Adherence to the Mediterranean diet is associated with lower platelet and leukocyte counts: results from the Moli-sani study

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Key Points

- Adherence to a Mediterranean diet is associated with reduced platelet and leukocyte counts.
- The observed associations are partially explained by the high dietary fiber and antioxidant content of the Mediterranean diet.

Platelet (PLT) and white blood cell (WBC) counts are 2 markers of inflammation and have been linked to the risk for cerebrovascular and coronary heart disease. A Mediterranean diet (MD) has been associated with reduced inflammation and mortality for major chronic diseases. We aimed at evaluating the association between the MD and both PLT and WBC counts. This cross-sectional analysis in a population-based cohort study included 14 586 healthy Italian citizens enrolled within the Moli-sani study. Adherence to MD was appraised by either the MD Score (MDS) or the Italian Mediterranean Index (IMI). PLT and WBC counts were both inversely related to MD adherence (MDS: *P* < .0001 and *P* = .008, respectively). As compared with those with poorer MD adherence, subjects with greater adherence had both reduced odds of being in the highest PLT-count group (MDS: odds ratio = 0.50; 95% confidence interval, 0.31-0.80) and increased odds of being in the lowest WBC-count group (IMI: odds ratio = 1.41; 95% confidence interval, 1.07-1.86). The association between WBC count and MDS disappeared when further adjusted for PLT count, whereas the association between PLT count and the MD was not affected by adjustment for WBCs. Food antioxidant and dietary fiber content modified the inverse association between MDS and WBC count and partially accounted for the association with PLTs. (*Blood*. 2014;123(19):3037-3044)

Introduction

Increased platelet (PLT) and white blood cell (WBC) counts have been recognized as possible markers of inflammation and of greater risk for cerebrovascular and coronary heart disease. In particular, PLT count has been associated with vascular[1] and nonvascular death, including cancer.[2] WBC count is a predictor of fatal and nonfatal ischemic vascular disease independent of other traditional cardiovascular disease (CVD) risk factors[3,4] and is a broadly used marker of systemic inflammation.[5]

Conversely, a Mediterranean dietary pattern has been associated with a reduction of low-grade inflammation[6] and mortality for cardiovascular and neurodegenerative diseases.[7] The Mediterranean diet (MD) is an eating pattern typical of the Mediterranean basin and is characterized by a wide consumption of plant foods, cereals, legumes, fish, olive oil as main fat source, and moderate (red) wine consumption.[8]

The beneficial effects of MD on health have been ascribed to its high content of antioxidants, fiber, monounsaturated fatty acids, and polyunsaturated fatty acids (PUFAs).[9] In particular, antioxidants and polyphenols have been shown to exert a positive role against ischemic vascular disease mainly due to their anti-inflammatory properties.[10,11]

Dietary fiber intake was associated with benefits for cardiovascular health and reduced risk of cardiovascular mortality[12,13]; similarly, a balanced ratio of ω-6 and ω-3 essential fatty acids has been suggested to yield favorable effects on cardio- and cerebrovascular health.[14]

The aim of this study was to investigate the association of an MD with PLT or WBC count based on the hypothesis that a diet rich in healthy compounds could favorably influence these 2 cellular biomarkers of low-grade inflammation in subjects without any overt chronic or hematologic disease.

Because adherence to the MD and both cell counts appeared to be inversely associated, we tested the possible role played by food antioxidant content (FAC), dietary fiber, and PUFA intake in accounting for the observed relationship.

Methods

Study population

A population-based cohort of 24 325 citizens of Molise, a region located between central and southern Italy, were enrolled in the Moli-sani study. Between March 2005 and April 2010, men and women aged ≥35 years were randomly recruited from subjects included in the city-hall registries of Molise.[15]

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For the present study, individuals from the Moli-sani cohort reporting at baseline a personal history of CVD (angina, myocardial infarction, heart failure, revascularization procedures, and stroke; 6.0%) or cancer (3.1%), those for whom there were no data on PLT (2.7%) or WBC count (2.7%), those for whom there were unreliable dietary or medical questionnaires (3.9% and 1%) or missing values for the FAC score (3.5%), those reporting hepatitis B or C or an hematologic disease (2.9% and 2.2%, respectively), and individuals recruited at the site of Termoli (due to a different cell counter used; n = 5107; 21.0%) were excluded from the analysis. The final sample comprised 14 586 individuals. Comparison between the whole Moli-sani cohort (n = 24 325) and the analyzed sample showed homogeneity for sex, PLT and WBC counts, and both the dietary scores (all P values $> .05$), but the sample was slightly younger (54.2 ± 11.5 vs 55.8 ± 12; $P < .0001$).

