Dietary folate intake and Metabolic Syndrome in participants of Predimed-Plus study: A cross-sectional study

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Abstract

Background: Folate is involved in different metabolic processes such as the homocysteine metabolism, regulatory changes in genes related to lipid metabolism and the endothelial function, and its deficiency has been related to the risk of Metabolic Syndrome (MetS). We examined the association between folate intake and a score of MetS and its components among older adults at higher cardiometabolic risk participating in the PREDIMED-Plus trial.

Methods: A cross-sectional analysis with 6633 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; CI 95%: -0.19 to -0.05), and plasma fasting glucose (β=-0.03; CI 95%: -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; CI 95%:0.04 to 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, a lower plasma fasting glucose, and a greater HDL cholesterol in high-risk cardio-metabolic subjects.

Key words: folate, cardiometabolic risk, metabolic syndrome score, diabetes, cholesterol.

Introduction

Cardiovascular disease (CVD) is the major leading cause of death and disability in the world, according to the 2015 Global Burden of Disease study (1). On current projections, it is expected that the global burden of CVD will continue to increase in the coming decades as result of the aging of the population. Metabolic syndrome (MetS) is a complex metabolic disorder including abdominal obesity, impaired glucose, dyslipidemia and hypertension, all of which can lead to many complications including CVD. Importantly, Its prevalence in the adult population has rapidly increased over the last two decades in developed countries (2–5). Therefore, the early identification of modifiable determinant of MetS including dietary factors have become a worldwide priority to prevent these metabolic complications in order to reduce the risk of CVD (6,7).

Folate is an essential micronutrient which is involved in several relevant physiological functions, including synthesis of methionine from homocysteine, synthesis of nucleic acids, amino acids, cell division, and methylation of DNA (8). The role of folate in MetS has been poorly investigated, although elevated homocysteine levels or changes in the expression of genes involved in lipid metabolism have been previously linked to MetS (9,10). In addition, it has been recently suggested that folate can intervene in nitric oxide synthesis and bioavailability, which is independent of its homocysteine-reducing effect (11). Indeed, several studies have shown that nitric oxide may play a protective effect as a potent vasodilator against the pathogenesis of endothelial dysfunction, which might be more prevalent in patients with MetS (11,12).

The present evidence suggests that the use of folic acid supplements is associated with a positive influence on risk factors of MetS such as a better lipid profile, glycemic control, or a reduction of hypertension (13–17); however, it is unknown its effect on the MetS analyzed as a whole. Moreover, little is understood on whether the effect of the natural form of this vitamin may be similar to the observed in the synthetic form due to the fact that these forms have different metabolic pathways or absorption processes (18,19). Therefore, the aim of this study was to examine the association between folate intake and a score based on the components of MetS (MetS score) and its components among older adults with overweight/obesity and MetS participating in the PREDIMED-Plus trial.

Material and Methods

Study population

This study is based on the cross-sectional analysis of baseline data collected from the 6874 participants recruited in the PREDIMED-Plus trial. This is a 6-year parallel-group, multicenter and randomized clinical trial designed to evaluate the effect of an energy-restricted Mediterranean diet, physical exercise and behavioral therapy compared to a 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) and the protocol with more detailed information is available on the website http://www.predimedplus.com/ and in previous publications (20,21). Briefly, participants included men aged 55-75 years and women aged 60-75 years, with body mass index (BMI) ≥27 to <40 kg/m^2 complying with at least three criteria of MetS and not suffering of CVD at enrollment (22). After excluding participants with missing data for the main variables and with implausible values for the 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. All participants provided written informed consent, and the trial was approved by de Intuitional Review Board of all the recruitment centers where the study was conducted.

Folate intake assessment

Dietary folate intake was assessed with a validated 143-item semi-quantitative food frequency questionnaire (FFQ) (23). The FFQ was administered to participants at baseline by trained interviewers. Participants were asked about the frequency of consumption of each food item during the previous year. The questionnaire included 9 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 per day, 4-6 times a day, and more than 6 times a day). Nutrient values and energy content of food were obtained from the Spanish food composition tables (24,25). To estimate folate intake and total energy intake, the frequency of use for each food item was multiplied 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 calculate residual (26). The folate intake was analyzed as a continuous variable (per 100 mcg/d increment) and categorized into quintiles.

