Egg consumption and cardiovascular disease according to diabetic status: the PREDIMED study

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Short running head:

Eggs and cardiovascular disease in diabetes

Abreviatures

FFQ**:** food-frequency questionnaire (FFQ)

CVD: Cardiovascular Disease (CVD)

PREDIMED: PREvención con DIeta MEDiterránea

HDL: High-Density Lipoprotein

LDL: Low-Density Lipoprotein

PREDIMED trial was registered at controlled-trials.com as ISRCTN35739639.

ABSTRACT

Background: Eggs are a major source of dietary cholesterol and their consumption has been sometimes discouraged. A relationship between egg consumption and the incidence of cardiovascular disease (CVD) has been suggested to be present exclusively among patients with type-2 diabetes.

Aims: To assess the association between egg consumption and CVD in a large Mediterranean cohort where approximately 50% of participants had type 2 diabetes.

Methods: We prospectively followed 7216 participants (55-80 years old) at high cardiovascular risk from the PREDIMED (PREvención con DIeta MEDiterránea) study for a mean of 5.8 years. All participants were initially free of CVD. Yearly repeated measurements of dietary information with a validated 137-item food-frequency questionnaire were used to assess egg consumption and other dietary exposures. The endpoint was the rate of major cardiovascular events (myocardial infarction, stroke or death from cardiovascular causes).

Results: A major cardiovascular event occurred in 342 participants. Baseline egg consumption was not significantly associated with cardiovascular events in the total population. Non-diabetic participants who ate on average >4 eggs/week had a hazard ratio (HR) of 0.96 (95% confidence interval, 0.33-2.76) in the fully adjusted multivariable model when compared with non-diabetic participants who reported the lowest egg consumption (<2 eggs/week). Among diabetic participants, the HR was 1.33 (0.72-2.46). There was no evidence of interaction by diabetic status. HRs per 500 eggs of cumulative consumption during follow-up were 0.94 (0.66-1.33) in non-diabetics and 1.18 (0.90-1.55) in diabetics.

Conclusions: Low to moderated egg consumption was not associated with an increased CVD risk in diabetic or non-diabetic individuals at high cardiovascular risk. This trial was registered at controlled-trials.com as ISRCTN35739639.

Keywords: Egg consumption, cardiovascular disease, diabetes, PREDIMED study.

Introduction

Eggs are a major source of dietary cholesterol. However, they are also an inexpensive source of unsaturated fat, high-quality protein, folate, and other vitamins and minerals (1,2). Dietary cholesterol contributes modestly to plasma concentration of low-density lipoprotein (LDL) cholesterol (3), an well-known risk factor for cardiovascular disease (CVD) (4). Dietary cholesterol has also been directly associated with a higher risk of CVD (5). Because egg yolk is rich in cholesterol, egg consumption is usually not recommended to subjects with hypercholesterolemia, high cardiovascular risk or established CVD (6,7).

Egg consumption was directly associated with carotid plaque area in a cross-sectional study conducted among Canadian subjects at high cardiovascular risk (8), but not in a cohort of middle-aged Finnish participants without coronary heart disease (9). On the other hand, the association between egg consumption and the risk of CVD is controversial. Li et al (10), found in a meta-analysis that egg consumption was directly associated with CVD. However, in another meta-analysis of prospective cohort studies, Shin et al (11), found that egg consumption was not associated with CVD risk in the general population. In a third meta-analysis of prospective cohort studies, Rong et al (12), reported that higher consumption of eggs was also unassociated with coronary heart disease or stroke. Interestingly, the three meta-analyses consistently found a direct association between egg consumption and CVD in diabetic individuals. However, most studies included in these meta-analyses were from the U.S. and other Western countries, but studies from Mediterranean countries are scarce (13).

The PREDIMED study provides a unique opportunity to assess prospectively the association between egg comsumption and CVD in participants who were at high cardiovascular risk, nearly 50% of whom had type 2 diabetes. This half-and-half distribution of exposure to diabetes allowed us to obtain an ideal setting to ascertain the association between egg consumption and CVD incidence according to diabetic status. We aimed to examine the association between egg consumption and CVD in a high cardiovascular risk cohort stratified by diabetic status.