The Moli-sani study was approved by the ethics committee of the Catholic University of Rome. All participants signed an informed consent before taking part in the study. This study was conducted in accordance with the Declaration of Helsinki.

Dietary information

Food intake was determined by the validated Italian version of the "European project investigation into cancer and nutrition" food frequency questionnaire.[16,17] The questionnaire, computerized with tailor-made software, allowed us to interview participants in an interactive way, including illustrations of sample dishes of definite sizes or by reference to standard portion sizes. To simplify the interpretation of data and to minimize within-person variations in intake of individual foods, 188 food items were classified into 45 predefined food groups on the basis of similar nutrient characteristics or culinary usage.

We evaluated the adherence to the MD by using the MD Score (MDS) developed by Trichopoulou et al.[18] Briefly, scoring was calculated in a population free from CVD, cancer, or diabetes and was based on the intake of the following 9 items: vegetables, legumes, fruits and nuts, dairy products, cereals, meat and meat products, fish, alcohol, and monounsaturated to saturated fat ratio. For most items, consumption above the study median received 1 point; all other intakes received 0 points. For dairy products, meat, and meat products, consumption below the median received 1 point. Medians were gender specific. For ethanol, men who consumed 10 to 50 g/day and women who consumed 5 to 25 g/day received 1 point; otherwise, the score was 0. The possible scores ranged between 0 and 9, the latter reflecting the maximal adherence to the MD.

In addition, we used the Italian Mediterranean Index (IMI), recently proposed to better capture some healthy foods more typically consumed in Italy, such as pasta.[19] This IMI score was based on the intake of 11 items: high intake of 6 typical Mediterranean foods (pasta; typical Mediterranean vegetables such as raw tomatoes, leafy vegetables, onion and garlic, salad, and fruiting vegetables; fruit; legumes; olive oil; and fish), low intake of 4 non-Mediterranean foods (soft drinks, butter, red meat, and potatoes), and alcohol consumption. If consumption of typical Mediterranean foods was in the third tertile of the distribution, the person received 1 point; all other intakes received 0 points. If consumption of non-Mediterranean foods was in the first tertile of the distribution, the person received 1 point. Ethanol received 1 point for intake up to 12 g/day; abstainers and persons who consumed >12 g/day received 0 points. Possible scores ranged from 0 to 11, the latter reflecting the maximal adherence to MD.[19]

Total FAC score was used to measure the antioxidant content of the diet, as previously described.[20] Briefly, the content in antioxidant vitamins and phytochemicals of each food group was derived by using the food composition tables from the Italian Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione and the US Department of Agriculture. Healthy foods, according to a Mediterranean eating pattern, were categorized into either high or low antioxidant content. The total FAC score was constructed for a comparative evaluation of the consumption of these 2 groups. Coffee, chocolate, and wine were not included in the analysis, despite their high antioxidant content, because their healthiness is reportedly limited to moderate consumption only.[21] The following items were considered as main antioxidant components of a healthy diet: selenium; vitamins C, A, and E; tocopherol-β, -γ, and -δ; carotene-β and -α; lycopene; lutein; and all types of flavonoids. PUFAs (evaluated as linoleic acid ω-6 to linolenic acid ω-3 ratio) or fiber intake from the diet was expressed as g/day.

Anthropometric measurements and assessment of risk factors

Body mass index (BMI) was calculated as kg/m^2. Waist circumferences were measured according to the National Heart, Lung and Blood Institute guidelines.[22] High blood pressure (BP) was defined as systolic BP ≥140 mm Hg and/or diastolic BP ≥90 mm Hg or by pharmacologic treatment.[23] Hyperglycemia was defined if glucose level was ≥126 mg/dL or by pharmacologic treatment. Hypercholesterolemia was defined if total cholesterol level was ≥240 or by antihyperlipidemic treatment. C-reactive protein (CRP) was measured by a high-sensitivity assay (IL-Coagulation Systems ACL9000; IL, Milan, Italy).