Metabolic syndrome score and its components.

Weight, height, waist (at the midpoint between the lowest rib and the iliac crest in a horizontal plane) and hip circumference were measured in duplicated with light clothing and no shoes using a calibrate 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) as waist circumference (cm) divided by hip circumference (cm). Blood pressure was measured three times with a validated semiautomatic oscillometer after 5 minutes of rest in-between measurement (Omron HEM-705CP, Hoofddorp, The Netherlands), and the mean of the three readings was used. After an overnight fast, blood samples were collected at baseline 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 by standard enzymatic methods in automatic analyzers in local laboratories.

A MetS score was based on the definition by the World Health Organization (22) and was computed based on Franks et al formula (27). 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 due to the fact that this information was not analyzed in this study. In parallel, standardized components of 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 METS-min/day using the validated Regicor Short Physical Activity Questionnaire (28), information regarding medication use (antihypertensive, hypolipidemic, diabetes, and vitamin supplement), 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 the validated 14-item questionnaire (29). Alcohol intake in grams per day were estimated using the validated FFQ (23).

Statistical analysis.

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

Multiple robust linear regression using 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 (30).

Models were adjusted for potential confounders based on previous literature, and for those variables related to the outcome (based on likelihood ratio: tests with a p value of < 0.10) or if the effect estimates for the exposure of interest changed by ≥ 10% when they were excluded from the model. Finally, four models were examined: Model 1 was adjusted for sex, age (continuous) and total energy intake; Model 2 included the variables in model 1 plus 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), and alcohol intake in grams per day; Model 3 included he variables in model 2 plus antihypertensive (no/yes), hypolipidemic (no/yes) and diabetes (no/yes) medication use and vitamin supplements use (no/yes), and Model 4 accounting for the variables in model 3 plus 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, several sensitivity analyses were conducted: a) excluding patients with prevalent diabetes; b) excluding patients with familiar history of stroke; c) excluding patients with familiar history of cardiac disease; d) excluding patients with vitamin supplements use; e) stratifying by sex; and g) stratifying by median value of vitamin B12. Statistical interactions were tested by means of likelihood ratio test, comparing the full adjusted model of the linear robust regression with and without cross-product terms between the aforementioned variables and per 100-mcg/d increment of energy-adjusted folate intake.

Statistical analyses were conducted with R 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria; http://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 on March 2019.

Results

Baseline characteristics according to quintiles of energy-adjusted folate intake are shown in the table 1. The mean of age, physical activity, adherence to energy-restricting Mediterranean diet, and vitamin B12, and the percentage of women, hypolipidemic medication use, and vitamin supplement use increased across quintiles of folate intake. In contrast, the percentage of current smoker and the mean of alcohol intake decreased across 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 2 presents the results of the multiple robust linear regression analysis for the association between energy-adjusted folate intake (in quintiles and in continuous) and MetS score and its components after adjusting for potential confounders. We observed a reduction of MetS score, expressed in units of SD, according to quintiles of energy-adjusted folate intake (p<0.001). Compared with 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 (IC 95%: -0.54 to -0.20) in 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 17-point screener of Mediterranean diet adherence did not change the statistical association between quintile of energy-adjusted folate intake and z-MetS 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 MetS score in the multiple adjusted model 2 and model 3 respectively.

Regarding, the components of MetS score, 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 (i.e. analyzed as continuous as well as quintiles of intake) when performing model 2. However, when the model was adjusted for the 17-point screener of Mediterranean diet adherence (i.e. model 3), 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 with plasma fasting glucose according to quintiles of energy-adjusted folate intake (p-trend <0.001). The results of model 3 also showed that the fifth quintile of energy-adjusted folate intake (>416 mcg per day) compared to that of the first quintile

(>275 mcg per day) was associated an increase of 0.13 points (IC 95%: 0.06 to 0.21) in SD of HDL-cholesterol and a decrease of 0.10 points (IC 95%: -0.15 to -0.04) in SD of plasma fasting glucose. Moreover, an increase of 0.07 points (IC 95%: 0.04 to 0.10) in SD of HDL-cholesterol and decrease of 0.03 points (IC 95%: -0.05 to -0.02) in SD of plasma fasting glucose per 100 mcg per day increase of energy-adjusted folate intake were observed. The effect of dietary folate intake (not energy-adjusted) on MetS score, HDL-cholesterol and plasma fasting glucose was on average similar to that observed when including energy-adjusted folate intake (Figure 1).

