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ORIGINAL CONTRIBUTION

Dietary folate intake and metabolic syndrome in participants of PREDIMED-Plus study: a cross-sectional study

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Abstract

Purpose We examined the association between dietary folate intake and a score of MetS (metabolic syndrome) and its components among older adults at higher cardiometabolic risk participating in the PREDIMED-Plus trial.

Methods A cross-sectional analysis with 6633 with overweight/obesity participants with MetS was conducted. Folate intake (per 100 mcg/day and in quintiles) was estimated using a validated food frequency questionnaire. We calculated a MetS score using the standardized values as shown in the formula: [(body mass index + waist-to-height ratio)/2] + [(systolic blood pressure + diastolic blood pressure)/2] + plasma fasting glucose–HDL cholesterol + plasma triglycerides. The MetS score as continuous variable and its seven components were the outcome variables. Multiple robust linear regression using MM-type estimator was performed to evaluate the association adjusting for potential confounders.

Results We observed that an increase in energy-adjusted folate intake was associated with a reduction of MetS score (β for 100 mcg/day = − 0.12; 95% CI: − 0.19 to − 0.05), and plasma fasting glucose (β = − 0.03; 95% CI: − 0.05 to − 0.02) independently of the adherence to Mediterranean diet and other potential confounders. We also found a positive association with HDL-cholesterol (β = 0.07; 95% CI: 0.04–0.10). These associations were also observed when quintiles of energy-adjusted folate intake were used instead.

Conclusion This study suggests that a higher folate intake may be associated with a lower MetS score in older adults, a lower plasma fasting glucose, and a greater HDL cholesterol in high-risk cardio-metabolic subjects.

Keywords Folate · Cardiometabolic risk · Metabolic syndrome score · Diabetes · Cholesterol

Introduction

Cardiovascular disease (CVD) is a major cause of death and disability in the world, according to the 2015 Global Burden of Disease study [1]. The global burden of CVD is expected to increase in the coming decades as a result of the aging of the population. Metabolic syndrome (MetS) is a complex metabolic disorder involving abdominal

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obesity, impaired glucose, dyslipidemia, and hypertension, all of which can lead to complications including CVD. The prevalence of MetS in developed countries has been also increasing in the adult population over the last 2 decades [2–5], particularly in older adults [6, 7]. Therefore, the early identification of modifiable determinants of MetS, including dietary factors, has become a priority to prevent metabolic complications and to reduce the risk of CVD [8, 9].

Folate is an essential micronutrient involved in several important physiological functions, including synthesis of methionine from homocysteine, synthesis of nucleic acids, amino acids, cell division, and methylation of DNA [10]. A meta-analysis of 11 randomized clinical trials of a dose of 5000 µg/d of the synthetic form of folate (folic acid), showed a beneficial effect on flow-mediated dilation (1.42; 95% CI: 1.26; 1.58) and other in cutaneous vasodilatation in older people [11, 12]. This suggests that nitric oxide may play a protective role as a potent vasodilator against the pathogenesis of endothelial dysfunction, which could be more prevalent in patients with MetS [13, 14]. It has been also shown that the combination of folic acid supplementation (5 mg/d) and vitamin B12 improves endothelial dysfunction in patients with MetS [15]. There is insufficient research on the relationship between the natural form of folate and MetS, despite the fact that elevated homocysteine levels or changes in the expression of genes involved in lipid metabolism have been previously linked to MetS [16, 17]. In a cross-sectional study of a nationally representative sample of 8077 adults in the US, a high dietary folate intake was associated with lower MetS risk [18]. In another study of 2800 Iranian adults aged 35–65, no association was found [19].

Current evidence suggests that the use of folic acid supplements, the synthetic form of the vitamin, is associated with a positive effect on risk factors for MetS, including a better lipid profile, glycemic control and a lower risk of hypertension [20–24]. However, little is known about the independent effect of dietary folate, the natural form of folate, and whether it has an effect that is similar to that observed for its synthetic form. This is of interest because there are differences between the two forms of folate, in terms of metabolic pathways and absorption processes [25, 26]. As far as we know, only two studies that have explored the association between dietary equivalent folate (DEF) intake and MetS, both reported a protective effect, although no distinction was made between the natural and synthetic forms of the vitamin [18, 19]. Since folate food fortification is not compulsory in Spain, we had the opportunity to examine the association between the dietary folate intake from natural sources and a score based on the components of MetS (MetS score) in older adults with overweight/obesity and MetS participating in the PREDIMED-Plus trial.

Materials and methods

Study population

This study was based on the cross-sectional analysis of baseline data collected from the 6874 participants recruited for the PREDIMED-Plus trial. This is a 6-year, parallel-group, multi-center and randomized clinical trial designed to evaluate the effect of an energy-restricted Mediterranean diet, physical exercise and behavioral therapy compared to usual care with an energy unrestricted Mediterranean diet for the primary prevention of CVD. The trial was registered at the International Standard Randomized Controlled Trial (ISRCTN89898870). The study protocol includes more detailed information and is available at the website https://www.predimedplus.com/ and in previous publications [27, 28]. Participants included men ages 55–75 years and women ages 60–75 years, with a body mass index (BMI) of ≥ 27 to < 40 kg/m^2 complying with at least three MetS criteria and not suffering from CVD at the time of enrollment [29]. After excluding participants with missing data for the main variables and with implausible values for mean daily energy intake (< 500 and > 3500 kcal/day for women, < 800 and > 4000 kcal/day for men), 6633 participants were included in the present analysis. Figure 1 shows the flowchart for the population sample in our study. All participants provided written informed consent, and the trial was approved by the Intuitional Review Board of the recruitment centers where the study was conducted.

