Intake of Total Polyphenols and Some Classes of Polyphenols Is Inversely Associated with Diabetes in Elderly People at High Cardiovascular Disease Risk[1–3]

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Abstract

Background: Higher consumption of some polyphenols has been associated with a reduced risk of diabetes. However, no studies have evaluated the relation between all polyphenol subclasses and the incidence of diabetes.

Objective: We aimed to prospectively examine the associations between the intake of total polyphenols and different groups of polyphenols (flavonoids, phenolic acids, stilbenes, lignans, and others) on the risk of incident diabetes in the PREDIMED (Prevención con Dieta Mediterránea) trial.

Methods: This was an observational cohort analysis of the nondiabetic participants in the PREDIMED trial. This study was a multicenter, controlled, randomized, parallel-group feeding trial to assess the effects of either a Mediterranean diet that was supplemented with extra-virgin olive oil or nuts or advice to adhere to a low-fat control diet on cardiovascular outcomes in elderly men and women at high cardiovascular disease risk. From the 7447 randomly assigned participants, 3430 were selected because they were free of diabetes at baseline and filled out the food-frequency questionnaires (FFQs). Polyphenol intake was calculated by matching food consumption data from repeated FFQs with the Phenol-Explorer database on the polyphenol content of each reported food. HRs and 95% CIs for diabetes according to tertiles of polyphenol intake were estimated with the use of time-dependent Cox proportional hazards models.

Results: Over a mean of 5.51 y of follow-up (18,900 person-years), there were 314 new cases of diabetes. After multivariable adjustment, we observed a 28% reduction in new-onset diabetes in the highest compared with the lowest tertile of total polyphenol intake (HR: 0.72; 95% CI: 0.52, 0.99; *P*-trend = 0.05). The intake of subclasses of polyphenols also was inversely associated with diabetes risk, including for total flavonoids (HR: 0.67; 95% CI: 0.48, 0.93; *P*-trend = 0.02), stilbenes (HR: 0.57; 95% CI: 0.38, 0.84; *P*-trend = 0.003), dihydroflavonols (HR: 0.59; 95% CI: 0.40, 0.88; *P*-trend = 0.003), and flavanones (HR: 0.69; 95% CI: 0.49, 0.97; *P*-trend = 0.03).

Conclusions: A high intake of total polyphenols, total flavonoids (specifically flavanones and dihydroflavonols), and stilbenes is associated with a reduced risk of diabetes in elderly persons at high risk of cardiovascular disease. This trial was registered at http://www.controlled-trials.com as ISRCTN35739639. *J Nutr* 2016;146:767–77.

Keywords: chronic disease, cox regression, epidemiology, glucose, observational study

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Introduction

In 2014, the global prevalence of diabetes was estimated to be 9% in adults, and it was the direct cause of 1.5 million deaths in 2012. In recent decades, the prevalence of this disease and its modifiable risk factors (overweight/obesity, dyslipidemia, hypertension, and physical inactivity) has been increasing globally, particularly in low- and middle-income countries (1). The incidence of type 2 diabetes could be reduced by the adoption of a healthier lifestyle. Weight loss, regular exercise, a healthy diet, and abstinence from smoking are all recognized as important lifestyle factors that condition the risk of diabetes. A diet including whole grains, fruits, vegetables, legumes, nuts, and moderate alcohol consumption has been shown to decrease the risk of the onset of type 2 diabetes, whereas consumption of refined grains, red or processed meats, and sugar-sweetened beverages increases the risk (2). Polyphenol dietary intake has been associated with a reduced incidence of type 2 diabetes in humans (3–5). The protective activity of these bioactive compounds, widespread in foods from plants, also has been demonstrated in animal models (6–10). Thus, polyphenols may influence glycemia through different mechanisms, including the inhibition of glucose absorption in the gut or inhibition of its uptake in peripheral tissues (11). However, the influence of the different subgroups of polyphenols on diabetes in humans has not been completely studied. Indeed, epidemiologic and clinical studies have focused attention only on lignans, flavanols, flavonols, flavones and anthocyanins, or individual polyphenols such as resveratrol or quercetin.

To our knowledge, no prospective research has comprehensively quantified the association between the intake of all polyphenol subgroups and the risk of diabetes; therefore, we aimed to prospectively examine whether polyphenol intake is associated with a risk of incident diabetes and which polyphenol subgroups may be involved in the possible association.

Methods

The study design was an observational cohort analysis of the nondiabetic participants in the PREDIMED (Prevención con Dieta Mediterránea) trial. This study, which took place from October 2003 to December 2010, was a multicenter, controlled, randomized, parallel-group feeding trial to assess the effects of either a Mediterranean diet (MedDiet) supplemented with extra-virgin olive oil or nuts or advice to adhere to a low-fat control diet on cardiovascular outcomes in individuals at high cardiovascular disease risk. Details of the recruitment method and study design have been described elsewhere (12) and also are available at www.predimed.es. The trial was stopped after a median follow-up of 4.8 y because of the benefit of the MedDiets on the prevention of major cardiovascular events (myocardial infarction, stroke, or death from cardiovascular disease) compared with the control low-fat group (13). Although the trial was completed in 2010, the ascertainment of the endpoints was extended until June 2012, and the results of the present analysis are based on an extended follow-up that used the same methods as those used during the trial to obtain updated information on diabetes. The study protocol was approved by the institutional review boards of the participating centers (ISRCTN35739639).

Study population. From the 7447 randomly assigned PREDIMED participants, we excluded 3614 who reported diabetes (including types 1 and 2) at baseline. We also excluded 371 participants who did not complete the FFQs at baseline, and 27 who had an extreme energy intake (i.e., energy intake <500 or >3500 kcal/d for women and <800 or >4000 kcal/d for men) (14). When some dietary data were missing, the imputed values were the mean between the previous and the following available FFQs. The percentages of missing dietary data in different years were as follows: 8% (year 1), 13% (year 2), 7% (year 3), 4% (year 4), 2% (year 5), and 0% (year 6). Finally, we also excluded 5 participants for whom the PREDIMED Clinical Event Ascertainment Committee had not confirmed the diagnosis of diabetes. After exclusions, data from 3430 participants were available for this analysis.