Methods

Study design and subjects

The current cohort study was conducted within the framework of the PREDIMED study (PREvención con DIeta MEDiterránea). The PREDIMED study is a parallel-group, multicenter, randomized, and controlled field trial. Details of the trial design have been published elsewhere (14,15). The primary aim of the trial was to test the efficacy of two Mediterranean diets (enriched with extra-virgin olive oil or mixed nuts), compared to advice on a control (low-fat) diet, on primary cardiovascular events (16) (www.predimed.es) (registered in controlled-trials: ISRCTN35739639). A total of 7447 participants were enrolled between October 2003 and June 2009 in primary care centers by their family practitioners.

Eligible participants were men aged 55-80 years and women aged 60-80 years who were free of CVD at baseline and had either type 2 diabetes or at least three of the following cardiovascular risk factors: hypertension (blood pressure >140/90 or treatment with antihypertensive medication); elevated LDL cholesterol concentration (≥160mg/dl or lipid lowering therapy); low high-density lipoprotein (HDL) cholesterol concentration (≤ 40mg/dl in men or ≤50 mg/dl in women); obesity or overweight; current smoking; and family history of premature coronary heart disease. All participants provided written informed consent to a protocol approved by Institutional Review Boards of all participating PREDIMED centers at study inception.

We excluded participants with missing baseline dietary information (n=78) or whose caloric intakes were outside of predefined limits (<800 or >4000 kcal/day for men and <500 or >3500 kcal/day for women; n=153). Thus, the final sample analyzed was 7216 participants (48.9% of them with type 2 diabetes).

Clinical and dietary measurements

Baseline dietary intake was ascertained with a 137-item semi-quantitative food-frequency questionnaire (FFQ) with 137 items, validated in an old population with high cardiovascular risk in Spain (17). The FFQ was administered at baseline and yearly during the trial. We used an incremental scale with 9 levels, which ranged from “never or almost never” to “>6 times/day”, to collect information on the frequencies of consumption of food items. Energy and nutrient intakes were computed by using Spanish food composition tables (18).

Participants underwent a baseline interview that included the assessment of cardiovascular risk factors and physician diagnosis of hypertension, diabetes and hypercholesterolemia. At the same time, we collected information about anthropometric, socio-demographic, medical, and lifestyle variables.

Adherence to Mediterranean diet was quantified with a validated 14-point Mediterranean diet score (19). Each question was scored 0 or 1. If the condition was met, 1 point was recorded for the category, but if not, 0 points were recorded. We estimated physical activity using the Minnesota leisure-time physical activity questionnaire (20, 21).

Ascertainment of cardiovascular events and mortality

The primary end point was a composite of myocardial infarction, stroke and death from cardiovascular causes. Four different sources of information were used: repeated contacts with participants, general practitioners who were responsible for the clinical care of the participants, yearly review of medical records, and consultation of the National Death Index. Medical records of deceased participants were requested. The endpoint adjudication committee, whose members were blinded to treatment allocation, examined information about cardiovascular events and mortality. This committee adjudicated the cardiovascular events and the cause of death. Only endpoints that were confirmed by the event adjudication committee and that occurred between October 1st 2003 and June 30th 2012, were included in the analyses.

Statistical analyses

Participants were divided into 3 categories of egg consumption (<2, 2-4 and >4 eggs/week). The residuals method was used to adjust egg consumption for total energy intake (22). We summarized quantitative variables by their mean and standard deviation (SD), and categorical variables using percentages.

We fitted Cox regression models to calculate hazard ratios (HR) and their 95% confidence intervals (CI) between egg consumption and major CVD events. The group with the lowest consumption (<2 eggs/week) was used as the reference category. The entry time was the date at recruitment. The exit time was defined as the date at myocardial infarction, stroke, or death from cardiovascular causes or June 30th 2012, whichever came first.

To minimize any effects of a change in diet, we calculated a cumulative sum of egg consumption by using yearly updated information from repeated FFQs collected at baseline and yearly thereafter up to 8 years of follow-up for each participant. Besides the baseline questionnaire, our participants completed 5.3 yearly food-frequency questionnaires, on average, during follow-up. These data were used to fit Cox regression models to assess the association between the cumulative sum of egg consumptions and the risk of CVD in the subsequent year. First we adjusted only for age (continuous), sex, BMI (continuous), and intervention group and stratified by recruitment center. In a second model, additional adjustments for smoking status (never smoker, quitters, current smoker), physical activity during leisure time (continuous), and educational status (3 categories) were performed. Model 3 was further adjusted for diabetes (yes/no), hypertension (yes/no), hypercholesterolemia (yes/no), and family history of premature coronary heart disease (yes/no). In the fully adjusted model, we additionally adjusted for Mediterranean diet score (continuous), alcohol intake (continuous), and total energy intake (continuous).