Folate intake assessment

The FFQ was administered to participants at baseline by trained interviewers. Participants were asked about the frequency of their consumption of each food item during the

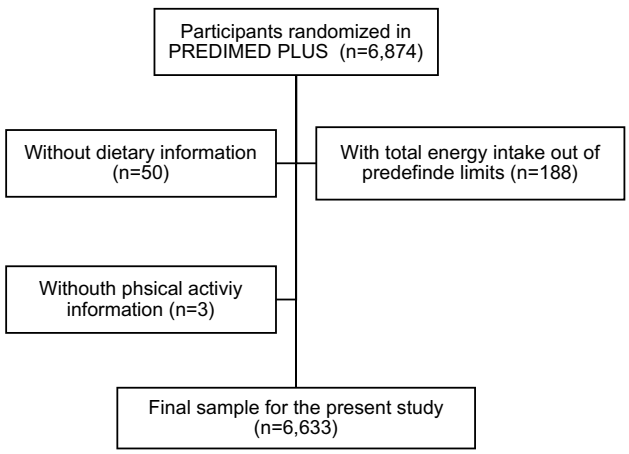

Fig. 1 Flowchart of participants

previous year. The questionnaire included nine frequency options for a specified serving size (never or almost never, 1–3 times a month, once a week, 2–4 times a week, 5–6 times a week, once a day, 2–3 times a day, 4–6 times a day, and more than 6 times a day). The nutrient and energy content of foods were obtained from Spanish food composition tables [31, 32]. Since folic acid fortification is not mandatory in Spain, we only used information on folate derived from natural, non-fortified foods. Thus, we estimated the mean daily folate intake and total energy intake by multiplying the frequency of use for each food item by folate and total energy intake content of the portion size and added the results across all foods to obtain a dietary folate and energy intake for each individual. Energy-adjusted folate intake was computed using the residual method, where dietary folate intake is regressed on total calories and the population mean was then added to the residual [33]. Folate intake was analyzed as a continuous variable (per 100 mcg/d increment) and categorized into quintiles. Participants were compared according to compliance with the average requirements (AR) of dietary folate intake according to the European Food Safety Authority recommendations [34].

Metabolic syndrome score and its components

Weight, height, waist (measured at the midpoint between the lowest rib and the iliac crest on a horizontal plane) and hip circumference were measured in duplicate with light clothing and no shoes using a calibrated scale, a wall-mounted stadiometer, and a non-elastic tape, respectively. Body mass index (BMI) was calculated as weight (kg) divided by height (squared meters), and waist–hip ratio (WHR) [waist circumference (cm) divided by hip circumference (cm)]. Blood pressure was measured three times with a validated semiautomatic oscillometer after 5 min of rest in-between measurements (Omron HEM-705CP, Hoofddorp, The Netherlands), and the mean of the three readings was used. Blood samples were collected at baseline after an overnight fast, and aliquots of serum and EDTA plasma were immediately processed, coded, and stored at − 80 °C in a central laboratory until analysis. High-density lipoprotein (HDL), serum glucose and triglyceride levels were determined using standard enzymatic methods in automatic analyzers in local laboratories of the National Health System hospitals.

A MetS score was compiled based on World Health Organization's definition of MetS [29] and was computed based on the formula devised by Franks et al. [35]. This variable was derived by standardizing and then summing the following continuously distributed indexes of obesity (BMI + WHR/2), hypertension (systolic blood pressure + diastolic blood pressure/2), hyperglycemia (plasma fasting glucose), inverted fasting HDL cholesterol, and hypertriglyceridemia to create a *z* score. A little variation in the formula was introduced: we used the sex-specific *z* score for WHR and HDL components instead of *z* score was used and insulin was not included because this information was not collected for the study. In parallel, standardized components of the MetS score (i.e. indexes of obesity, hypertension, hyperglycemia, inverted fasting HDL cholesterol, and hypertriglyceridemia) were also calculated.

Covariates

The following information was also collected at baseline age, sex, educational level, smoking, total physical activity in Metabolic Equivalents (METS)-min/day using the validated Regicor Short Physical Activity Questionnaire [36], information regarding medication use (antihypertensive, hypolipidemic, diabetes, and vitamin supplementation), and family history of illness (i.e. stroke and cardiac disease). Adherence to an energy-restricted Mediterranean diet (MedDiet) was assessed using a 17-item questionnaire, a modified version of a validated 14-item questionnaire [37]. Alcohol intake in grams per day was estimated using the validated FFQ [30].

