36/2443	
Multivariable-adjusted model 1†	1 (Ref.)	1.19 (0.73–1.94)	0.80 (0.47–1.36)	0.73 (0.42–1.26)	0.90 (0.51–1.58)	0.39

Time-dependent Cox regression models with outcome of diabetes complications and yearly updated information on diastolic blood pressure and high-density lipoprotein cholesterol (assessed as the average of all available repeated measurements at baseline and during follow-up) as exposure. Abbreviations: Q, quintiles; HDL, high-density lipoprotein. The model was adjusted for age, sex, baseline body-mass index (continuous variable), waist circumference (continuous variable), smoking (never, current or former smoker), physical activity in MET-min/day (continuous variable), education 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), dietary intervention group and hypertension (yes or no) and dyslipidemia (yes or no) adjusted for each other. All models were stratified by recruitment center.

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