Subjects were classified as nonsmokers, ex-smokers (having smoked cigarettes in the past and having quitted for at least 1 year), and current smokers.[24]

Physical activity was assessed by a structured questionnaire (24 questions on working and leisure time and sport participation) and expressed as daily energy expenditure in metabolic equivalent task-hours.

Global individual cardiovascular risk was calculated applying the risk equations of the CUORE project.[25] It predicts the 10-year risk of vascular fatal or nonfatal events. For men, the cardiovascular risk was categorized as low (<3%), medium (≥3% and <20%), or high (≥20%) risk. For women, low CVD risk was set at <3%, whereas the high-risk group was defined as CVD risk ≥3%.

PLT and WBC count ranges

Low or high PLT count were set according to age/sex-specific cutoffs.[26] Low PLT count (thrombocytopenia) was defined as PLT $<156 \times 10^9$/L or $<140 \times 10^9$/L for women aged <64 years or >64 years, respectively; for men, low PLT count was defined as PLT $<141 \times 10^9$/L or $<122 \times 10^9$/L for men aged <64 years or >64 years, respectively.

Cutoffs for high PLT count (thrombocytosis) were set when PLT $>405 \times 10^9$/L or $>379 \times 10^9$/L for women aged <64 years or >64 years, respectively; high PLT count was defined as PLT $>362 \times 10^9$/L or $>350 \times 10^9$/L for men aged <64 or >64 years, respectively.

High (leukocytosis) or low (leukopenia) WBC categories were defined as WBC $<4 \times 10^9$/L or WBC $>10 \times 10^9$/L, respectively.[27]

Neutrophil granulocytes to lymphocyte ratio (NLR) was also evaluated as a marker of inflammation.[28]

Statistical analyses

Values for continuous variables are means ± standard deviation (SD). Analysis of variance for continuous or categorical variables was applied to test the associations in Tables 1 and 2. Multivariable linear regression analysis was used for testing the association between PLT or WBC count with adherence to the MDS, FAC, or dietary fiber (considered as continuous independent variables; Table 3).

By using multivariable logistic regression analysis, odds ratios (ORs) with corresponding 95% confidence intervals (CIs) were calculated to quantify the association between PLT or WBC categories and adherence to a Mediterranean eating pattern (Tables 4 and 5). Four categories of adherence to MD were considered, ranging from low (0-2 points, both for MDS and IMI) to high (≥7 points for MDS and ≥6 points for IMI) adherence. An ordinal logistic regression model was used to test whether the impact of diet could be influenced by the cutoff used in defining PLT or WBC groups. Because the assumption of proportional odds failed ($P < .0001$ for each test), we analyzed data by using 2 logistic regression models: one contrasting high PLT (WBC) vs the other group containing normal and low PLT (WBC), and the other contrasting low PLT (WBC) vs the other group containing normal and high PLT (WBC). FAC and dietary fiber were considered either as continuous variables or quartiles.

Both for linear and logistic regression analysis, covariates included in the models were age, sex, BMI, total energy intake, total physical activity, education, smoking, hyperglycemia, high BP, hypercholesterolemia, and