Statistical analysis

The descriptive analysis of participants' characteristics according to energy-adjusted folate intake quintiles was displayed as means and standard deviations (SD) for quantitative variables, and as percentages for categorical variables. An analysis of variance (ANOVA) test was used for quantitative variables and the Chi-square test was used for qualitative variables to compare the sample characteristics between quintiles of levels of intake.

We used a stepwise multiple linear regression analysis to estimate the cumulative coefficient of determination to identify the main food sources of dietary folate intake among the study participants [38].

A robust multiple linear regression using an MM-type estimator was performed to evaluate the association between energy-adjusted folate intake (in quintiles and per 100-mcg/d increment) and MetS score and its components [39]. Regression coefficients represent the change in each outcome per one unit of dietary folate intake, where 1 unit is equivalent to a 1-SD difference in *z* scores, or a 1-unit difference in the CM-risk score, either in the continuous or quintile form of the variable folate intake.

Models were adjusted for potential confounders based on previous literature, and for those variables related to the outcome (based on the likelihood ratio: tests with a p value of < 0.10) or if the effect estimates for the exposure of interest changed by $\geq 10\%$ when they were excluded from the model. Finally, four models were examined: Model 1 was adjusted for sex, age (continuous), total energy intake,

educational level (illiterate or primary education, secondary education, academic or graduate, and missing information), total physical activity (METS-min/day), smoking status (current smoker, former smoker, and never smoker), alcohol intake in grams per day, antihypertensive (no/yes), hypolipidemic (no/yes) and diabetes (no/yes) medication use and vitamin supplements use (no/yes), and Model 2 accounted for the variables in model 1 plus the 17-score energy-restricted Mediterranean diet.

To assess the possible effect of dose–response, linear trend tests were applied for quintiles of energy-adjusted folate intake as continuous variable. The median consumption level within a quintile was assigned to all people within that quintile. Finally, to check the robustness of our findings, we conducted several sensitivity analyses: (a) excluding patients with prevalent diabetes; (b) excluding patients with a family history of stroke; (c) excluding patients with family history of cardiac disease; (d) excluding patients using vitamin supplements; (e) stratifying by sex; and (g) stratifying by median value of vitamin B2, B6 and B12 intake.

Statistical interactions were tested by the means of likelihood ratio test and compared the full adjusted model of the linear robust regression with and without cross-product terms between the aforementioned variables and per 100-mcg/d increments of energy-adjusted folate intake.

Statistical analyses were conducted using R 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria; https://www.R-project.org). For the robust linear regression analyses, we also used the "robustbase" package of R statistical software. We used the PREDIMED-Plus database update of March 2019.

Results

The mean daily folate intake among participants was 351 mcg/day, and 86 percent of participants exceeded the EFSA AR recommendations of 250 mcg/day. Table 1 shows the main food groups and individual foods contributing at least 80 percent to dietary folate intake. Vegetables, fruits, cereals, and legumes were the main food groups, and spinach, orange and natural juices were the main individual food sources.

Table 2 shows the baseline characteristics according to quintiles of energy-adjusted folate intake. The mean age, physical activity, adherence to an energy-restricting Mediterranean diet, and vitamin B12, percentage of women, hypolipidemic medication use, and vitamin supplement use increased across the quintiles of folate intake. In contrast, the percentage of current smokers and the mean alcohol intake decreased across the quintiles of folate intake. Compared with the first quintile of energy-adjusted folate intake, participants in the fifth quintile had higher HDL-cholesterol levels, and lower plasma triglycerides, WHR, and slightly lower systolic and diastolic blood pressure.

Table 1 Main sources of variability concerning dietary folate intake in the PREDIMED-Plus participants ($n = 6633$)

	R^2	Cumulative R^2
Food groups		
Vegetables	0.453	0.453
Fruit	0.155	0.608
Fruit juice	0.065	0.673
Cereals	0.065	0.738
Legumes	0.036	0.774
Nuts	0.022	0.796
Dairy products	0.012	0.808
Meat	0.008	0.816
Fish	0.006	0.822
Foods		
Spinach	0.241	0.241
Oranges	0.165	0.406
Natural juice	0.085	0.491
Other vegetables	0.072	0.563
Tomato	0.059	0.622
Dry beans	0.052	0.674
Muesli	0.048	0.722
Melon	0.042	0.764
Green beans	0.030	0.794
White bread	0.021	0.815

Table 3 presents the results of the multiple robust linear regression analysis for the association between energy-adjusted folate intake (in quintiles and in continuous) and the MetS score and its components after adjusting for potential confounders. We observed a reduction in MetS score, expressed in units of SD, according to quintiles of energy-adjusted folate intake (p trend < 0.001). Compared with the first quintile of energy-adjusted folate intake (< 275 mcg per day), the participants in the fifth quintile (> 416 mcg per day) had a reduction of − 0.37 points (95% CI: − 0.54 to − 0.20) in the SD of the MetS score after adjusting for age, sex, energy intake, educational level, smoking status, alcohol intake, total physical activity, hypertension, diabetes, cholesterol medication, and vitamin supplement use. Additional adjustment for the 17-point screener for Mediterranean diet adherence did not change the statistical association between quintile of energy-adjusted folate intake and MetS z score, but the magnitude of the association was slightly lower (− 0.29 vs. − 0.37 points, respectively). The increment in 100 mcg per day in energy-adjusted folate intake showed a reduction of − 0.15 (95% CI: − 0.21 to − 0.00) and − 0.12 (95% CI: 0.19 to − 0.05) points in the SD MetS score in the multiple adjusted model 1 and 2 respectively.