Assessment of diet, polyphenol intake, and lifestyle. At baseline and yearly, participants filled out the following validated questionnaires: a 137-item semiquantitative FFQ (15), a 14-point score questionnaire on adherence to the traditional MedDiet (16), and the Spanish version of the Minnesota Leisure Time Physical Activity Questionnaire (17). In addition, participants filled out a general questionnaire to provide data on lifestyle habits, concurrent diseases, and medication use.

Total energy and nutrient intake were calculated on the basis of Spanish food composition tables (18). Individual polyphenol intake was calculated by multiplying the content of each polyphenol in a particular food item (milligrams per gram) by the consumption of this food item (grams per day) and then summing the product across all food items. We obtained the polyphenol content of foods with the use of the Phenol-Explorer database (www.phenol-explorer.eu). The correspondence between food items in the FFQ and the database was previously described (19). In previous studies, our group validated the FFQ to assess total polyphenol intake in both clinical

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[3] Supplemental Table 1 is from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.
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(r = 0.48, P < 0.01) and cross-sectional (r = 0.26, P = 0.04) studies (20, 21).

Assessment of diabetes. For the present analysis, the main endpoint was incidence of type 2 diabetes diagnosed according to the American Diabetes Association criteria (22), namely, fasting plasma glucose concentrations of ≥7.0 mmol/L (≥126.1 mg/dL) or 2-h plasma glucose concentrations of ≥11.1 mmol/L (≥200.0 mg/dL) after an oral dose of 75 g glucose. A review of all medical records of participants was completed yearly in each center by physician investigators who were blinded to the intervention. When new-onset diabetes cases were identified on the basis of a medical diagnosis reported in the medical charts or by a glucose test during routine biochemical analyses (done ≥1 time/y), these reports were sent to the PREDIMED Clinical Events Ascertainment Committee, whose members also were blinded to treatment allocation. Only when a second test that used the same criteria and repeated within the next 3 mo was available and confirmed the new diabetes case was the endpoint definitively confirmed by the adjudication committee (23).

Statistical analysis. Baseline characteristics are presented as means ± SDs for continuous variables and frequencies (and percentages) for categorical variables across tertiles of total polyphenol intake at baseline adjusted for energy intake (with the use of the residual method) (24). Differences between tertiles were tested by a 1-factor ANOVA test for continuous variables and by the chi-square test for categorical variables.

We calculated person-years of follow-up for each individual from the date of inclusion to the date of diagnosis of type 2 diabetes, death, or end of the follow-up, whichever came first. We used time-dependent Cox proportional hazards regression models with updated diet and covariate information to estimate the HRs for polyphenol intake in relation to type 2 diabetes risk, while using the lowest tertiles of intake as the reference group. Tertiles were used to avoid assumptions about linearity and also to reduce the effect of potential outliers. Then, the median intake value and the 25th and 75th percentiles, the number of cases, and the median years of follow-up (with the 25th and 75th percentiles) were assigned to each tertile. A test for linear trend was performed with the use of the resulting variable as a continuous one.

Total polyphenols and subclasses were previously adjusted for total energy intake with the use of the residual method (24). To assess long-term polyphenol intake and reduce within-person variation, we also calculated the weighted cumulative mean of polyphenol intake at each yearly visit, i.e., polyphenol intake for a given year was the mean between the intake for that year and the mean of the previous years. Nondietary covariates such as smoking, BMI, physical activity, and medication use, as well as dietary covariates, were updated yearly.

In multivariable models, we adjusted for age (<60, 60–64.9, 65–69.9, 70–74.9, and ≥75 y), BMI (continuous), smoking status (never, current, or former), physical activity (continuous), education (primary education, secondary education, or academic/graduate), fasting blood glucose concentrations at baseline (continuous), prevalence of dyslipidemia (yes/no) and hypertension (yes/no), alcohol consumption (continuous in grams per day, adding a quadratic term), energy intake (continuous), and adherence to the traditional MedDiet (14-point score). We also stratified for sex, recruitment center, and intervention group in all models. Stratification allows for the assessment of modifying effects, as well as controlling for confounding factors. The stratums were then pooled by the software (SAS) to give an overall estimate of the RR adjusted for other potential confounders.

We conducted additional stratified analyses for sex, age, alcohol intake, smoking, physical activity, intervention group, and fasting glucose concentrations at baseline to evaluate potential effect modification. We present the HRs and 95% CIs for each risk factor category, comparing the third tertile with the first tertile and using the fully-adjusted model, taking out the risk factor that we were evaluating. We also included the number of cases and median years of follow-up for each category. To test for linearity, we used the median intake in each tertile as a continuous variable. To test for statistical interactions, we also added to the model interaction terms between total polyphenol intake and each of these factors.

All statistical analyses were conducted with the use of SAS software, version 9. All t tests were 2-sided and P values below 0.05 were considered to be significant.

Results

The present study was conducted on 3430 subjects: 1314 men aged 65.2 ± 6.3 y and 2116 women aged 67.5 ± 5.6 y. We present baseline characteristics by tertiles of baseline total polyphenol intake in **Table 1**. At baseline, participants in the third tertile were more likely to be men (P < 0.001), younger (P < 0.001), and current (P < 0.001) or former smokers (P = 0.03); have a lower BMI (in kg/m^2; P = 0.02); and be more physically active (P < 0.001). They also had a higher adherence to the traditional MedDiet (P < 0.001) and tended to consume foods with a high polyphenol content, such as fruits and vegetables, nuts, coffee, and wine (P < 0.001). Those with a lower intake of polyphenols had a lower education level (P < 0.001), were more hypertensive (P = 0.003), and had a higher waist-to-height ratio (P < 0.001).

During a median of 5.5 ± 2.0 y of follow-up (18,900 person-years), a total of 314 incident cases of diabetes were diagnosed (9.1%). The Cox proportional HRs for type 2 diabetes according to tertiles of cumulative intake of total polyphenols (adjusted for calories) and the main polyphenol groups are shown in **Table 2**. After adjustment for anthropometric, sociodemographic, lifestyle, and dietary variables (fully adjusted model) and stratifying by sex, recruitment center, and intervention group, significant and linear inverse associations were found for total polyphenols (HR: 0.72; 95% CI: 0.52, 0.99; P-trend = 0.05), total flavonoids (HR: 0.67; 95% CI: 0.48, 0.93; P-trend = 0.02), and stilbenes (HR: 0.57; 95% CI: 0.38, 0.84; P-trend = 0.003), whereas nonsignificant results were found for other polyphenol groups.