Table 3 displays sensitivity analyses of the association between energy adjusted folate intake per 100 mcg per 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 familiar 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, and vitamin B12 intake and the observed association between energy adjusted folate intake and MetS score, HDL-cholesterol and plasma fasting glucose was no statistically significant. Nonetheless, the magnitude of association between energy-adjusted folate intake per 100 mcg per day increase and MetS score, HDL-cholesterol and plasma fasting glucose was greater in men than in women (β=-0.15, 95% CI: -0.26 to -0.04; β=0.09, 95% CI: 0.05 to 0.13; and β=-0.05, 95% CI: -0.08 to -0.02, respectively), and in those with vitamin b12 intake equal or less than median (β=-0.18, 95% CI: -0.28, -0.08; β=0.07, 95% CI: 0.03, 0.12; and β=-0.04, 95% CI: -0.07, -0.01, respectively).

Discussion

This study suggests that an increase of folate intake (in quintiles or per 100 mcg per day) was associated with a reduction in MetS score after adjusting for potential confounders, including the adherence to Mediterranean diet. Moreover, a higher folate intake was associated with a lower plasma fasting glucose and a higher plasma concentration of HDL-cholesterol independently of Mediterranean diet and other factors.

As far as we know, no previous published studies conducted in adults have explored the effect of the folate intake on a score based on the components of MetS calculated as a continuous variable. The MetS score has emerged as an alternative to the definition based on dichotomous variables and it is considered a valid tool for research evaluating cardiometabolic risk in different age groups including adults (31). However, this score has not been sufficiently used yet, and the evidence of the association between folate intake and specific components of MetS is still scarce and inconclusive. Most of the performed studies carried out have been clinical trials focused on the therapeutic effect of folic acid supplementation (13–17,32).

In our study, we observed that a higher folate intake is associated with a lower concentration of plasma fasting glucose. In concordance with our findings, a meta-analysis conducted by Zhao and colleagues with 10 randomized clinical trials reported that folic acid supplements were associated with a reduction of plasma fasting glucose compared to placebo (17). In addition, a meta-analysis conducted by Akbari and colleagues among patients with metabolic diseases supported that, compared to placebo, the folic acid supplementation reduced fasting plasma glucose, although the association was not statistically significant (standardized mean difference –0.30; 95 % CI, –0.63, 0.02) (33).

Regarding the effect of folic acid supplementation on HDL cholesterol, a recent meta-analysis including 10 clinical trials with patients suffering of metabolic diseases concluded that the effect was not sufficiently consistent between studies due to the high heterogeneity observed (34). The main reasons reported were the presence of differences in the patient characteristics, dosages and timing of folic acid supplements. However, in agreement with our findings, two double-blind randomized placebo-controlled trials, conducted with 60 patients with MetS and 74 obese women respectively, found that those participants receiving 5mg of folic acid supplements for 12 weeks had higher HDL cholesterol levels compared with those in the placebo group (15,35).

The biological mechanisms by which the folate (natural and synthetic form) may be related to MetS score, plasma fasting glucose and HDL-cholesterol are not still fully understood. One possible explanation may be due to the fact that folate can reduce circulation concentrations of homocysteine, which may be a potential mediator improving lipid metabolism and endothelial dysfunction (9–13). Moreover, it has been speculated that methyl donors as folate may reduce oxidative stress and systemic inflammation, which can have a positive effect on the normal regulation of insulin secretion from the pancreatic β-cells and glycemic control (36–38). A tentative explanation about the positive relationship observed between folate intake and HDL-cholesterol might be given by the fact that both factors take part in the improvement of the synthesis and bioavailability of oxide nitric (11,12). Furthermore, it has been also documented that the defects in DNA methylation are associated with metabolic diseases, suggesting that our findings could be explained by the crucial role of folate in DNA metabolism (39). In addition, a previous study conducted by Ramos-Lopez et al showed a folate deficiency can be related with insulin resistance in people with obesity(38).