We assigned the median of the category to all participants in the category and treating it as a continuous variable to conduct tests of linear trend across increasing categories of egg consumption.

All P values were 2-sided, and significance was set at $p<0.05$. Analyses were performed using STATA software, version 12.0 (Stata Corp, College Station, TX, USA).

Results

A total of 8713 candidates were screened for eligibility, and 7447 participants were randomly assigned to one of the three intervention groups. Of them, 153 subjects who were outside the limits for total energy intake at baseline were excluded. We also excluded 78 participants with missing baseline dietary information. Thus the analyses were carried out on 7216 participants.

After a mean follow-up of 5.8 years, 342 participants had a myocardial infarction, a stroke or died from a cardiovascular cause. The main characteristics of the 7216 participants of the PREDIMED study according to baseline egg consumption are shown in Table 1. In this cohort, 3.0% of the participants consumed >4 eggs/week and 34.8% consumed <2 eggs/week. Participants reporting >4 eggs/week were younger and had a higher physical activity level, better educational attainment, and higher total energy and alcohol intake. They were also more likely to be male and current smokers, and to take antiplatelet drugs or oral hypoglycemic drugs, but less likely to be treated with insulin than participants reporting consumption of less than 2 eggs/week. Participants with the lowest consumption (<2 eggs/week) were more likely to have dyslipidemia or hypertension and to be treated with statins, β-blockers or calcium channel blockers than participants reporting the highest level of consumption (>4 eggs/week). During follow-up, 61% of the participants in the <2 eggs/week category at baseline, stayed always in the lowest category of egg consumption. On the other hand, 44% of the participants in the >4 eggs/week category at baseline, stayed always in the highest category of egg consumption during follow-up. Finally, those participants with baseline egg consumption between 2 and 4 eggs/week, increased ever their consumption in 2% of the cases and decreased it ever in 17% of the cases.

Using as the reference category the group of participants who reported the lowest egg consumption (<2 eggs/week), participants who reported a higher consumption did not show a significantly higher risk of CVD (Table 2). Participants who ate 2-4 eggs/week had a HR of primary-outcome events of 0.95 (95% CI: 0.75-1.19) and participants who ate >4 eggs/week had a HR of 1.22 (95% CI: 0.72-2.07) in the fully adjusted multivariable model.

Among 3527 participants who had diabetes at study inception (48.9%), we identified 225 primary outcomes. Using as reference category diabetics who had the lowest egg consumption (<2 eggs/week), those who consumed 2-4 eggs/week had a HR of the primary cardiovascular end-point of 0.86 (95% CI: 0.65-1.14), while those consuming >4 eggs/week had a HR of 1.33 (95% CI: 0.72-2.46) in the fully adjusted multivariable model (Table 2).

A total of 117 primary-outcome events occurred in participants without diabetes at baseline (n=3689). Using as reference category non-diabetics who had the lowest egg consumption (<2 eggs/week), those who reported a consumption of 2-4 eggs/week had a HR of 1.09 (95% CI: 0.73-1.62) and those with a consumption >4 eggs/week had a HR of 0.96 (95% CI: 0.33-2.76) in the fully adjusted model (Table 2). The test for interaction between egg consumption and diabetes at baseline was not significant (P=0.80).

Neither intervention groups (Mediterranean diet) nor the control group showed an association of egg consumption with the primary outcome (Table 2). Interaction between intervention group (Mediterranean diet groups versus control) was not significant (P=0.49)

In the cumulative dietary analyses using sum of repeated measurements of egg consumption we found similar results with no significant association between egg consumption and CVD (Table 3). The test for interaction between sum of repeated measurements of egg consumption and baseline diabetes was not significant (P=0.80).

Discussion

Higher baseline egg consumption was not associated with CVD in our Mediterranean cohort of older subjects at high cardiovascular risk. Egg consumption in this cohort was lower than that reported in others studies (7,8,9). Indeed, only 3% of the participants in our study consumed >4 eggs/week at baseline. In our study, egg consumption was unrelated to an increased risk of CVD in patients with diabetes and in those without diabetes. However, when we compared extreme categories of consumption we found higher point estimates for diabetics than for non-diabetics. Therefore, our results are compatible with the hypothesis that diabetic patients may be more sensitive to egg consumption and that very high egg consumption among diabetics may predispose them to a higher risk of developing CVD.