Table 1. Characteristics of the population sample by PLT or WBC count categories

	PLT count					WBC count			
Characteristics	All (n = 14 586)	Low (n = 272, 1.9%)	Normal (n = 13 946, 95.6%)	High (n = 368, 2.5%)	*P**	Low (n = 554, 3.8%)	Normal (n = 13 690, 93.9%)	High (n = 342, 2.3%)	*P**
Age (y; mean ± SD)	54.2 (11.5)	55.5 (11.7)	54.2 (11.5)	53.2 (11.8)	.020	55.2 (11.9)	54.2 (11.5)	52.9 (11.0)	.003
Sex (men; n, %)	6975 (47.8)	135 (49.6)	6670 (47.8)	170 (46.2)	.70	139 (25.1)	6627 (48.4)	209 (61.1)	<.0001
Body mass index (mean ± SD)	27.9 (4.7)	28.1 (4.7)	27.9 (4.7)	27.5 (4.9)	.15	26.0 (4.1)	28.0 (4.7)	28.6 (5.3)	<.0001
Physical activity (mean ± SD)	43.1 (9.0)	43.9 (10.8)	43.1 (9.0)	43.3 (9.5)	.35	43.5 (9.2)	43.1 (9.0)	43.2 (9.1)	.04
Smokers (n, %)	3507 (24.0)	68 (25.0)	3335 (23.9)	104 (28.3)	.07	58 (10.5)	3232 (23.6)	217 (63.4)	<.0001
Education (secondary or higher; n,%)	7212 (49.4)	114 (41.9)	6919 (49.6)	179 (48.6)	.07	285 (51.4)	6762 (49.4)	165 (48.2)	.08
Hypertension (n, %)	8069 (55.3)	144 (52.9)	7726 (55.4)	199 (54.0)	.41	248 (44.8)	7630 (55.7)	191 (55.8)	<.0001
Diabetes (n, %)	1196 (8.2)	28 (10.3)	1144 (8.2)	24 (6.5)	.24	18 (3.2)	1128 (8.3)	50 (14.6)	<.0001
Hypercholesterolemia (n, %)	4746 (32.5)	50 (18.4)	4578 (32.8)	118 (32.1)	<.0001	164 (29.6)	4464 (32.6)	118 (34.5)	.02
CRP levels (mg/dL)†	1.42 (0.73-2.60)	1.43 (0.61-2.35)	1.41 (0.73-2.59)	1.65 (0.76-3.38)	.0004	0.87 (0.48-1.78)	1.43 (0.74-2.59)	2.64 (0.37-5.14)	<.0001

**P* value adjusted for age and sex.
†CRP values are reported as median and lower-upper quartiles.

CRP. Further adjustments for FAC and dietary fiber were considered for testing the role of these compounds in explaining the association among PLTs, WBCs, and the MD.

Data were analyzed using SAS/STAT software version 9.1.3 of the SAS System for Windows 2009 (SAS Institute, Cary, NC).

Results

Table 1 shows the main characteristics of the population sample according to levels of PLT or WBC count. Subjects in the high-PLT group were younger and reported higher prevalence of hypercholesterolemia and increased CRP levels compared with those in the normal- or low-PLT count categories. Individuals in the high-WBC category were mainly men, younger, smokers, had a higher BMI and higher CRP levels, and showed a higher prevalence of high BP, hyperglycemia, and hypercholesterolemia (all $P < .05$). Both blood cell counts decreased by increasing levels of adherence to a Mediterranean eating pattern (Table 2). Mean PLT or WBC count slightly decreased according to quartiles of FAC ($P = .05$ and $P < .0001$ for PLTs and WBCs, respectively; Table 2) and to quartiles of dietary fiber intake ($P = .03$ and $P < .0001$ for PLT and WBC count, respectively; Table 2). PLT count was inversely associated either with MDS or IMI scores (multivariable adjusted model 2: $\beta = -1.21$; $P < .0001$ and $\beta = -1.28$; $P < .0001$, respectively; Table 3). Similarly, WBC count was inversely linked to an MD ($\beta = -0.022$ and $P = .008$; $\beta = -0.039$ and $P < .0001$ for MDS and IMI, respectively; Table 3). Further adjustment for CRP did modify the association for PLT or WBC count (multivariable model 3; Table 3).

Additional adjustments for FAC and fiber intake simultaneously were performed in order to account for the observed associations between PLTs or WBCs and adherence to the MD. The inclusion of these 2 dietary components in the multivariable models slightly reduced the association between PLT count and adherence to the MD (Table 3), whereas the relationship between WBC and MDS was reduced and no longer statistically significant (Table 3). On the contrary, further adjustment for the ω-6 (linoleic acid) to ω-3 (linolenic acid) fatty acids ratio did not modify the association of adherence to the MD with either PLT or WBC count (data not shown).

Multivariable logistic regression analysis was used to test the odds of being in the low or high category of WBC or PLT counts according to levels of adherence to a Mediterranean dietary pattern. Subjects with very high adherence to the MD reported significantly lower odds of being in the high-PLT count group compared with individuals with poor adherence (OR = 0.50; 95% CI: 0.31-0.80 and OR = 0.73; 95% CI: 0.52-1.02 for MDS and IMI, respectively; Table 4). The associations were partially explained by FAC and dietary fiber intake when the MD was measured according to IMI, because the strength of the association was reduced or was no longer statistically significant.

No significant relationship was found for the odds of being in the low-PLT-count groups (Table 4).