Strengths of the present study include the large sample size, as well as the detailed and quality information collected by trained interviewers. Additionally, the associations found remained after adjusting for Mediterranean diet adherence and other potential confounders and the results obtained from sensitivity analysis reinforced the strength of these findings.

Nevertheless, our study has also some limitations. The cross-sectional analysis of our data prevents us from establishing a causal link between folate intake and MetS score, plasma fasting glucose and HDL-cholesterol; however, it does constitute a suitable rationale for replicating in other samples using a longitudinal study design. Moreover, it should be noted that possible reverse causation must not be disregarded. Another limitation is that the participants from the PREDIMED-plus study were elderly individuals with specific clinical conditions, thereby avoiding us to extrapolate the findings of this study to the general population. Although we adjusted for a wide range of potential confounding factors including the adherence to Mediterranean diet, residual confounding by unknown factors cannot be ruled out. Regarding the dietary data, the use of a food frequency questionnaire to estimate folate and energy intake is subject to possible misclassification errors but any inaccuracy in reporting should be non-differential. This potential bias can be minimized by using of a carefully designed and validated food-frequency questionnaire such as ours (23). 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 MetS patients. Pending confirmation from further observational longitudinal or experimental studies, investigating the effect of a higher intake of vegetables, fruits, legumes and cereals as main sources of folate may represent an approach to reduce the risk of cardiovascular disease and diabetes.

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Table 1. 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

Abbreviations: BMI, Body mass index; HDL, High-density lipoprotein-cholesterol; MET, metabolic equivalent of task. [a] From the $\chi 2$ test (categorical variables), and analysis of variance (continuous variables)

Table 2. 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]							
Age, energy & sex adjusted	Ref.	-0.14 (-0.31; 0.03)	-0.09 (-0.26; 0.09)	-0.33 (-0.51; -0.15)	-0.36 (-0.53; -0.18)	<0.001	-0.15 (-0.21; -0.08)
Multiple adjusted 1	Ref.	-0.13 (-0.31; 0.04)	-0.07 (-0.24; 0.10)	-0.30 (-0.48; -0.12)	-0.31 (-0.49; -0.14)	<0.001	-0.13 (-0.20; -0.07)
Multiple adjusted 2	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 3	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]							
Age, energy & sex adjusted	Ref.	-0.02 (-0.10; 0.06)	-0.03 (-0.11; 0.05)	-0.07 (-0.16; 0.01)	-0.05 (-0.14; 0.03)	0.115	-0.03 (-0.06; 0.00)
Multiple adjusted 1	Ref.	-0.02 (-0.10; 0.06)	-0.02 (-0.10; 0.06)	-0.05 (-0.13; 0.04)	-0.02 (-0.10; 0.06)	0.574	-0.01 (-0.04; 0.02)
Multiple adjusted 2	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 3	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]							
Age, energy & sex adjusted	Ref.	-0.07 (-0.15; 0.00)	-0.10 (-0.18; -0.03)	-0.13 (-0.20; -0.05)	-0.14 (-0.22; -0.17)	<0.001	-0.06 (-0.09; -0.03)
Multiple adjusted 1	Ref.	-0.07 (-0.14; -0.01)	-0.09 (-0.17; -0.02)	-0.11 (-0.18; -0.03)	-0.12 (-0.20; -0.05)	0.002	-0.05 (-0.08; -0.02)
Multiple adjusted 2	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 3	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]							
Age, energy & sex adjusted	Ref.	0.00 (-0.08; 0.07)	0.08 (0.01; 0.16)	-0.05 (-0.13; 0.02)	-0.07 (-0.14; 0.01)	0.020	-0.03 (-0.05; 0.00)
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)	0.074	-0.02 (-0.05; 0.01)