The HRs for new-onset type 2 diabetes and tertiles of cumulative flavonoid class intake are shown in **Table 3**. Dihydroflavonols and flavanones were significantly associated with the risk of type 2 diabetes in the fully adjusted model when comparing the third with the first tertile (HR: 0.59; 95% CI: 0.40, 0.88; P-trend = 0.003; and HR: 0.69; 95% CI: 0.49, 0.97; P-trend = 0.03, respectively). Nevertheless, it is worth mentioning that, for catechins, the middle tertile was significantly associated with the risk of type 2 diabetes compared with the first tertile, even in the fully adjusted model (HR: 0.61; 95% CI: 0.44, 0.85). This association was not observed for the group with the highest intake (HR: 0.84; 95% CI: 0.60, 1.17; P-trend = 0.45).

There were substantial changes from model 2 to model 3; for instance, HRs for stilbenes changed from 0.84 to 0.57, and for dihydroflavonols, from 0.87 to 0.59, when comparing the third to the first tertile, and flavonols changed from 0.93 to 0.77 when comparing the second to the first tertile. This was due to the inclusion of both alcohol and fasting glucose concentrations at baseline in the model.

The HRs and 95% CIs of diabetes risk, comparing the highest with the lowest tertile of intake of total polyphenols and subclasses after adjustment for all potential confounders, are shown in **Figure 1**.

We also conducted stratified analyses by different predictors of diabetes (results shown in **Table 4**) and total polyphenol intake. None of the stratified results had a significant interaction term; therefore, we cannot draw conclusions.

TABLE 1 Baseline characteristics of the PREDIMED study cohort according to tertiles of calorie-adjusted total polyphenol intake at baseline[1]

Characteristics	T1 (n = 1143)	T2 (n = 1144)	T3 (n = 1143)	P^2
Polyphenol intake (cutoff values), mg/d	554 ± 103 (<701)	805 ± 63 (701–914)	1131 ± 203 (>914)	
Female	722 (63.2)[b]	778 (68.0)[b]	616 (53.9)[a]	<0.001
Age, y	66.9 ± 6.1[a]	67.1 ± 5.9[a]	66.0 ± 5.9[b]	<0.001
BMI, kg/m^2	30.2 ± 3.5[a]	29.8 ± 3.4[b]	29.8 ± 3.5[b]	0.02
Leisure-time physical activity, MET-min/d	211 ± 214[b]	223 ± 205[b]	263 ± 248[a]	<0.001
Smoking				<0.001
Never	746 (65.3)[b]	774 (67.7)[b]	613 (53.6)[a]	
Current	196 (17.1)[b]	174 (15.2)[b]	283 (24.8)[a]	
Former	201 (17.6)[b]	196 (17.1)[b]	247 (21.6)[a]	
Education				<0.001
Primary	906 (79.3)[b]	885 (77.3)[b]	802 (70.2)[a]	
Secondary	174 (15.2)	179 (15.7)	201 (17.6)	
Academic/graduate	63 (5.5)[b]	80 (7.0)[b]	104 (12.2)[a]	
Intervention group				<0.001
MedDiet–EVOO	384 (33.6)	380 (33.2)	364 (31.9)	
MedDiet–nuts	374 (32.7)[a]	387 (33.8)[a,b]	437 (38.2)[b]	
Low-fat diet (control group)	385 (33.7)	377 (33.0)	342 (29.9)	
Drug use				
Hypolipidemic	539 (47.2)[a]	591 (51.7)[b]	579 (50.9)[a,b]	0.07
Antihypertensive	907 (79.5)[a]	878 (76.8)[a,b]	863 (75.8)[b]	0.09
Aspirin	174 (15.3)	211 (18.4)	201 (17.7)	0.11
Multivitamins	152 (13.3)	165 (14.5)	142 (12.5)	0.36
Mean intake				
Total energy intake, kcal/d	2372 ± 621[a]	2196 ± 513[b]	2310 ± 558[c]	<0.001
Carbohydrates, g/d	258 ± 87[a]	235 ± 71[b]	248 ± 75[c]	<0.001
Protein, g/d	94.5 ± 22.4[a]	89.3 ± 19.0[b]	91.0 ± 20.7[c]	<0.001
SFAs, g/d	26.6 ± 9.7[a]	23.9 ± 7.7[b]	23.9 ± 8.7[b]	<0.001
MUFAs, g/d	49.9 ± 15.4[a]	46.6 ± 13.6[b]	47.3 ± 14.4[b]	<0.001
PUFAs, g/d	16.2 ± 6.7[a]	14.9 ± 6.0[b]	15.3 ± 6.3[b]	<0.001
Fiber, g/d	23.2 ± 7.6[a]	24.7 ± 7.7[b]	28.7 ± 9.7[c]	<0.001
Total cholesterol, mg/d	378 ± 135[a]	357 ± 117[b]	352 ± 112[b]	<0.001
Alcohol, g/d	6.2 ± 10.9[a]	7.4 ± 12.2[b]	14.4 ± 19.3[c]	<0.001
Vegetables, g/d	303 ± 125[a]	322 ± 132[b]	359 ± 152[c]	<0.001
Fruits, g/d	279 ± 152[a]	359 ± 170[b]	480 ± 227[c]	<0.001
Legumes, g/d	20.8 ± 15.7	20.3 ± 11.3	20.5 ± 12.3	0.63
Cereals, g/d	256 ± 117[a]	220 ± 95[b]	217 ± 94[b]	<0.001
Dairy products, g/d	389 ± 228[b]	371 ± 212[b]	350 ± 220[a]	<0.001
Meat or meat products, g/d	139 ± 61[a]	129 ± 51[b]	129 ± 52[b]	<0.001
Fish, g/d	96.0 ± 44.5[b]	96.3 ± 45.3[b]	101 ± 45.5[a]	0.01
Sugar-sweetened soft drinks, g/d	28.9 ± 88.9[a]	21.1 ± 69.4[b]	17.9 ± 60.1[b]	0.001
Nuts, g/d	9.7 ± 13.1[b]	8.7 ± 1.9[b]	11.4 ± 14.0[a]	0.003
Coffee, mL/d	45.2 ± 42.6[a]	65.8 ± 44.9[b]	90.8 ± 58.8[c]	<0.001
Tea, mL/d	4.9 ± 20.1	5.2 ± 18.0	6.5 ± 25.2	0.17
Wine, mL/d	36.5 ± 74.0[a]	52.3 ± 94.2[b]	110.3 ± 154.0[c]	<0.001
14-point MedDiet score	8.24 ± 1.89[a]	8.67 ± 1.93[b]	9.08 ± 1.84[c]	<0.001
Clinical variables				
Hypertension	1076 (94.1)[a]	1049 (91.7)[b]	1034 (90.5)[b]	<0.001
Hypercholesterolemia	925 (80.9)[a]	999 (87.3)[b]	991 (86.7)[b]	<0.001
Waist-to-height ratio	0.63 ± 0.06[a]	0.62 ± 0.06[b]	0.62 ± 0.06[b]	<0.001
Systolic BP, mm Hg	149 ± 19[a]	148 ± 19[b]	148 ± 18[b]	0.02
Diastolic BP, mm Hg	84 ± 10	84 ± 10	84 ± 10	0.41
Glucose,[3] mg/dL	98 ± 15	98 ± 16	99 ± 16	0.57
Total cholesterol,[3] mg/dL	210 ± 37	214 ± 39	214 ± 38	0.09
HDL cholesterol,[3] mg/dL	52 ± 12	53 ± 11	53 ± 11	0.05
LDL cholesterol,[3] mg/dL	139 ± 34	140 ± 33	140 ± 34	0.83
TGs,[3] mg/dL	128 ± 73	129 ± 71	129 ± 63	0.94