Table 2. Mean PLT and WBC count according to categories of adherence to the MD and FAC or dietary fiber quartiles

	n (%)	PLT count	WBC count
MDS			
Low (0-2)	1866 (12.8)	252.8 (64.2)	6.31 (1.60)
Low-medium (3-4)	5789 (39.7)	250.3 (63.3)	6.24 (1.66)
Medium-high (5-6)	5381 (36.9)	249.4 (61.5)	6.20 (1.57)
High (7-9)	1550 (10.6)	246.0 (57.2)	6.17 (2.19)
P for trend		.0013	.004
IMI			
Low (0-2)	3128 (21.4)	252.0 (62.4)	6.33 (1.72)
Low-medium (3)	2995 (20.5)	252.1 (64.4)	6.31 (1.97)
Medium-high (4-5)	5584 (38.3)	249.0 (62.0)	6.18 (1.55)
High (≥6)	2879 (19.7)	246.8 (59.9)	6.15 (1.56)
P for trend		.0001	<.0001
FAC quartiles			
First	3619 (24.8)	250.1 (63.5)	6.31 (1.74)
Second	3674 (25.1)	250.8 (64.1)	6.24 (1.55)
Third	3631 (24.9)	251.3 (60.6)	6.21 (1.56)
Fourth	3662 (25.1)	247.1 (60.5)	6.15 (1.86)
P for trend		.054	<.0001
Dietary fiber quartiles			
First	3646 (25.0)	250.6 (64.6)	6.35 (1.65)
Second	3647 (25.0)	251.3 (62.1)	6.21 (1.64)
Third	3647 (25.0)	249.6 (62.0)	6.18 (1.57)
Fourth	3646 (25.0)	247.9 (59.9)	6.19 (1.86)
P for trend		.03	<.0001

PLT and WBC counts are reported as mean (±SD). Means and *P* for trend value adjusted for age and sex. All the analyses with PLTs were further controlled for hematocrit.

Table 3. Multivariable linear regression analysis regarding the association between PLT or WBC and the MD and further adjusted for FAC or dietary fiber intake

	Model 1		Model 2		Model 3		Model 4	
Count	β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*
PLT								
MDS	−1.05 (−1.65 to −0.46)	.0005	−1.21 (−1.81 to −0.61)	<.0001	−1.15 (−1.75 to −0.55)	.0002	−0.85 (−1.56 to −0.135	.020
IMI	−1.21 −1.76 to −0.66)	<.0001	−1.28 (−1.83 to −0.72)	<.0001	−1.18 (−1.73 to −0.62)	<.0001	−0.98 (−1.66 to −0.29)	.005
WBC								
MDS	−0.024 (−0.04 to −0.01)	.004	−0.022 (−0.04 to −0.01)	.008	−0.019 (−0.03 to −0.003)	.019	−0.009 (−0.03 to 0.01)	.33
IMI	−0.042 (−0.06 to −0.03)	<.0001	−0.039(−0.05 to −0.02)	<.0001	−0.033 (−0.05 to −0.02)	<.0001	−0.033 (−0.05 to −0.01)	.0003

Model 1 included age and sex. Model 2 included age, sex, energy intake, BMI, physical activity, smoking, education, hypertension, diabetes, and hypercholesterolemia. Model 3 as in model 2 but further adjusted for CRP. Model 4 as in model 3 but further adjusted for FAC and dietary fiber intake. All the analyses with PLTs were further controlled for hematocrit.

Concerning WBC count, a very good adherence to the MD was significantly associated with an increased chance of being in the bottom-WBC-count group (Table 5), and the association was fairly well explained by FAC and dietary fiber intake, especially when adherence to the MD was measured by the MDS (Table 5). No significant relationship was found regarding the association between adherence to the MD and the high-WBC group.

NLR was significantly and inversely associated with adherence to the MD ($P = .0010$ and $P < .0001$ for MDS and IMI, respectively). In addition, leukocyte subgroup analysis showed an inverse relationship between adherence to the MD and either granulocyte ($P = .17$ and $P = .0001$ for MDS and IMI respectively) or monocyte ($P < .0001$ for both MDS and IMI, respectively) count and a positive link with lymphocyte count ($P < .0001$ for both dietary scores).