Multiple adjusted 2	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 3	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)

Continued table 2

	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**
Diastolic blood pressure[b]							
Age, energy & sex adjusted	Ref.	0.02 (-0.06; 0.10)	0.14 (0.06; 0.22)	0.01 (-0.07; 0.09)	0.02 (-0.06; 0.10)	0.990	0.01 (-0.01; 0.04)
Multiple adjusted 1	Ref.	0.02 (-0.05; 0.10)	0.15 (0.07; 0.23)	0.02 (-0.05; 0.10)	0.02 (-0.05; 0.10)	0.621	0.02 (-0.01; 0.05)
Multiple adjusted 2	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 3	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]							
Age, energy & sex adjusted	Ref.	0.00 (-0.07; 0.08)	0.07 (0.00; 0.14)	0.08 (0.01; 0.16)	0.08 (0.01; 0.16)	0.008	0.05 (0.02; 0.07)
Multiple adjusted 1	Ref.	0.01 (-0.06; 0.08)	0.10 (0.03; 0.17)	0.12 (0.05; 0.19)	0.13 (0.05; 0.20)	<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 (0.06; 0.20)	0.14 (0.06; 0.21)	<0.001	0.07 (0.04; 0.09)
Multiple adjusted 3	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]							
Age, energy & sex adjusted	Ref.	-0.02 (-0.08; 0.03)	-0.03 (-0.08; 0.03)	-0.03 (-0.08; -0.01)	-0.07 (-0.12; -0.01)	0.012	-0.03 (-0.05; -0.01)
Multiple adjusted 1	Ref.	-0.01 (-0.07; 0.05)	-0.01 (-0.07; 0.05)	-0.04 (-0.07; 0.05)	-0.04 (-0.10; 0.01)	0.127	-0.02 (-0.04; 0.00)
Multiple adjusted 2	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 3	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]							
Age, energy & sex adjusted	Ref.	0.00 (-0.05; 0.05)	-0.01 (-0.06; 0.04)	-0.03 (-0.08; 0.02)	-0.07 (-0.12; -0.01)	0.005	-0.03 (-0.04; -0.01)
Multiple adjusted 1	Ref.	0.00 (-0.05; 0.05)	0.00 (-0.05; 0.05)	-0.01 (-0.07; 0.05)	-0.05 (-0.10; 0.00)	0.047	-0.02 (-0.04; 0.00)
Multiple adjusted 2	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 3	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)

Abbreviations: 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; Multiple adjusted 1: Additionally adjusted for educational level (primary, secondary or university/graduate), smoking status (never, former or current), alcohol intake (grams per day), and total physical activity (METS-min/day); Multiple adjusted 2 Additionally adjusted for antihypertensive medication (yes/no), diabetes medication (yes/no), and hypolipidemic medication (yes/no), and vitamin supplements use (yes/no); Multiple adjusted 3: Additionally adjusted for 17 point screener of Mediterranean diet adherence (continuous)