[1] Values are frequencies (percentages) for categorical variables or means ± SDs for continuous variables; n = 3430. Values in a row without a common superscript letter are significantly different, $P < 0.05$. BP, blood pressure; MedDiet–EVOO, Mediterranean diet supplemented with extra-virgin olive oil; MedDiet–nuts, Mediterranean diet supplemented with nuts; MET, metabolic equivalent task; PREDIMED, Prevención con Dieta Mediterránea, T, tertile.
[2] Calculated by ANOVA or χ^2 tests.
[3] Measured in plasma.

TABLE 2 Cox proportional HRs for new-onset diabetes in the PREDIMED cohort by cumulative intake of polyphenols, adjusted for energy intake and divided into tertiles[1]

	T1	T2	T3	*P*-trend[2]	*P*[3]
Total polyphenols, mg/d	600 (518, 653)	781 (739, 825)	1002 (929, 1119)		
Cases, *n*	117	103	94		
Person-years, *n*	5910	6785	6205		
Follow-up, y	5.6 (4.0, 7.2)	5.4 (4.0, 7.2)	5.3 (3.9, 7.2)		0.70
Incidence, %	10.9	8.4	8.3		0.06
Model 1	1.00 (ref)	0.82 (0.62, 1.07)	0.81 (0.61, 1.08)	0.15	
Model 2	1.00 (ref)	0.78 (0.59, 1.04)	0.74 (0.55, 0.99)	0.04	
Model 3	1.00 (ref)	0.74 (0.54, 1.00)	0.72 (0.52, 0.99)	0.05	
Flavonoids, mg/d	291 (236, 334)	425 (392, 462)	596 (533, 698)		
Cases, *n*	133	91	90		
Person-years, *n*	5659	6685	6556		
Follow-up, y	5.0 (3.9, 7.1)	5.9 (4.0, 7.3)	5.8 (4.0, 7.3)		<0.0001
Incidence, %	12.4	7.7	7.7		<0.0001
Model 1	1.00 (ref)	0.66 (0.50, 0.87)	0.69 (0.52, 0.92)	0.01	
Model 2	1.00 (ref)	0.64 (0.48, 0.85)	0.69 (0.51, 0.93)	0.02	
Model 3	1.00 (ref)	0.60 (0.44, 0.82)	0.67 (0.48, 0.93)	0.02	
Phenolic acids, mg/d	164 (130, 192)	256 (234, 279)	381 (342, 442)		
Cases, *n*	101	109	104		
Person-years, *n*	6577	6555	6767		
Follow-up, y	6.0 (4.1, 7.3)	5.8 (4.0, 7.2)	4.9 (3.8, 7.1)		<0.0001
Incidence, %	8.7	9.3	9.5		0.83
Model 1	1.00 (ref)	1.03 (0.78, 1.36)	1.03 (0.78, 1.37)	0.84	
Model 2	1.00 (ref)	1.06 (0.80, 1.41)	0.96 (0.71, 1.29)	0.73	
Model 3	1.00 (ref)	0.89 (0.65, 1.21)	0.85 (0.62, 1.17)	0.34	
Stilbenes, mg/d	0.04 (0, 0.17)	1.01 (0.73, 1.35)	3.89 (2.77, 6.95)		
Cases, *n*	102	115	97		
Person-years, *n*	3141	6519	6238		
Follow-up, y	5.4 (4.0, 7.3)	5.1 (4.0, 7.1)	6.0 (4.0, 7.3)		0.003
Incidence, %	9.2	9.5	8.8		0.85
Model 1	1.00 (ref)	1.08 (0.82, 1.42)	0.81 (0.60, 1.09)	0.09	
Model 2	1.00 (ref)	1.01 (0.76, 1.34)	0.84 (0.62, 1.14)	0.23	
Model 3	1.00 (ref)	0.90 (0.64, 1.27)	0.57 (0.38, 0.84)	0.003	
Lignans, mg/d	0.42 (0.35, 0.47)	0.59 (0.56, 0.63)	0.78 (0.73, 0.88)		
Cases, *n*	111	96	107		
Person-years, *n*	5401	6345	7153		
Follow-up, y	4.9 (3.6, 7.0)	5.4 (4.0, 7.1)	6.1 (4.4, 7.4)		<0.0001
Incidence, %	10.5	8.3	8.8		0.17
Model 1	1.00 (ref)	0.73 (0.55, 0.97)	0.78 (0.58, 1.05)	0.12	
Model 2	1.00 (ref)	0.68 (0.51, 0.92)	0.75 (0.55, 1.01)	0.08	
Model 3	1.00 (ref)	0.68 (0.48, 0.94)	0.82 (0.58, 1.15)	0.35	
Others,[4] mg/d	41.3 (32.8, 47.3)	63.3 (58.1, 69.5)	96.5 (85.3, 115.0)		
Cases, *n*	90	113	111		
Person-years, *n*	5701	7143	6055		
Follow-up, y	5.1 (3.9, 7.2)	5.9 (4.0, 7.3)	5.4 (4.0, 7.2)		0.004
Incidence, %	8.5	8.9	10.1		0.43
Model 1	1.00 (ref)	0.97 (0.72, 1.29)	1.08 (0.81, 1.45)	0.51	
Model 2	1.00 (ref)	0.95 (0.71, 1.28)	1.06 (0.79, 1.44)	0.60	
Model 3	1.00 (ref)	0.98 (0.71, 1.36)	0.97 (0.70, 1.36)	0.89	