A mechanism that might explain the lack of association between egg consumption and incident CVD is that egg consumption promotes the creation of large LDL particles that are less atherogenic (23). These changes have also been observed in elderly individuals (24). Additionally, in hyperlipidemic adults treated with lipid-lowering drugs (around 40% of our participants were statin users) the consumption of 3 additional eggs/day increased HDL-cholesterol and decreased the LDL:HDL ratio (25). Furthermore, egg yolk contains inhibitors of platelet activating factor that reinforce their value in CVD protection (26). In a 12-week randomized trial the effect of a hypoenergetic high-protein high-cholesterol (2 eggs/day) diet versus a hypoenergetic high-protein and low cholesterol diet was assessed. A high cholesterol diet (from eggs) improved glycaemic control, plasma lipids and blood pressure in participants with type 2 diabetes or impaired glucose tolerance (27). Recently, a trial randomized a total of 140 participants, with diabetes or prediabetes, to a high-egg diet (2 eggs/day) or a low-egg diet (<2 eggs/week). High egg consumption did not have an adverse effect on the lipid profile in a context of high MUFA and PUFA consumption (28).

On the other hand, eggs are a major source of dietary cholesterol and also contain other nutrients such as high-quality proteins, unsaturated fat, minerals, vitamins, and carotenoids (1, 2, 29). However, eggs have low amounts of antioxidants (30).

Food comes from living organisms where different components interact. These interactions are relevant when hen eggs are consumed as food (31). A study in rats found that egg white proteins may have a cholesterol-lowering action because egg white proteins lower cholesterol absorption by interfering the micellar formation in the intestines (32). The context of individual foods in whole diets, food processing and cuisine must also be considered. Our cohort participants had a high adherence to a Mediterranean diet pattern that is rich in olive oil, nuts, green vegetables, fruits, poultry and fish. Therefore, their diet was rich in polyunsaturated and monounsaturated fat but low in saturated fat. This high-quality food pattern perhaps may have modulated the potential adverse effects of a single nutrient such as dietary cholesterol, therefore explaining the lack of association between egg consumption and CVD. In fact, adherence to a Mediterranean dietary pattern has beneficial effects on cardiovascular risk factors, lowers the total cholesterol: HDL cholesterol ratio and reduces the incidence of major cardiovascular events (11,13).

We recognize that our study has some limitations. First, only 3% of our participants consumed >4 eggs/week, which hampers the capacity to detect associations between higher egg consumption and CVD. Second, the effect of the dietary intervention may have modulated the effect of egg consumption on CVD incidence. The method used for dietary assessment was a food-frequency questionnaire, with the potential for misclassification bias. However, the FFQ was extensively validated, and the estimated cumulative sum of consumption may have been more robust than with a one-time assessment. Third, although we adjusted for many covariates, residual confounding cannot be completely excluded. Finally, the generalizability of these results is limited because our study is a non-representative sample of the general Spanish population, as it is a high-cardiovascular risk cohort. However, having around 50% of diabetic patients, allowed us to study the association between egg consumption and cardiovascular risk in people with and without type 2 diabetes.

Our study also has strengths: the prospective design, a large sample size, a long period of follow-up, the use of repeated dietary egg consumption measures during follow-up, and adjustment for a wide array of confounders.

Conclusions

In this cohort of elderly subjects at high cardiovascular risk and with high adherence to a Mediterranean dietary pattern, low to moderated egg consumption was unrelated to CVD risk in both diabetic and non-diabetic individuals. The relationship between egg consumption and CVD remains inconclusive.

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The autors´ responsibilities were as follows JD-E, FJB-G, PB-C, JS-S, ER, RE, DC and ET: conceived the project; JD-E, FJB-G, PB-C, JS-S, MF, ER, RE, DC, EG-G, FA, MF, JL, LS-M, XP, NB, LQ, MF, AM and ET: conducted the research; JD-E, FJB-G, PB-C and ET: analyzed the data; JD-E, FJB-G, PB-C and ET: wrote the manuscript and had primary responsibility for the final content