Table 2 Baseline characteristics of the study population by quintiles of energy-adjusted folate intake in the PREDIMED-Plus study ($n = 6633$)

	Folate intake (mcg/day)					
	Q1: < 275 ($n = 1327$)	Q2: 275–315 ($n = 1327$)	Q3: 316–357 ($n = 1326$)	Q4: 358–416 ($n = 1327$)	Q5: > 416 ($n = 1326$)	p value[a]
Age in years, mean (SD)	64.5 (5.0)	65.5 (5.0)	65.6 (4.8)	65.9 (4.9)	66.5 (4.6)	< 0.001
Sex, % women	32.9	40.2	49.7	55.7	63.5	< 0.001
Education level, % academic or graduate	25.5	19.8	19.2	23.1	21.7	< 0.001
Smoking status, % current smoker	18.6	13.2	10.3	9.7	10.1	< 0.001
Physical activity (METS-min/day), mean (SD)	304.2 (291.3)	343.1 (330.2)	353.1 (318.8)	374.6 (345.2)	384.9 (349.6)	< 0.001
Adherence to Mediterranean diet (0–17 points), mean (SD)	7.1 (2.5)	8.0 (2.4)	8.5 (2.5)	9.1 (2.5)	9.9 (2.5)	< 0.001
Vitamin B12 intake (mcg/day)	8.6 (3.6)	9.5 (3.9)	10.0 (4.7)	10.4 (4.4)	11.1 (5.3)	< 0.001
Alcohol intake (g/day), mean (SD)	15.9 (18.5)	13.7 (17.0)	10.0 (13.6)	8.6 (12.2)	6.9 (10.5)	< 0.001
HDL-cholesterol (mg/dL), mean (SD)	46.2 (11.5)	47.1 (12.1)	48.4 (11.8)	49.0 (11.6)	49.8 (11.9)	< 0.001
Plasma triglycerides (mg/dL), mean (SD)	159.9 (86.7)	153.2 (76.9)	151.6 (74.5)	148.3 (76.8)	147.0 (72.0)	< 0.001
Plasma fasting glucose (mg/dL), mean (SD)	114.3 (29.1)	113.6 (28.2)	114.6 (30.6)	113.4 (29.3)	111.6 (28.1)	0.078
BMI (kg/m^2), mean (SD)	32.6 (3.4)	32.5 (3.4)	32.6 (3.4)	32.5 (3.5)	32.6 (3.5)	0.900
Waist circumference (cm), mean (SD)	109.3 (9.4)	108.1 (9.7)	107.4 (9.4)	106.7 (9.9)	106.3 (9.6)	< 0.001
Hip circumference (cm), mean (SD)	109.4 (8.3)	109.5 (8.3)	110.1 (8.5)	110.1 (8.5)	110.8 (8.8)	< 0.001
Waist-to-hip ratio, mean (SD)	1.001 (0.073)	0.989 (0.076)	0.989 (0.076)	0.970 (0.077)	0.961 (0.076)	< 0.001
Systolic blood pressure (mmHg), mean (SD)	139.8 (16.7)	140.1 (17.2)	141.2 (17.4)	138.6 (16.9)	138.2 (16.2)	< 0.001
Diastolic blood pressure (mmHg), mean (SD)	81.3 (10.2)	80.9 (10.0)	81.8 (10.3)	80.3 (9.6)	80.0 (9.5)	< 0.001
Familiar history of stroke, %	26.2	25.2	28.4	27.7	27.0	0.590
Familiar history of cardiac disease, %	38.1	38.3	41.9	41.9	43.1	0.168
Prevalent diabetes, %	28.5	31.2	32.2	30.9	31.1	0.315
Antihypertensive medication use, %	75.7	77.5	79.6	76.6	79.1	0.179
Hypolipidemic medication use, %	47.9	50.7	52.0	52.8	53.5	0.018
Diabetes medication use, %	17.6	20.5	21.2	20.2	20.6	0.158
Vitamin supplement use, %	8.6	9.9	12.0	14.3	15.2	< 0.001

BMI body mass index, *HDL* high-density lipoprotein-cholesterol, *MET* metabolic equivalent of task

[a]From the χ^2 test (categorical variables), and analysis of variance (continuous variables)

Regarding, the components of the MetS score, in Model 1 we observed that three (i.e. WHR, HDL-cholesterol, and plasma fasting glucose) of the seven individual risk factors were associated with the energy-adjusted folate intake (analyzed as continuous as well as quintiles of intake). However, when the model was adjusted for the 17-point screener for Mediterranean diet adherence (Model 2), the association remained significant only for HDL-cholesterol and plasma fasting glucose. A positive dose–response was observed for the association with HDL-cholesterol and a negative dose–response was observed for the association with plasma fasting glucose according to quintiles of energy-adjusted folate intake (p trend < 0.001). The results of model 2 also showed that, compared to the first quintile (> 275 mcg per day), the fifth quintile of energy-adjusted folate intake (> 416 mcg per day) was associated with an increase of 0.13 (IC 95%: 0.06–0.21) points in SD of HDL-cholesterol and a decrease of 0.10 (IC 95%: − 0.15 to − 0.04) points in SD of plasma fasting glucose. Moreover, we observed an increase of 0.07 (IC 95%: 0.04–0.10) points in SD of HDL-cholesterol and a decrease of 0.03 (IC 95%: − 0.05 to − 0.02) points in SD of plasma fasting glucose per 100 mcg/day increase in energy-adjusted folate intake.