[1] Values are HRs (95% CIs), unless otherwise indicated. Polyphenol intake and follow-up values are medians (25th, 75th percentiles). Model 1 is adjusted for age and stratified by sex, recruitment center, and intervention group. Model 2 is adjusted for factors in model 1, in addition to smoking, BMI, physical activity, dyslipidemia, hypertension, and education level. Model 3 is adjusted for factors in model 2, in addition to total energy intake, alcohol intake, adherence to the Mediterranean diet, and fasting glucose concentrations at baseline. PREDIMED, Prevención con Dieta Mediterránea; ref, reference; T, tertile.

[2] Based on tests for trend across tertiles of polyphenol intake by assigning the median value of each tertile.

[3] Calculated by ANOVA (continuous variables) or χ^2 tests (categorical variables).

[4] Includes alkylmethoxyphenols, alkylphenols, curcuminoids, furanocoumarins, hydroxybenzaldehydes, hydroxybenzoketones, hydroxycinnamaldehydes, hydroxycoumarins, hydroxyphenylpropenes, methoxyphenols, naphtoquinones, phenolic terpenes, and tyrosols.

TABLE 3 Cox proportional HRs for new-onset diabetes in the PREDIMED cohort by cumulative intake of flavonoid classes, adjusted for energy intake and divided into tertiles[1]

	T1	T2	T3	*P*-trend[2]	*P*[3]
Anthocyanidins, mg/d	14.9 (8.6, 19.6)	30.9 (26.9, 35.3)	58.9 (48.3, 77.1)		
Cases, *n*	104	97	113		
Person-years, *n*	6642	6485	5772		
Follow-up, y	5.2 (3.9, 7.2)	5.9 (4.0, 7.3)	5.6 (4.0, 7.2)		0.02
Incidence, %	8.5	8.4	10.7		0.11
Model 1	1.00 (ref)	0.84 (0.63, 1.12)	0.99 (0.75, 1.30)	0.89	
Model 2	1.00 (ref)	0.84 (0.63, 1.13)	0.96 (0.72, 1.27)	0.89	
Model 3	1.00 (ref)	0.82 (0.59, 1.13)	0.88 (0.62, 1.24)	0.54	
Catechins, mg/d	13.8 (10.5, 16.4)	23.2 (20.8, 26.2)	39.4 (33.7, 48.2)		
Cases, *n*	125	83	106		
Person-years, *n*	6080	6593	6227		
Follow-up, y	5.4 (4.0, 7.3)	5.5 (4.0, 7.2)	5.4 (4.0, 7.2)		0.35
Incidence, %	11.3	7.0	9.3		0.002
Model 1	1.00 (ref)	0.64 (0.48, 0.85)	0.85 (0.64, 1.11)	0.37	
Model 2	1.00 (ref)	0.64 (0.47, 0.85)	0.84 (0.63, 1.11)	0.35	
Model 3	1.00 (ref)	0.61 (0.44, 0.85)	0.84 (0.60, 1.17)	0.45	
Dihydrochalcones, mg/d	0.99 (0.41, 1.38)	2.40 (2.00, 2.77)	3.96 (3.48, 6.18)		
Cases, *n*	117	97	100		
Person-years, *n*	5477	6906	6516		
Follow-up, y	5.1 (3.9, 7.1)	6.0 (4.1, 7.4)	5.3 (3.9, 7.2)		<0.0001
Incidence, %	11.3	8.1	8.4		0.22
Model 1	1.00 (ref)	0.77 (0.58, 1.01)	1.00 (0.75, 1.01)	0.99	
Model 2	1.00 (ref)	0.79 (0.60, 1.06)	1.00 (0.74, 1.35)	0.92	
Model 3	1.00 (ref)	0.88 (0.64, 1.19)	1.15 (0.83, 1.61)	0.44	
Dihydroflavonols, mg/d	0 (0, 0.16)	1.48 (1.03, 2.03)	6.09 (4.31, 11.0)		
Cases, *n*	100	117	97		
Person-years, *n*	6132	6546	6220		
Follow-up, y	5.6 (4.0, 7.3)	5.1 (3.9, 7.1)	6.0 (4.0, 7.3)		0.002
Incidence, %	9.0	9.6	8.8		0.8
Model 1	1.00 (ref)	1.13 (0.86, 1.49)	0.83 (0.62, 1.12)	0.12	
Model 2	1.00 (ref)	1.05 (0.79, 1.39)	0.87 (0.64, 1.17)	0.27	
Model 3	1.00 (ref)	0.99 (0.70, 1.38)	0.59 (0.40, 0.88)	0.003	
Proanthocyanidins, mg/d	74.5 (54.3, 87.4)	122 (111, 134)	187 (164, 228)		
Cases, *n*	122	102	90		
Person-years, *n*	5778	6831	6290		
Follow-up, y	5.1 (3.9, 7.2)	5.8 (4.0, 7.3)	5.6 (4.0, 7.2)		0.003
Incidence, %	11.3	8.4	7.9		0.01
Model 1	1.00 (ref)	0.78 (0.59, 1.02)	0.73 (0.55, 0.97)	0.04	
Model 2	1.00 (ref)	0.80 (0.61, 1.06)	0.70 (0.52, 0.95)	0.02	
Model 3	1.00 (ref)	0.75 (0.55, 1.02)	0.75 (0.54, 1.04)	0.09	
Flavanones, mg/d	43.4 (15.9, 63.8)	114 (96.4, 132)	197 (166, 292)		
Cases, *n*	121	105	88		
Person-years, *n*	5206	6670	7024		
Follow-up, y	5.1 (3.9, 7.1)	6.0 (4.1, 7.3)	5.6 (3.9, 7.3)		<0.0001
Incidence, %	12.3	8.9	7.0		<0.0001
Model 1	1.00 (ref)	0.77 (0.59, 1.01)	0.73 (0.55, 0.98)	0.04	
Model 2	1.00 (ref)	0.81 (0.61, 1.07)	0.74 (0.54, 1.00)	0.05	
Model 3	1.00 (ref)	0.87 (0.65, 1.17)	0.69 (0.49, 0.97)	0.03	
Flavones, mg/d	21.5 (16.6, 25.1)	34.7 (31.5, 38.0)	56.8 (48.4, 71.2)		
Cases, *n*	116	94	104		
Person-years, *n*	5328	6474	7098		
Follow-up, y	5.1 (3.9, 7.1)	5.9 (4.0, 7.2)	5.8 (4.0, 7.3)		<0.0001
Incidence, %	11.5	8.1	8.3		0.009
Model 1	1.00 (ref)	0.78 (0.59, 1.03)	0.87 (0.66, 1.15)	0.46	
Model 2	1.00 (ref)	0.79 (0.49, 1.06)	0.87 (0.65, 1.17)	0.46	
Model 3	1.00 (ref)	0.78 (0.56, 1.06)	0.98 (0.71, 1.35)	0.92	