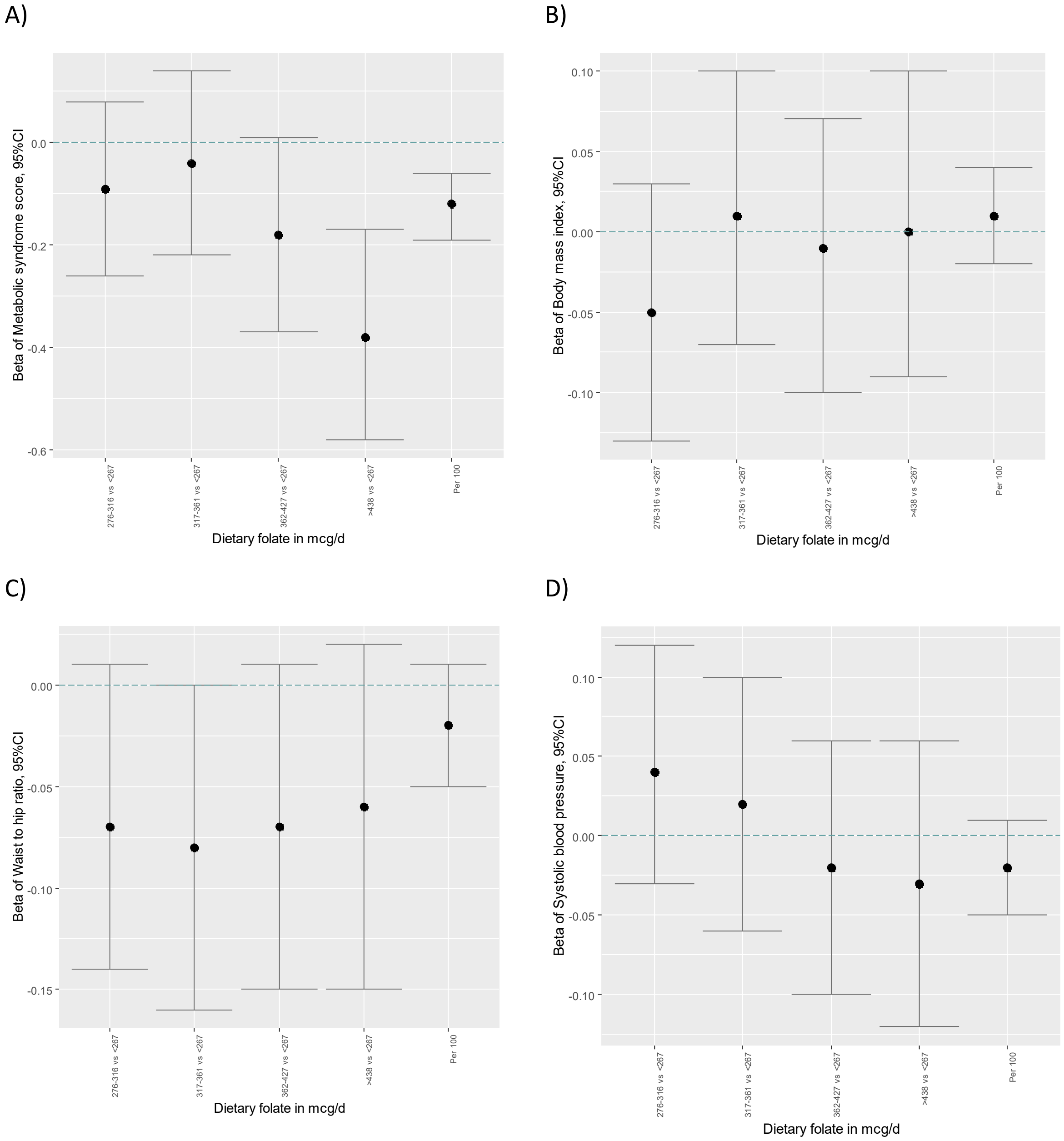
Table 3. Sensitivity analyses exploring the association between 100 mcg/d increment of energy-adjusted folate intake and z metabolic syndrome score, HDL cholesterol and plasma glucose components at baseline in participants PREDIMED-PLUS study (n=6633)

	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

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

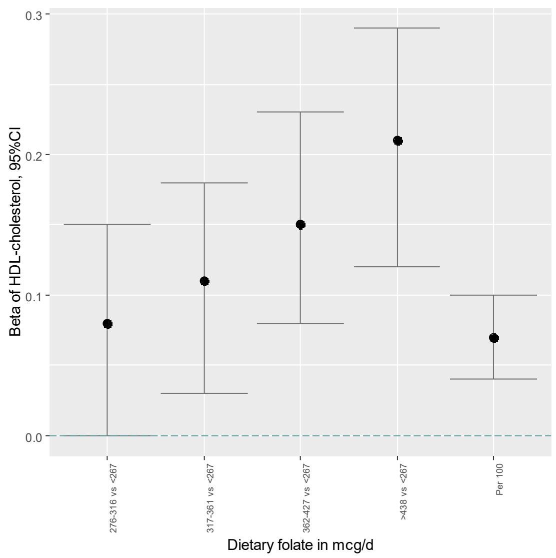
(METS-min/day), antihypertensive medication (yes/no), diabetes medication (yes/no), and hypolipidemic medication (yes/no), vitamin supplements use (yes/no); and 17 point screener of Mediterranean diet adherence (continuous)