Table 4 shows the sensitivity analyses of the association between energy-adjusted folate intake per 100 mcg/day of increase and MetS score, HDL-cholesterol and plasma fasting glucose after excluding those participants with potentially relevant conditions for the association. Excluding prevalent diabetes ($n = 2042$), patients with a family history of stroke ($n = 1785$), cardiac disease ($n = 2697$) or vitamin supplement use ($n = 802$) did not change the main findings. Furthermore, the interaction (i.e. effect modification) between sex, vitamin B12 intake and the observed association between energy-adjusted folate intake and MetS score, HDL cholesterol and plasma fasting glucose was not statistically significant. Nonetheless, the magnitude of the association between energy-adjusted folate intake per 100 mcg/day increase and MetS score, HDL-cholesterol and

Table 3 Multiple adjusted β[a] (95% CI) for *z* metabolic syndrome score and their individual components according to energy-adjusted folate intake (in quintiles and continuous) at baseline in participants PREDIMED-Plus study ($n=6633$)

	Folate intake (mcg/day)						
	Q1: < 275 ($n=1327$)	Q2: 275–315 ($n=1327$)	Q3: 316–357 ($n=1326$)	Q4: 358–416 ($n=1327$)	Q5: > 416 ($n=1326$)	*p* trend[d]	Per 100-mcg/d increment
Metabolic syndrome score[b]							
Multiple adjusted 1	Ref	− 0.17 (− 0.34; − 0.01)	− 0.11 (− 0.28; 0.06)	− 0.32 (− 0.50; − 0.15)	− 0.37 (− 0.54; − 0.20)	< 0.001	− 0.15 (− 0.21; − 0.08)
Multiple adjusted 2	Ref	− 0.14 (− 0.31; 0.02)	− 0.07 (− 0.24; 0.10)	− 0.27 (− 0.45; − 0.09)	− 0.29 (− 0.48; − 0.11)	0.001	− 0.12 (− 0.19; − 0.05)
Body mass index[b]							
Multiple adjusted 1	Ref	− 0.02 (− 0.10; 0.05)	− 0.03 (− 0.11; 0.05)	− 0.04 (− 0.12; 0.04)	− 0.02 (− 0.10; 0.06)	0.641	− 0.01 (− 0.04; 0.02)
Multiple adjusted 2	Ref	− 0.00 (− 0.08; 0.08)	− 0.01 (− 0.08; 0.09)	− 0.01 (− 0.08; 0.09)	− 0.04 (− 0.04; 0.13)	0.300	− 0.01 (− 0.02; 0.05)
Waist-to-hip ratio[c]							
Multiple adjusted 1	Ref	− 0.08 (− 0.16; − 0.01)	− 0.11 (− 0.18; − 0.04)	− 0.12 (− 0.19; − 0.04)	− 0.14 (− 0.21; − 0.06)	< 0.001	− 0.05 (− 0.08; − 0.03)
Multiple adjusted 2	Ref	− 0.06 (− 0.13; 0.02)	− 0.07 (− 0.14; 0.01)	− 0.06 (− 0.13; 0.02)	− 0.05 (− 0.13; 0.03)	0.378	− 0.02 (− 0.05; 0.01)
Systolic blood pressure[b]							
Multiple adjusted 1	Ref	− 0.01 (− 0.08; 0.07)	0.08 (0.01; 0.16)	− 0.04 (− 0.12; 0.03)	− 0.05 (− 0.12; 0.03)	0.080	− 0.02 (− 0.05; 0.01)
Multiple adjusted 2	Ref	− 0.01 (− 0.08; 0.07)	0.08 (0.00; 0.16)	− 0.04 (− 0.12; 0.04)	− 0.05 (− 0.13; 0.03)	0.084	− 0.02 (− 0.05; 0.01)
Diastolic blood pressure[b]							
Multiple adjusted 1	Ref	0.03 (− 0.04; 0.11)	0.16 (0.08; 0.24)	0.04 (− 0.04; 0.12)	0.06 (− 0.02; 0.13)	0.354	0.03 (0.00; 0.06)
Multiple adjusted 2	Ref	0.01 (− 0.07; 0.08)	0.12 (0.04; 0.20)	− 0.01 (− 0.09; 0.07)	− 0.02 (− 0.10; 0.07)	0.343	0.00 (− 0.03; 0.03)
HDL-cholesterol[c]							
Multiple adjusted 1	Ref	0.02 (− 0.05; 0.09)	0.11 (0.04; 0.18)	0.13 (0.06; 0.20)	0.14 (0.06; 0.21)	< 0.001	0.07 (0.04; 0.09)
Multiple adjusted 2	Ref	0.02 (− 0.05; 0.09)	0.11 (0.04; 0.18)	0.13 (0.05; 0.20)	0.13 (0.06; 0.21)	< 0.001	0.07 (0.04; 0.10)
Plasma triglycerides[b]							
Multiple adjusted 1	Ref	− 0.01 (− 0.07; 0.04)	− 0.01 (− 0.07; 0.05)	− 0.05 (− 0.11; 0.01)	− 0.04 (− 0.10; 0.02)	0.088	− 0.02 (− 0.04; 0.00)
Multiple adjusted 2	Ref	0.00 (− 0.06; 0.06)	0.01 (− 0.05; 0.07)	− 0.01 (− 0.07; 0.05)	0.00 (− 0.06; 0.06)	0.964	0.00 (− 0.03; 0.02)
Plasma fasting glucose[b]							
Multiple adjusted 1	Ref	− 0.04 (− 0.09; 0.00)	− 0.03 (− 0.08; 0.02)	− 0.05 (− 0.09; 0.00)	− 0.09 (− 0.14; − 0.04)	< 0.001	− 0.03 (− 0.05; − 0.01)
Multiple adjusted 2	Ref	− 0.04 (− 0.09; 0.00)	− 0.03 (− 0.08; 0.01)	− 0.05 (− 0.10; 0.00)	− 0.10 (− 0.15; − 0.04)	< 0.001	− 0.03 (− 0.05; − 0.02)