(Continued)

TABLE 3 *Continued*

	T1	T2	T3	*P*-trend[2]	*P*[3]
Flavonols, mg/d	57.5 (46.8, 64.5)	82.9 (76.9, 87.9)	106 (99.1, 122)		
Cases, *n*	114	100	100		
Person-years, *n*	6242	6279	6378		
Follow-up, y	5.0 (3.9, 7.0)	5.7 (4.0, 7.3)	6.0 (4.2, 7.4)		<0.0001
Incidence, %	9.6	8.9	9.0		0.84
Model 1	1.00 (ref)	0.94 (0.71, 1.25)	1.03 (0.75, 1.42)	0.88	
Model 2	1.00 (ref)	0.93 (0.69, 1.24)	1.04 (0.75, 1.45)	0.96	
Model 3	1.00 (ref)	0.77 (0.56, 1.06)	0.97 (0.68, 1.39)	0.77	

[1] Values are HRs (95% CIs), unless otherwise indicated. Polyphenol intake and follow-up values are medians (25th, 75th percentiles). Model 1 is adjusted for age and stratified by sex, recruitment center, and intervention group. Model 2 is adjusted for factors in model 1, in addition to smoking, BMI, physical activity, dyslipidemia, hypertension, and education level. Model 3 is adjusted for factors in model 2, in addition to total energy intake, alcohol intake, adherence to the Mediterranean diet, and fasting glucose concentrations at baseline. PREDIMED, Prevención con Dieta Mediterránea; ref, reference; T, tertile.
[2] Based on tests for trend across tertiles of polyphenol intake by assigning the median value of each tertile.
[3] Calculated by ANOVA (continuous variables) or χ^2 tests (categorical variables).

When we removed fasting glucose concentrations at baseline from the model (**Supplemental Table 1**), some associations were no longer significant (dihydroflavonols and flavanones), whereas others became significant (proanthocyanidins and lignans).

Discussion

In this observational and longitudinal study within the PREDIMED trial, we found that a higher intake of total polyphenols, total flavonoids, stilbenes, and some flavonoid subclasses (dihydroflavonols and flavanones) was inversely and linearly associated with incidence of type 2 diabetes. Even though previous studies have investigated the association between the intake of specific groups of polyphenols and type 2 diabetes, to our knowledge, this is the first study that comprehensively quantified the association between the intake of all polyphenol subgroups and the risk of type 2 diabetes.

Some of our results agree with previous studies, whereas others are contradictory or cannot be compared because of a lack of previously reported data. In 1983, Thompson et al. (25) found for the first time, to our knowledge, an inverse correlation between the glycemic index of the diet and total intake of polyphenols in both healthy and diabetic individuals, especially the large polymeric type or condensed tannins. Other epidemiologic studies that mainly focused on lignans, flavanols, flavonols, anthocyanins and individual polyphenols have associated these polyphenols with a lower incidence of diabetes (3–5).

Several mechanisms have been invoked to explain the inverse associations between polyphenol consumption and diabetes incidence. Long-term cellular inflammation plays an important role in the metabolic consequences of diabetes and other chronic diseases. Indeed, classic cases of hormonal disruption are insulin resistance, which causes hyperinsulinemia, or the β cell burnout in type 2 diabetes that results in chronic hyperglycemia (26). Some polyphenols can inhibit cellular inflammation through the activation of PPARγ and AMPK (Adenosine Monophosphate-activated Protein Kinase), an upstream activator of the anti-inflammatory gene transcription factors SIRT1 (Sirtuin 1) and FOX (Forkhead box) (26, 27). Two flavanones, naringin and hesperidin, showed antidiabetic properties partially mediated through the regulation of PPARγ (6). In a prospective study conducted in 2 cohorts of US women, urinary excretion of hesperetin, another flavanone, was associated with a decreased risk of type 2 diabetes. Other polyphenol metabolites, including naringenin, quercetin, isorhamnetin, and caffeic acid, were associated only during the early follow-up period (28). Consistent with these results, the intake of flavanones in our population was inversely associated with diabetes risk in the fully adjusted model. It is important to note that 99% of flavanone intake came from the consumption of oranges and orange juice (19).