of the manuscript; and all authors: read and approved the final manuscript. ER reported serving on the scientific board of and receiving travel support as well as grant support through his institution from the California Walnut Commission. JS-S reported serving on the scientific committee of and receiving grant support through his institution from the International Nut and Dried Fruit Council, receiving consulting fees from Danone, and receiving grant support through his institution from Eroski and Nestlé. LS-M reported serving on the boards of the Mediterranean Diet Foundation and the Beer and Health Foundation. XP reported serving on the board of and receiving grant support through his institution from the Residual Risk Reduction Initiative Foundation, serving on the board of OMegafort, serving on the board of and receiving payment for the development of educational presentations, receiving lecture fees from Danone, and receiving grant support though his institution from Unilever and Karo Bio. RE reported serving on the board of and receiving lecture fees from the Research Foundation on Wine and Nutrition, serving on the boards of the Beer and Health Foundation, and the European Foundation for Alcohol Research, and receiving lecture fees from Cerveceros de España. JD-E, FJB-G, PB-C, MF, DC, EG-G, FA, JL, NB, LQ, MF, AM, and ET had no conflicts of interests.

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Table 1. Baseline characterystics of the 7216 participants of the PREDIMED study according to baseline egg consumption (Mean and s.d. or percentages).

	Egg consumption		
	<2 eggs/week (n = 2509)	**2-4 eggs/week** (n = 4493)	**>4 eggs/week** (n = 214)
Age	67.1 (6.1)	67.0 (6.3)	65.6 (5.8)
Male %	39.3	43.3	65.4
BMI Kg/m^2	30.0 (3.9)	29.9 (3.8)	30.3 (3.7)
Physical activity (METS-min/day)	254 (208)	260 (232)	292 (262)
Smoker status %			
Never Smoker	61.5	62.2	48.1
Quitters	25.6	23.7	32.2
Current Smoker	13.0	14.2	19,6
Educational level %			
University	3.4	3.8	5.6
High School	15.9	19.9	24.3
Hypertension %	83.5	82.6	75.7
Dyslipidemia %	76.8	70.4	55.6
Diabetes %	47.6	49.5	50,9
Family history premature CHD (%)	22.8	22.5	12.6
Carbohydrates intake (% total energy)	42.3 (7.3)	41.6 (7.0)	39.9 (7.4)
Protein intake (% total energy)	16.4 (2.9)	16.7 (2.7)	16.2 (2.8)
Fat total (% total energy)	39.1 (7.0)	39.2 (6.7)	40.1 (6.9)
Monounsaturated (% total	19.7 (4.8)	19.4 (4.5)	19.8 (4,3)
energy)	6.2 (2.2)	6.2 (2.0)	6.2 (2.1)
Polyunsaturated (% total energy)	9.8 (2.3)	10.1 (2.2)	10.6 (2.4)
Saturated(% total energy)			

Cholesterol intake (mg/d)	294 (98)	392 (86)	529 (146)
Score for adherence to Mediterranean Diet	8.6 (1.9)	8.7 (1.9)	8.5 (1.9)
Fiber (g/d)	25.7 (7.4)	25.1 (7.4)	23.5 (7.5)
Alcohol (g/d)	7.9 (12.9)	8.3 (13.6)	12.1 (18.7)
Total energy intake (kcal/d)	2106 (531)	2289 (532)	2645 (566)
Medication			
ACE/ARB_II (%)	47.5	49.1	48.6
Diuretics (%)	21.2	21.4	19.2
Calcium channel blockers (%)	13.3	13.6	11.2
B blockers (%)	13.3	11.0	10.8
Statins (%)	43.9	38.8	28.0
Insulin (%)	5.2	5.3	2.8
Oral hypoglycaemic agents (%)	28.8	30.3	32.7
Antiplatelet(%)	19.7	19.7	25.2

Table 2. HRs and (95% CIs) of CVD according to baseline egg consumption in the PREDIMED trial

Participants		**Egg consumption**			p for trend
		<2/week	**2-4/week**	**>4/week**	
Total					
	Incident cases of CVD	121	204	17	
	N	2509	4493	214	
	Persons-years	14,793	25,674	1,305	
	Multivariable 1	1 (ref)	0.96 (0.76-1.20)	1.34 (0.80-2.25)	0.73
	Multivariable 2	1 (ref)	0.97 (0.77-1.22)	1.36 (0.81-2.28)	0.65
	Multivariable 3	1 (ref)	0.95 (0.75-1.19)	1.26 (0.75-2.13)	0.86
	Multivariable 4	1 (ref)	0.95 (0.75-1.19)	1.22 (0.72-2.07)	0.92
Diabetics					
	Incident cases of CVD	81	131	13	
	N	1,193	2,225	109	
	Persons-years	7,076	12,850	682	
	Multivariable 1	1 (ref)	0.88 (0.67-1.17)	1.42 (0.78-2.60)	0.94
	Multivariable 2	1 (ref)	0.89 (0.67-1.18)	1.41 (0.77-2.58)	0.90
	Multivariable 3	1 (ref)	0.87 (0.65-1.15)	1.39 (0.76-2.55)	0.98
	Multivariable 4	1 (ref)	0.86 (0.65-1.14)	1.33 (0.72-2.46)	0.89
Non-diabetics					
	Incident cases of CVD	40	73	4	
	N	1316	2268	105	
	Persons-years	7717	12,824	622	