The association between WBC count and MDS (but not IMI) disappeared when further adjusted for PLT count ($\beta = -0.009$ and $P = .22$ in the multivariate model including CRP). On the contrary, the association between PLT count and both dietary scores remained significant after further controlling for WBC count.

Mean PLT count increased with increasing predicted CVD risk in men (low CVD risk: 236.5 ± 54.7, medium CVD risk: 239.6 ± 57.1, and high CVD risk: 247.1 ± 58.7; *P* for trend = .027 in multivariable analysis of variance). In women, we failed to observe any difference in PLT count within the predicted CVD risk groups.

Regarding WBC, we found a clear association with estimated CVD risk in men (low CVD risk: 5.88 ± 1.43, medium CVD risk: 6.79 ± 2.02, and high CVD risk: 7.59 ± 1.71; *P* for trend < .0001 in multivariable analysis of variance). The same trend was observed for women ($P < .0001$).

Discussion

Recent studies have suggested a significant relationship between the type of diet and chronic low-grade inflammation, an underlying pathophysiological mechanism linking behavioral factors and oxidative stress to the risk of developing chronic disease.[29] Increased WBC

Table 4. Odds of being in the high- or low-PLT category according to adherence to the MD and further adjusted for FAC or dietary fiber intake

	Model 2		Model 3		Model 4	
Category	ORs (95%CI)	*P**	ORs (95%CI)	*P**	ORs (95%CI)	*P**
High PLT (n = 368)						
MDS		.0013		.0016		.056
Low (0-2)	1		1		1	
Low-medium (3-4)	0.79 (0.59-1.07)		0.80 (0.59-1.09)		0.85 (0.62-1.16)	
Medium-high (5-6)	0.67 (0.49-0.92)		0.68 (0.50-0.94)		0.67 (0.54-1.09)	
High (7-9)	0.50 (0.31-0.80)		0.50 (0.31-0.81)		0.60 (0.36-1.02)	
IMI		.048		.064		.64
Low (0-2)	1		1		1	
Low-medium (3)	0.99 (0.74-1.36)		1.01 (0.74-1.37)		1.05 (0.77-1.43)	
Medium-high (4-5)	0.87 (0.66-1.14)		0.88 (0.67-1.16)		0.97 (0.72-1.31)	
High (≥6)	0.73 (0.52-1.02)		0.74 (0.53-1.04)		0.92 (0.61-1.36)	
Low PLT (n = 272)						
MDS		.31		.33		.84
Low (0-2)	1		1		1	
Low-medium (3-4)	0.98 (0.66-1.45)		0.97 (0.65-1.44)		0.92 (0.61-1.38)	
Medium-high (5-6)	1.03 (0.69-1.55)		1.02 (0.69-1.53)		0.91 (0.59-1.41)	
High (7-9)	1.28 (0.78-2.10)		1.27 (0.78-2.08)		1.08 (0.62-1.87)	
IMI		.09		.10		.39
Low (0-2)	1		1		1	
Low-medium (3)	1.27 (0.86-1.87)		1.25 (0.85-1.85)		1.22 (0.82-1.81)	
Medium-high (4-5)	1.24 (0.88-1.77)		1.23 (0.87-1.75)		1.15 (0.79-1.68)	
High (≥6)	1.45 (0.98-2.14)		1.43 (0.96-2.11)		1.27 (0.80-2.01)	

Model 2 included age, sex, energy intake, BMI, physical activity, smoking, education, hypertension, diabetes, and hypercholesterolemia. Model 3 as in model 2 but further adjusted for CRP. Model 4 as in model 3 but further adjusted for FAC and dietary fiber intake.

**P* for trend value.

Table 5. Odds of being in the high- or low-WBC category according to adherence to the MD and further adjusted for FAC or dietary fiber intake