Previous results from animal and cell-cultured studies have shown that flavan-3-ols, especially epigallocatechin gallate, which belongs to the family of catechins, have antidiabetic effects. According to these studies, epigallocatechin gallate acts through multiple signaling pathways, leading to improvements in insulin secretion, glucose uptake, insulin resistance, glucose tolerance, oxidative stress, inflammation, and mitochondrial function (6). A substantial reduction in estimated peripheral insulin resistance and an improvement in insulin sensitivity have also been demonstrated after the consumption of flavonoid-enriched chocolate (containing flavan-3-ols and isoflavones) in individuals with type 2 diabetes (29). In our study, catechins also were significantly associated with a decreased risk of type 2 diabetes when comparing the second to the first tertile. However,

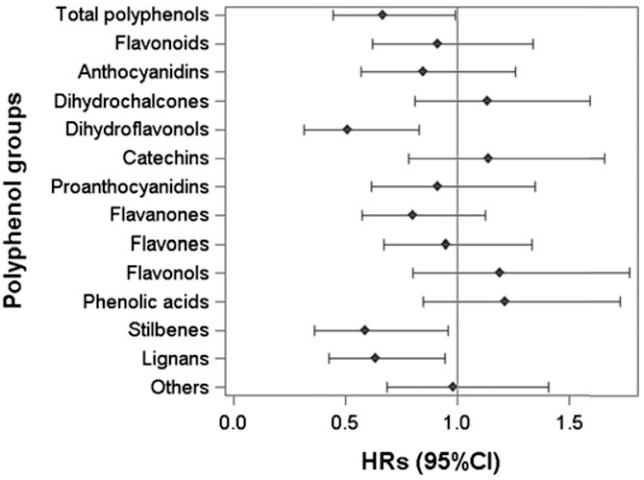

FIGURE 1 HRs (95% CIs) of diabetes incidence for the highest compared with the lowest tertile of polyphenol intake (fully adjusted model) in the Prevención con Dieta Mediterránea study cohort (*n* = 3430).

TABLE 4 Cox proportional HRs for new-onset diabetes in the PREDIMED cohort by cumulative intake of total polyphenols, adjusted for energy intake and stratified by risk factors[1]

Risk factors	Cases, *n*	Person-years	Follow-up,[2] y	T3 vs. T1 multivariable-adjusted HR (95% CI)[3]	*P*-trend[4]	*P*-interaction
Sex						0.96
M	140	7169	5.4 (3.9, 7.3)	0.73 (0.44, 1.19)	0.18	
F	174	11,731	5.5 (4.0, 7.2)	0.77 (0.49, 1.20)	0.22	
Age, y						0.30
<65	154	8608	5.1 (3.9, 7.2)	0.79 (0.48, 1.29)	0.30	
≥65	160	10,292	5.9 (4.0, 7.3)	0.69 (0.43, 1.09)	0.09	
Alcohol intake						0.11
Nondrinkers[5]	123	7117	5.3 (4.0, 7.3)	0.55 (0.30, 0.88)	0.04	
Drinkers	191	11,783	5.6 (4.0, 7.2)	0.89 (0.60, 1.34)	0.59	
Smoking						0.16
Never	173	12,190	5.8 (4.0, 7.3)	0.59 (0.37, 0.94)	0.03	
Former	63	3857	5.1 (3.9, 7.2)	0.97 (0.46, 2.04)	0.94	
Current	78	2853	5.2 (3.8, 7.2)	1.40 (0.69, 2.88)	0.35	
Physical activity						0.25
<median	200	11,940	5.1 (3.9, 7.1)	0.80 (0.49, 1.30)	0.82	
≥median	114	6945	6.0 (4.1, 7.4)	0.47 (0.28, 0.79)	0.006	
Intervention group						0.74
MedDiet–EVOO	91	6435	6.0 (4.2, 7.3)	0.84 (0.45, 1.55)	0.57	
MedDiet–nuts	107	6497	5.2 (3.8, 7.2)	0.77 (0.41, 1.43)	0.38	
Low-fat diet	116	5968	5.2 (3.9, 7.3)	0.83 (0.49, 1.41)	0.48	
Fasting glucose concentrations at baseline, mg/dL						0.54
≤100	78	13,109	6.0 (4.2, 7.3)	0.87 (0.45, 1.68)	0.67	
>100	236	5791	4.9 (3.6, 7.1)	0.63 (0.44, 0.91)	0.02	

[1] MedDiet–EVOO, Mediterranean diet supplemented with extra-virgin olive oil; MedDiet–nuts, Mediterranean diet supplemented with nuts; PREDIMED, Prevención con Dieta Mediterránea; T, tertile.
[2] Medians (25th, 75th percentiles).
[3] Analyses were stratified by sex, recruitment center, and intervention group and adjusted for age, smoking, BMI, physical activity, dyslipidemia, hypertension, education level, fasting glucose concentrations at baseline, total energy intake, alcohol intake, and adherence to the Mediterranean diet.
[4] Highest compared with lowest groups with the use of continuous variables.
[5] Alcohol intake of 0 g/d.

proanthocyanidins, which are polymers of the flavan-3-ols found in grapes, red wine, apples, berries, chocolate, seeds, and legumes, were only inversely associated with diabetes risk when glucose concentrations were taken out of the model.

Similar results were found for anthocyanidins. These colorful compounds traditionally have received special attention because of their antioxidant capacity. Although their bioavailability seems to be low compared with other flavonoids, results from human and animal trials have shown that anthocyanidins improve glucose homeostasis through different mechanisms (5, 6).

In a cross-sectional study conducted by Jennings et al. (30), the intake of different flavonoids was calculated with the use of FFQs and the USDA database. After adjusting for potential confounders, higher anthocyanin and flavone intake and consumption of anthocyanin-rich food were associated with improvements in insulin resistance and high-sensitivity C-reactive protein. Flavones also increased adiponectin secretion, as described by Liu et al. (31).

There is a lack of consensus on the antidiabetic properties of flavonols, the most consumed flavonoids, and flavones. We did not find any relation between flavonols or flavones and diabetes in our study, and neither did Kataja-Tuomola et al. (32) in a large cohort of male smokers aged 50–69 y. On the contrary, flavonols and myricein were significantly associated with a lower risk of developing type 2 diabetes in participants of the European Prospective Study into Cancer and Nutrition study (3, 33). Flavonol intake was also associated with a lower incidence of type 2 diabetes in participants of the Framingham Heart Study Offspring Cohort (34). Moreover, some animal and cell-culture studies support the hypoglycemic effects of quercetin (7, 8).