	Multivariable 1	1 (ref)	1.08 (0.73-1.59)	0.99 (0.35-2.81)	0.79
	Multivariable 2	1 (ref)	1.10 (0.75-1.63)	1.03 (0.36-2.93)	0.69
	Multivariable 3	1 (ref)	1.07 (0.72-1.58)	0.95 (0.33-2.70)	0.85
	Multivariable 4	1 (ref)	1.09 (0.73-1.62)	0.96 (0.33-2.76)	0.79
Mediterranean diet groups					
	Incident cases of CVD	79	124	12	
	N	1652	3035	147	
	Persons-years	9808	17542	913	
	Multivariable 1	1 (ref)	0.85 (0.64-1.13)	1.43 (0.77-2.65)	0.90
	Multivariable 2	1 (ref)	0.86 (0.65-1.14)	1.40 (0.75-2.61)	0.90
	Multivariable 3	1 (ref)	0.83 (0.62-1.10)	1.28 (0.68-2.40)	0.67
	Multivariable 4	1 (ref)	0.81 (0.61-1.08)	1.19 (0.63-2.26)	0.54
Control group					
	Incident cases of CVD	42	80	5	
	N	857	1458	67	
	Persons-years	4985	8132	391	
	Multivariable 1	1 (ref)	1.18 (0.80-1.72)	1.10 (0.43-2.85)	0.49
	Multivariable 2	1 (ref)	1.19 (0.81-1.74)	1.12 (0.43-2.91)	0.45
	Multivariable 3	1 (ref)	1.18 (0.80-1.72)	1.12 (0.43-2.91)	0.48
	Multivariable 4	1 (ref)	1.20 (0.81-1.76)	1.13 (0.43-2.97)	0.45

Multivariable 1: adjusted for age, sex, BMI and intervention group, and stratified by recruitment center.
Multivariable 2: additionally adjusted for smoking status (never smokers, quitters, current smokers), physical activity during leisure time (MET-min/day, continuous), and educational status (3 categories).
Multivariable 3: additionally adjusted for diabetes (yes/no), hypertension (yes/no), hypercholesterolemia (yes/no) and family history of CVD (yes/no).
Multivariable 4: additionally adjusted for Mediterranean food pattern (continuous), alcohol intake (continuous), total energy intake (continuous).

Table 3. HRs (and 95% CIs) of CVD according to cumulative sum of egg consumption in the PREDIMED trial. Adjusted HRs per 500 eggs of cumulative consumption

Participants		HR (95% CI)	P for trend
Total			
	Multivariable 1	1.12 (0.91-1.37)	0.30
	Multivariable 2	1.12 (0.91-1.38)	0.27
	Multivariable 3	1.08 (0.88-1.34)	0.46
	Multivariable 4	1.08 (0.88-1.34)	0.46
Diabetics			
	Multivariable 1	1.19 (0.91-1.56)	0.20
	Multivariable 2	1.20 (0.92-1.58)	0.18
	Multivariable 3	1.18 (0.89-1.55)	0.25
	Multivariable 4	1.18 (0.90-1.55)	0.22
Non-diabetics			
	Multivariable 1	0.96 (0.68-1.36)	0.84
	Multivariable 2	0.97 (0.69-1.37)	0.88
	Multivariable 3	0.94 (0.67-1.34)	0.75
	Multivariable 4	0.94 (0.66-1.33)	0.72

Multivariable 1: adjusted for age, sex, BMI and intervention group, and stratified by recruitment center.
Multivariable 2: additionally adjusted for smoking status (never smokers, quitters, current smokers), physical activity during leisure time (METs-min/day, continuous), and educational status (3 categories).
Multivariable 3: additionally adjusted for diabetes (yes/no), hypertension (yes/no), hypercholesterolemia (yes/no) and family history of CVD (yes/no).
Multivariable 4: additionally adjusted for Mediterranean food pattern (continuous), alcohol intake (continuous), total energy intake (continuous).