	Model 2		Model 3		Model 4	
Category	**ORs (95%CI)**	***P****	**ORs (95%CI)**	***P****	**ORs (95%CI)**	***P****
High WBC (n = 342)						
MDS		.18		.26		.70
Low (0-2)	1		1		1	
Low-medium (3-4)	1.05 (0.74-1.48)		1.05 (0.74-1.50)		1.10 (0.77-1.57)	
Medium-high (5-6)	0.90 (0.63-1.29)		0.94 (0.66-1.35)		1.03 (0.69-1.52)	
High (7-9)	0.80 (0.49-1.30)		0.80 (0.49-1.31)		0.92 (0.53-1.59)	
IMI		.12		.24		.69
Low (0-2)	1		1		1	
Low-medium (3)	0.87 (0.63-1.19)		0.92 (0.67-1.27)		0.95 (0.69-1.32)	
Medium-high (4-5)	0.62 (0.47-0.84)		0.64 (0.48-0.87)		0.70 (0.51-0.96)	
High (≥6)	0.92 (0.67-1.27)		0.99 (0.71-1.38)		1.18 (0.79-1.75)	
Low WBC (n = 554)						
MDS		.078		.093		.40
Low (0-2)	1		1		1	
Low-medium (3-4)	1.24 (0.92-1.66)		1.21 (0.90-1.63)		1.17 (0.87-1.58)	
Medium-high (5-6)	1.41 (1.05-1.90)		1.37 (1.02-1.86)		1.28 (0.92-1.76)	
High (7-9)	1.26 (0.85-1.85)		1.25 (0.84-1.84)		1.11 (0.72-1.71)	
IMI		.0031		.0052		.031
Low (0-2)	1		1		1	
Low-medium (3)	1.01 (0.76-1.35)		1.00 (0.75-1.34)		1.00 (0.74-1.33)	
Medium-high (4-5)	1.30 (1.01-1.66)		1.27 (0.99-1.63)		1.25 (0.96-1.63)	
High (≥6)	1.41 (1.07-1.86)		1.39 (1.05-1.83)		1.34 (0.97-1.87)	

Model 2 included age, sex, energy intake, BMI, physical activity, smoking, education, hypertension, diabetes, and hypercholesterolemia. Model 3 as in model 2 but further adjusted for CRP. Model 4 as in model 3 but further adjusted for FAC and dietary fiber intake.

**P* for trend value.

count is widely recognized as a reliable biomarker of inflammation,[30] but PLTs have also been ascribed a role in inflammation due to the production and release of prostaglandins and other substances causing either vasodilation or vasoconstriction.[31-34]

Our study has investigated whether the adherence to a Mediterranean-style diet is a determinant of PLT or WBC count in a healthy population. Our results show that a greater adherence to the MD, measured by 2 widely used dietary scores, is significantly associated with a reduction in either PLT or WBC count in multivariable models controlling for many possible confounders. In addition to lifestyle covariates, we considered a model further adjusted for CRP. The latter had been previously shown to be inversely associated with a Mediterranean eating pattern[35] and directly linked both to PLT and WBC count.[27] The link between PLTs and inflammation is also supported by our present findings due to the direct association of PLTs with CRP, which is a recognized inflammatory marker. Yet, we exclude a role of CRP in explaining the association of PLT or WBC with a Mediterranean-type diet because further controlling for CRP had a minimal impact on our findings.

Similarly, thrombopoietin levels are reportedly increased in inflammatory conditions, and this may result in higher PLT counts.[36] Lack of data on thrombopoietin levels in our population sample did not allow us to test for the possible involvement of this protein in the association between PLT count and an MD.

The present study refers to subjects apparently free from clinically overt chronic disease and major hematologic pathologies. Thus, the differences observed among categories of PLT or WBC counts indicate substantial changes within normal ranges of variability in a healthy general population. Individuals with high adherence to the MD reported significantly reduced odds of being in the high-PLT group compared with those with poor adherence and an indicative trend in being in the lowest PLT group. Similarly, top adherence to MD was linked to increased odds of being in the bottom WBC count group.

WBC subgroup analyses revealed an inverse link between greater adherence to the MD and neutrophils or monocytes, which are the first WBCs involved in the inflammatory response.[37] Conversely, a positive association was found with lymphocytes, which are involved in the immune reactivity and have been previously shown to be positively stimulated by some dietary components such as antioxidants.[38] Moreover, we found an inverse association between the MD and the NLR ratio, which has been recently reported as a prognostic marker for CVD,[28] further confirming that this dietary pattern is mainly effective on the inflammatory components of WBCs. The relationship between WBC count and adherence to the MD (when measured by the MDS score) disappeared after further controlling for PLT, suggesting the important assumption that the observed associations between a Mediterranean eating pattern and WBC and PLT counts are mainly ascribed to the PLT count.