Multiple adjusted 1: adjusted for age, energy, sex (male, women),educational level (primary, secondary or university/graduate), smoking status (never, former or current), alcohol intake (grams per day), total physical activity (METS-min/day), antihypertensive medication (yes/no), diabetes medication (yes/no), and hypolipidemic medication (yes/no), and vitamin supplements use (yes/no); multiple adjusted 2: additionally adjusted for 17-point screener of Mediterranean diet adherence (continuous)

HDL-c high-density lipoprotein-cholesterol

[a]MM-type estimators for linear robust regression models

[b]Data were standardized

[c]Data were sex-specific standardized

[d]*p* trend: test for linear trend were conducted using the median folate intake within a quintile was assigned to all people within that quintile and entered as continuous term in the robust linear regression models

plasma fasting glucose was greater in men than in women ($\beta=-0.15$, 95% CI: − 0.26 to − 0.04; $\beta=0.09$, 95% CI: 0.05–0.13; and $\beta=-0.05$, 95% CI: − 0.08 to − 0.02, respectively), and in those with vitamin b12 intake equal to or less than the median ($\beta=-0.18$, 95% CI: − 0.28, − 0.08; $\beta=0.07$, 95% CI: 0.03, 0.12; and $\beta=-0.04$, 95% CI: − 0.07, − 0.01, respectively). A significant interaction was also observed between folate and vitamin B6 ($p=0.028$), with a protective effect between folate and MetS score that was only present among participants with a median vitamin b6 intake higher than 2.4 mcg/d ($\beta=-0.13$, 95% CI: − 0.24, − 0.03). Interactions were also observed between folate and vitamin B2 ($p<0.001$) and vitamin B6 ($p=0.048$) for the protective association with plasma fasting glucose.

Table 4 Sensitivity analyses exploring the association[a] between 100 mcg/d increment of energy-adjusted folate intake and *z* metabolic syndrome score, HDL cholesterol and plasma glucose components at baseline in participants PREDIMED-Plus study ($n = 6633$)

	n Total	Metabolic syndrome score β^a (95% CI)	HDL-cholesterol β^a (95% CI)	Plasma fasting glucose β^a (95% CI)
Basal model	6633	− 0.12 (− 0.19; − 0.05)	0.07 (0.04; 0.10)	− 0.03 (− 0.05; − 0.02)
Excluding prevalent diabetes	4591	− 0.12 (− 0.20; − 0.05)	0.06 (0.03; 0.10)	− 0.03 (− 0.05; − 0.02)
Excluding patients with familiar history of stroke	4848	− 0.13 (− 0.21; − 0.05)	0.07 (0.04; 0.10)	− 0.03 (− 0.05; − 0.01)
Excluding patients with familiar history of cardiac disease	3936	− 0.12 (− 0.20; − 0.05)	0.09 (0.05; 0.12)	− 0.02 (− 0.05; 0.00)
Excluding patients with vitamin supplements use	5831	− 0.12 (− 0.19; − 0.04)	0.08 (0.05; 0.11)	− 0.03 (− 0.05; − 0.01)
Including only women	3209	− 0.10 (− 0.19; − 0.01)	0.05 (0.01; 0.09)	− 0.03 (− 0.05; 0.00)
Including only men	3424	− 0.15 (− 0.26; − 0.04)	0.09 (0.05; 0.13)	− 0.05 (− 0.08; − 0.02)
p-interaction		0.179	0.126	0.236
Including only people with vitamin b12 intake < 9.1 mcg/day (median value)	3317	− 0.05 (− 0.14; 0.04)	0.06 (0.02; 0.10)	− 0.03 (− 0.06; 0.00)
Including only people with vitamin b12 intake ≥ 9.1 mcg/day (median value)	3316	− 0.18 (− 0.28; − 0.08)	0.07 (0.03; 0.12)	− 0.04 (− 0.07; − 0.01)
p-interaction		0.105	0.994	0.319
Including only people with vitamin B2 intake < 2.0 mcg/day (median value)	3443	− 0.08 (− 0.18; 0.02)	0.10 (0.05; 0.14)	0.00 (− 0.03; 0.03)
Including only people with vitamin b2 intake ≥ 2.0 mcg/day (median value)	3190	− 0.11 (− 0.21; − 0.01)	0.03 (− 0.01; 0.07)	− 0.06 (− 0.09; 0.03)
p-interaction		0.407	0.082	< 0.001
Including only people with vitamin B6 intake < 2.4 mcg/day (median value)	3588	0.03 (− 0.09; 0.14)	0.05 (0.00; 0.10)	0.00 (− 0.3; 0.03)
Including only people with vitamin b6 intake ≥ 2.4 mcg/day (median value)	3045	− 0.13 (− 0.24; − 0.03)	0.06 (0.02; 0.10)	− 0.05 (− 0.08; − 0.02)
p-interaction		0.028	0.510	0.048