Figure 1. Multiple adjusted β[a] (95%CI) for z metabolic syndrome score and their individual components according to folate intake (in quintiles and continuous) at baseline in participants PREDIMED-PLUS study (n=6633)



Continued Figure 1

E)



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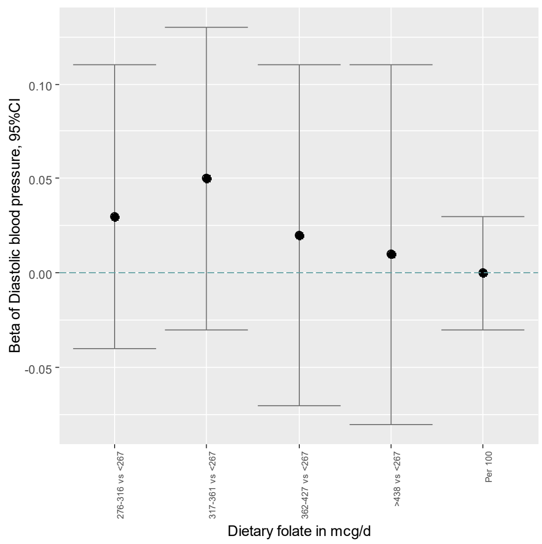

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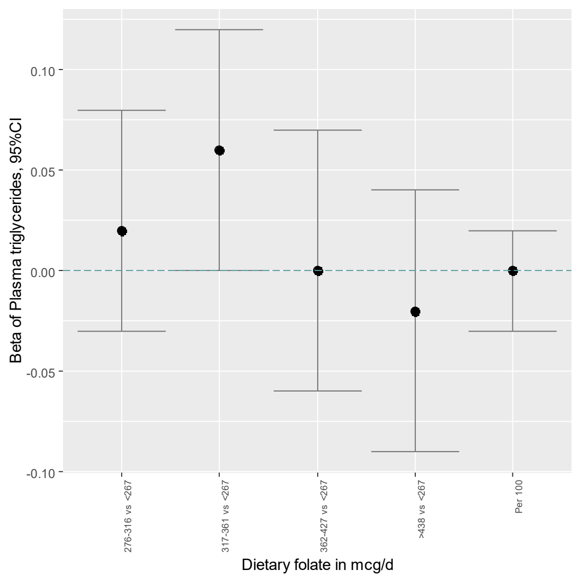
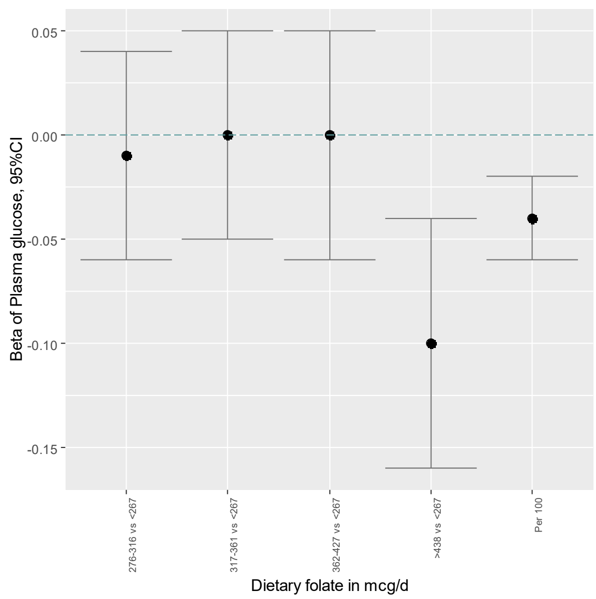


H)



a. Multiple adjusted for sex (male, female), energy(kcals/d), age (in years), 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) in A) Z metabolic syndrome score; B) Z body mass index; C) Z waist to hip ratio; D) Z systolic blood pressure; E) Z diastolic blood pressure; F) Z HDL-cholesterol; G) Z plasma triglycerides; H) Z plasma glucose.