These observations suggest a positive effect of a Mediterranean eating pattern in reducing either PLT or WBC count and possibly the extent of chronic low-grade inflammation. These are in agreement with an intervention study in healthy Swedish subjects reporting that a supplementation of a kind of MD significantly reduced the number of both PLTs and WBCs.[39]

Further analyses were performed to find possible variables accounting for the observed associations. In particular, we tested the role played by FAC, dietary fiber, and PUFA intake as possible mediators. Both dietary antioxidant content and fiber partially accounted for the association between adherence to the MD and WBC count, suggesting a role of these healthy food components in the effect of the MD. Antioxidants and fiber intake also reduced the strength of the association between PLTs and the MD, leading to similar conclusions. The ratio of dietary ω-6 (linoleic acid) to ω-3 fatty acids (linolenic acid), whose lower value is more desirable in reducing the risk of major chronic diseases,[40] was significantly associated with WBCs but did not account for the relationship between WBCs and the MD.

The biological plausibility of the observed associations may rely on the close relationship between oxidative stress and chronic low-grade inflammation (eg, increased CRP, WBCs, and PLTs).[41] Considering that the high antioxidant and fiber content of the MD has been linked to reduced oxidative stress,[42,43] it is likely that the antioxidant properties of the MD may contribute to the reduction of subclinical inflammation by reducing the number of leukocytes and PLTs. It has been suggested that the change in the hematology pattern (lower inflammatory blood cells count) observed after supplementation of a Mediterranean-type diet to Swedish subjects could be linked both to a lower inflammatory activity or to a consistent decrease in vasoregulation and in the vascular endothelial growth factor concentrations requiring reduced endothelial cell repair processes.[43] Previous studies have shown that antioxidants are related to the inhibition of vascular endothelial growth factor release[44] and to lower inflammation.[11] This may explain a possible role of these healthy compounds in modulating the number of PLTs or WBCs in response to a low-grade chronic inflammatory status.

However, the dietary antioxidant and fiber content only partially explains the observed associations. This leads us to confirm the assumption that the diet as a whole, not a single food item, is responsible for the beneficial health outcomes reportedly documented.[45]

To our knowledge, this is the first large epidemiological study considering the traditional MD as a possible determinant of the number of PLT and WBC in an adult healthy population.

The observed association between higher adherence to a Mediterranean pattern and lower PLT or WBC counts suggests that this dietary pattern may contribute to reducing 2 important cellular biomarkers of chronic low-grade inflammation. This beneficial effect can be partly ascribed to its pivotal components as food antioxidants and dietary fiber.

Limitations of this study

A major limitation of this study is its cross-sectional nature, which cannot allow the inference of possible causality. Caution is also needed in extending the results presented here to larger population contexts, because data were collected in a region located between central and southern Italy, Mediterranean by tradition and culture.[15] Yet, the main characteristics of our population sample are comparable to those of the Italian Cardiovascular Epidemiological Observatory[46]: our sample could therefore be considered representative at least of the Italian population.[26]

The possibility of residual confounding cannot be entirely excluded, although our analyses have been adjusted for a very large panel of potential confounders.

In addition, we cannot estimate the real improvement, in terms of reduction of the risk of major clinical outcomes, that could be ascribed to the relatively small difference in mean PLTs observed between the lowest and highest group of adherence to the MD.

However, we should recognize that, at least in men, the changes in PLT count within the low and high categories of adherence to an MD are comparable to those observed within the low and high predicted cardiovascular risk groups.

Because the MD has been related to reduced mortality,[7] we might speculate that this effect could be partly explained by the association of this dietary pattern with lower PLT and WBC counts. However, we cannot exclude that lower PLTs and leukocytes could simply be a marker of adherence to the MD without any clinical significance.

Future studies should estimate to what extent a reduced number of PLTs and/or WBCs, within normal-range values, may contribute to the lowered risk of thrombosis (PLT)- or inflammation (WBC)-related diseases that is associated with high adherence to the MD.[7]

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Authorship

Contribution: M.B. and C.C. designed the research; S.C., A. De Curtis, and M.P. managed data collection; M.B. and A. Di Castelnuovo analyzed the data; M.B. wrote the paper; L.I., M.B.D., and G.d.G. originally inspired the research, obtained the financial support, and critically reviewed the manuscript; and all authors had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Appendix

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