[a]MM-type estimators for linear robust regression models adjusted for age (continuous), sex (female, male), energy intake in kcals per day (continuous), educational level (primary, secondary or university/graduate), smoking status (never, former or current), alcohol intake (grams per day), total physical activity (METS-min/day), antihypertensive medication (yes/no), diabetes medication (yes/no), and hypolipidemic medication (yes/no), vitamin supplements use (yes/no); and 17-point screener of Mediterranean diet adherence (continuous)

This was only the case for participants with vitamin B2 intake > 2 mcg/d ($\beta = -0.06$, 95% CI: − 0.09, − 0.03) and vitamin B6 intake > 2.4 mcg/d ($\beta = -0.05$, 95% CI: − 0.08, − 0.02).

Discussion

In the present study, we observed that 86 percent of PREDIMED-Plus participants complied with the recommendation of 250 mcg/day of dietary folate intake, with spinach and oranges as the main food sources of dietary folate. In addition, this study suggests that an increase in folate intake by quintiles or per 100 mcg/day was associated with a reduction in the MetS score, after adjusting for potential confounders, including adherence to Mediterranean diet. Moreover, a higher folate intake was associated with lower plasma fasting glucose and higher plasma concentration of HDL-cholesterol independently of factors such as adherence to a Mediterranean diet and others. However, when we explored the effect for a 100 mcg/day increase of dietary folate intake on *z*-glucose or *z*-HDL, the effect was very small, and the results should be interpreted with caution regarding their clinical relevance.

The percentage of participants in compliance with the EFSA AR recommendations for dietary folate intake in the present study was high. This is similar to what has been reported in other populations of similar ages in Spain [40], a country where fortification is not mandatory. This percentage is also comparable to that found in other studies carried out in the US and Brazil, where fortification is mandatory [41, 42]. It could be due in part to the high consumption of vegetables, fruits, cereals, and legumes that is traditionally seen in Mediterranean populations such as those in Spain.

As far as we know, there are no previously published studies conducted in adults that have explored the effect of folate intake on a component-based MetS score, calculated as a continuous variable. The MetS score has emerged as an alternative to a definition based on dichotomous variables, and it is considered a valid tool for research that evaluates cardiometabolic risk in different age groups including adults [43]. However, it should be noted that the MetS score

has not been sufficiently used to date, and evidence of the association between dietary folate intake and MetS is still scarce and inconclusive. One study carried out with a representative sample of adults in the US also reported an inverse association between dietary folate and MetS similar to that shown in our study [18]. Another study conducted among Iranian adults aged 35–65 did not report any significant association [19]. Before comparing our results with these two studies, it should be taken into account that there was a different folate intake distribution in these studies; it ranged from the lowest intake in Iran to the highest intake in the US. Also, there is very scarce information related to the association between folate biomarkers and MetS risk, with only one study conducted that showed no association for serum folate levels above or below the normal clinical range and MetS risk [44].

In our study, we observed a significant protective effect of high intake of dietary folate intake (> 416 mcg/day) on plasma fasting glucose and HDL in older adults with MetS. A previous cross-sectional study based on a representative sample of adults in the US reported a slightly higher dietary folate intake than that found in our study, with a non-significant reduction in elevated fasting glucose and a significant reduction in HDL cholesterol [18]. Additionally, two studies conducted in patients with metabolic diseases reported that participants receiving 5 mg of folic acid supplements for 12 weeks had higher HDL cholesterol levels compared with those in the placebo group [22, 45]. A meta-analysis conducted by Akbari et al. concluded that, compared to placebo, folic acid supplementation was not associated with glucose [46]. On the other hand, the magnitude of association between additional 100 mcg/day of dietary folate and *z*-fasting plasma glucose and z-HDL found in our study was small and should be interpreted with caution. This result could be influenced by potential confounders, the effect of some fortified foods or the use of folic acid supplements, which this study was unable to take into account. Another factor to consider is that the association with higher folate intake could be due to a higher consumption of fruits and vegetables among the Mediterranean study population. Therefore, further research, especially longitudinal studies, is warranted to confirm these results.