Despite having a similar structure, dihydroflavonols and flavonols have different solubilities and antioxidant capacities and, therefore, different bioavailability and properties (35). We found a strong inverse association between dihydroflavonols and diabetes, which has been previously demonstrated in animal and in vitro models (9, 10).

Stilbenes, a group of polyphenols that includes the well-known resveratrol, were also strongly and inversely associated with type 2 diabetes in this PREDIMED cohort. The antidiabetic effects of resveratrol evidenced from in vitro and clinical studies have multiple mechanisms: improving insulin sensitivity, enhancing GLUT4 (Glucose transporter type 4) translocation, reducing oxidative stress, regulating carbohydrate metabolizing enzymes, activating SIRT1 and AMPK, and decreasing adipogenic genes (36, 37).

It is noteworthy that the main source of dihydroflavonols and stilbenes in the PREDIMED population was red wine (19). Indeed, Nettleton et al. (38) previously found that women who reported drinking red wine >1 time/wk had a 16% reduced risk of diabetes compared with those drinking wine less often. Nevertheless, these authors could not find any association for total flavonoid intake in this cohort of postmenopausal women. In a crossover trial with men at high risk of cardiovascular

disease, plasma insulin and HOMA-IR decreased after consumption of red wine and dealcoholized red wine, but not gin, suggesting that the nonalcoholic fraction of red wine (mainly polyphenols) was responsible for the effect (39).

Finally, our results suggest an inverse association between lignan intake and diabetes incidence, but only when glucose concentrations at baseline were not added to the model. Enterodiol and enterolactone, which are gut microbiota metabolites of dietary lignans, were associated with a lower type 2 diabetes incidence in US women after multivariable adjustment (4). In the European Prospective Study into Cancer and Nutrition study, however, intake of lignans was not associated with type 2 diabetes (33). The main sources of lignans in our population were olive oil and whole-grain wheat bread (19). These results agree with those from a randomized, placebo-controlled crossover trial in middle-aged overweight men who received capsules with olive-leaf extract or a placebo for 12 wk. The supplementation improved insulin sensitivity and pancreatic β cell secretory capacity (40).

Results from stratified analyses suggested that the association between total polyphenol intake and diabetes could be affected by alcohol intake and smoking. Those who did not drink alcohol and had never smoked appeared to have higher inverse associations. Indeed, an increasing number of epidemiologic studies show that the relation between the risk of the onset of type 2 diabetes and alcohol intake is *U*-shaped. Heavy alcohol consumption is related to obesity and impaired liver function, both of which are associated with an increased risk of diabetes, and active smoking is also positively associated with diabetes in a dose-dependent manner (41).

Foods high in polyphenols may provide multiple other beneficial food components, such as fiber, unsaturated FAs or magnesium (42), which have been associated with a decreased risk of type 2 diabetes. For instance, red wine was the main source of stilbenes and dihydroflavonols in the PREDIMED, but red wine also contains alcohol, and moderate wine or alcohol consumption was inversely associated with diabetes risk in an intervention (43) and 2 meta-analysis (44, 45). Muraki et al. (46) studied whether individual fruits were differentially associated with risk of type 2 diabetes in 3 prospective longitudinal cohort studies, and found that blueberries, grapes, and apples were inversely associated with type 2 diabetes. Grapes and blueberries are a good source of stilbenes, and apples were the third contributor to total polyphenol intake in the PREDIMED cohort (19). Olive oil is the main source of fat in the MedDiet, and it is rich in MUFAs and polyphenols (lignans, flavones, and other classes of polyphenols). It has been demonstrated that extra-virgin olive oil in the framework of a MedDiet can reduce the risk of diabetes in persons with a high risk of cardiovascular disease (23). Diets that are rich in whole grains and fiber can also decrease the risk of diabetes, according to different studies (47–49).

The main strengths of this study include the prospective design, a relatively large sample size, blinded assessment of the endpoints, and comprehensive information about risk factors and confounders for diabetes. Moreover, the use of a weighted cumulative mean of polyphenol intake, calculated with validated FFQs, is the best approach for reducing measurement error in nutritional epidemiology (50), and allowed us to control changes in the diet from the intervention. Finally, we also used the most comprehensive database currently available: the Phenol-Explorer database.

The study also has some limitations. First, this is an observational study within an intervention trial. Because polyphenol-rich foods, mainly extra-virgin olive oil and nuts, were recommended in both MedDiet intervention groups but not in the control group, the dietary advice could affect the accuracy of reporting the consumption of polyphenol-rich foods, mainly by over-reporting some of them. However, the distribution of intervention groups between tertiles was uniform ($P > 0.05$), because the foods that primarily contributed to polyphenol intake in the PREDIMED study were fruits, vegetables, and coffee, and there were no statistically significant differences in the intake of these foods after 5 y of follow-up. Moreover, even though we controlled for several confounders in multivariable models, other unknown or unmeasured confounders could exist. Other limitations refer to the estimation of polyphenol intake. Data were indirectly derived from FFQs; therefore, the bioavailability of the molecules was not taken into account. Moreover, intake could have been underestimated because not all foods from the FFQs were available in the database (e.g., honey) and not all polyphenol-rich foods were recorded in the FFQs (e.g., spices and seasonings). We also should mention that the Phenol-Explorer database contains data on foods from various countries that may lead to a misclassification of Spanish foods. We also did not take into account other factors that modify polyphenol content in foods, including ripeness, environmental factors, processing and storage, and variety (51). Because the intake of theaflavins and isoflavones was very low in our population, we could not study the associations for these polyphenol groups. Finally, these results might not be generalizable to other populations other than middle-aged to elderly people at high risk of cardiovascular disease.

In sum, our data suggest an inverse association between the intake of total polyphenols, total flavonoids (specifically dihydroflavonols and flavanones), and stilbenes and the risk of type 2 diabetes in an elderly Mediterranean population at high risk of cardiovascular disease. These associations were independent of other dietary and nondietary risk factors. Our findings could be the starting point of future randomized, controlled trials to clarify the promising benefits deriving from long-term consumption of polyphenol-rich diets, and to establish dietary recommendations.

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