The biological mechanisms by which folate (in its natural and synthetic forms) may be related to the MetS score, plasma fasting glucose and HDL-cholesterol are still not fully understood. One possible explanation could be related to the fact that folate can reduce circulation concentrations of homocysteine, which may be a potential mediator that improves lipid metabolism and endothelial dysfunction [13, 14, 16, 17]. It should be noted that there is debate in the literature about the relationship between the folate intake (both natural and synthetic form) and homocysteine concentration and endothelia function [13, 47]. It has been documented that folate (in the form of 5MTHF) can have a direct effect on endothelial function due to its role in nitric oxide synthesis and bioavailability, which is independent of its homocysteine-reducing effect [13]. Moreover, it has been postulated that methyl donors such as folate may reduce oxidative stress and systemic inflammation, which can have a positive effect on the normal regulation of insulin secretion from pancreatic β-cells and glycemic control [48–50]. A tentative explanation regarding the positive relationship observed between folate intake and HDL-cholesterol might be related to the fact that both factors improve the synthesis and bioavailability of oxide nitric [13, 14]. Furthermore, defects in DNA methylation are associated with metabolic diseases, which suggests that our findings could be explained by folate's crucial role in DNA metabolism [51]. In addition, a previous study conducted by Ramos-Lopez et al. showed that folate deficiency can be related to insulin resistance in people with obesity [50].

The interactions we observed between dietary folate intake and vitamin B6 intake for MetS score, and between dietary folate intake and vitamins B2 and B6 for fasting plasma glucose could be partly explained by the fact that vitamins B2 and B6 are cofactors for methylenetetrahydrofolate reductase, a critical enzyme in folate recycling. Methylenetetrahydrofolate reductase generates methyl groups for homocysteine remethylation to methionine, which is the precursor to the universal methyl donor S-adenosylmethionine [52, 53]. However, this finding warrants additional exploration in other studies.

Strengths of this study include the large sample size and, the detailed and high-quality information collected by trained interviewers. Additionally, the observed associations remained so after adjusting for Mediterranean diet adherence and other potential confounders. The results obtained from the sensitivity analysis reinforced the strength of these findings.

Nevertheless, this study has limitations. The cross-sectional analysis of our data prevents us from establishing a causal link and temporal direction in the association between folate intake and MetS score, plasma fasting glucose and HDL-cholesterol. However, our findings provide a rationale for potential replication in other samples using a longitudinal study design. Moreover, we should not disregard possible reverse causation. Another limitation is that the participants from the PREDIMED-Plus study were elderly individuals with specific clinical conditions. This prohibited us from extrapolating the findings of this study to the general population. Although we adjusted for a wide range of potential confounding factors including the adherence to a Mediterranean diet, residual confounding by unknown or unmeasured factors cannot be ruled out. For instance, we did not collect information about several genetic polymorphisms that are involved in folate metabolism and could affect folate status

in the participants. Regarding dietary data, the use of a food frequency questionnaire to estimate folate dietary intake is subject to possible misclassification errors, although any inaccuracy in reporting should be non-differential. This potential bias can be minimized using a carefully designed and validated FFQ that, in our case, showed good reproducibility and validity [30], particularly for folate intake ($r=0.86$ and $r=0.69$). It should also be mentioned that dietary folate intake did not include information for fortified foods since folate fortification is not mandatory in Spain. Therefore, the contribution of these foods to total folate intake should be minor, despite that some dairy and cereal brand names are fortified with folate and other vitamins. Unfortunately, detailed information on dosages and timing of folic acid supplements was not collected.

In conclusion, this study suggests that a higher folate intake was associated with a lower MetS score, a lower plasma fasting glucose and a higher plasma HDL cholesterol among older adults with MetS patients. Though further observational longitudinal or experimental studies are needed, investigating the effect of a higher intake of vegetables, fruits, legumes and cereals as main sources of folate may be a possible approach to reducing the risk of cardiovascular disease and diabetes.

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Author contributions ENM-M and JV conducted the statistical analyses and drafted the article. All authors contributed substantially to the acquisition of data or analysis and interpretation of data. All authors revised the article critically for important intellectual content. All authors approved the final version to be published.

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Compliance with ethical standards

Conflict of interest J.S.-S. reports serving on the board of the International Nut and Dried Fruit Council from which he has also received grant support through his institution, receiving consulting fees from Danone, Font Vella Lanjaron, Nuts for Life, and Eroski, and being given grant support through his institution from Eroski. J.S.-S is a member of the executive committee of the Instituto Danone Spain and a member of the scientific committee of the Institute Danone International. L.D. reports receiving a grant from the Fundación Cerveza y Salud. N-B. declares that she received payments from Danone S.A. for the purposes of scientific and technical consulting but not for preparing this study and grant support through his institution from Font Vella Lanjaron. No other potential conflicts of interest relevant to this article were reported.

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