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Higher adherence to the Mediterranean diet is associated with lower levels of D-dimer: findings from the MOLI-SANI study

D-dimer is a high molecular weight fibrinogen derivative resulting from the cleavage of cross-linked fibrin that reflects both thrombin production and/or activation of fibrinolysis.[1] Elevated D-dimer levels have been reportedly associated with increased risk of coronary artery disease[2,3] and more recently related to higher risk of ischemic stroke[4] and total mortality.[5]

Common cardiovascular risk factors cannot adequately explain the variability of D-dimer levels in a healthy population. So far, the relationship between D-dimer and dietary habits has not been well investigated, with the exception of a few intervention studies aimed at monitoring the effects of high-fat dietary supplementation on several coagulation factors, including D-dimer.[6]

The possible role of dietary habits on D-dimer on an epidemiological level has never been explored. The traditional Mediterranean diet (MD) is a healthy dietary pattern reportedly linked to lower mortality and risk of major chronic disease[7] and is characterized by a wide consumption of plant foods, cereals, legumes, fish, and olive oil. The anti-thrombogenic effect of a Mediterranean diet has been widely documented[7] and is partly ascribed to its high content of antioxidants, fiber, monounsaturated and polyunsaturated fatty acids.[7] In particular, antioxidants and polyphenols have been shown to exert a positive role against ischemic vascular disease mainly due to their anti-inflammatory properties. More recently, a Mediterranean diet has been associated with reduced platelet and leukocyte counts, two cellular biomarkers of low-grade inflammation.[8]

The aim of the present study is to investigate the association between D-dimer levels and adherence to a Mediterranean diet in a large community-based sample of the Italian population.

Analyses were conducted in the framework of the MOLI-SANI Project, a cohort study that from March 2005 to April 2010 had randomly enrolled 24,325 men and women aged 35 years or over from the general population of a Southern Italian region.[5,7,8] For the present study, individuals from the MOLI-SANI cohort reporting at baseline a personal history of cancer or cardiovascular disease (CVD), those for whom there were no data on D-dimer (10.3%) or C-reactive protein (CRP), or reporting CRP protein more than 10 mg/dL (4.0%), or unreliable dietary or medical questionnaires were excluded from the analysis. The final sample was made up of 17,403 subjects (47% men, mean age 54±11 years). Food intake was determined once at recruitment by the validated Italian version of the "European project investigation into cancer and nutrition" (EPIC) food frequency questionnaire.[9] Adherence to the Mediterranean diet was appraised by using the Mediterranean Diet Score developed by Trichopoulou *et al.*[10] D-dimer was measured once at recruitment on fresh citrated plasma by an automated latex-enhanced immunoassay (HemosIL-IL, Milan, Italy). Multivariable logistic regression was used to calculate the odds (and corresponding 95% confidence intervals) of having D-dimer more than 221 ng/mL in different categories of increasing adherence to MD. The cut off of 221 ng/mL was fixed according to previous studies in which this cut off discriminated between low and high risk of total mortality.[5]

Table 1 shows the main characteristics of the whole population sample according to quartiles of D-dimer levels. Subjects in the highest D-dimer categories were older, mainly women, non-smokers, with a low level of education. They reported higher prevalence of hypercholesterolemia, and increased CRP levels and Body Mass Index (BMI). In multivariable linear regression analysis adjusted for age, sex, BMI, total calories, hypertension, dyslipidemia, diabetes, smoking habits, education level, income, and total physical activity, the adherence to a Mediterranean diet was clearly associated with a decrease in D-dimer levels (Table 2). The difference in geometric means between individuals with the highest *versus* the lowest adherence to a Mediterranean diet was 15.1 ng/mL, i.e. 15.1% of the standard deviation of D-dimer distribution in the whole population. These findings did not change when D-dimer was further adjusted

Table 1. Characteristics of the study population according to quartiles of D-dimer levels.

	All	Quartiles of D-dimer				P**
		Q1	Q2	Q3	Q4	
N	17403	4392	4392	4396	4223	
D-dimer (mean±sd, ng/mL)	198 (100)	<142*	142-179*	179-1-220.9*	≥221*	
Age (mean±sd)	54.3 (11.3)	52.7 (9.6)	53.2 (10.7)	54.0 (11.1)	57.4 (13.0)	<0.0001
Male sex (n, %)	8135 (46.7)	2357 (53.7)	2238 (51.0)	2025 (46.1)	1515 (35.9)	<0.0001
Body mass index (mean±sd)	27.8 (4.6)	27.5 (4.4)	27.6 (4.5)	27.9 (4.6)	28.2 (5.0)	<0.0001
Physical activity (mean±sd)	43.3 (9.0)	43.6 (8.8)	43.4 (9.1)	43.4 (9.2)	42.9 (8.7)	0.79
Smokers (n, %)	4150 (23.9)	1181 (26.9)	1165 (26.6)	1025 (23.3)	779 (18.5)	<0.0001
Secondary or higher education (n, %)	8363 (48.1)	2313 (52.7)	2221 (50.6)	2025 (46.1)	1804 (42.8)	<0.0001
Hypertension (n, %)	9195 (52.8)	2200 (50.1)	2211 (50.3)	2337 (52.9)	2457 (58.1)	0.19
Diabetes (n, %)	1402 (8.1)	341 (7.8)	334 (7.6)	335 (7.6)	392 (9.3)	0.091
Hypercholesterolemia (n, %)	5203 (29.9)	1181 (26.9)	1335 (30.4)	1396 (31.8)	1291 (30.6)	<0.0001
C-reactive protein levels (mg/dL)	2.03 (1.89)	1.82 (1.74)	1.91 (1.79)	2.01 (1.86)	2.34 (2.06)	<0.0001
INFLA-Score	0.00 (1.00)	-0.13 (0.99)	-0.02 (0.98)	0.05 (1.00)	0.10 (1.02)	<0.0001

sd: standard deviation; INFLA-Score: low-grade inflammation score. *Adjusted for age and sex. **Minimum-Maximum.

Table 2. D-dimer levels and odds of having D-dimer 221 ng/mL or over according to Mediterranean Diet Score adherence.

Mediterranean Diet Score adherence	0-1 Low	2	3	4	5	6	7	8-9 High	*P*-trend*
No	613	1763	3073	3967	3696	2628	1270	393	
D-dimer (ng/mL)									
Geometric mean (95% CI)	189.3 (183.6-195.1)	187.5 (184.2-190.9)	185.5 (183.0-188.0)	183.4 (181.2-185.5)	180.0 (177.8-182.2)	175.9 (173.3-178.5)	175.6 (171.9-179.3)	174.2 (167.7-180.9)	<0.0001
D-dimer ≥221 ng/mL									
Prevalence (n, %)	173 (28.2%)	487 (27.6%)	788 (25.6%)	1011 (25.5%)	897 (24.3%)	552 (21.0%)	245 (19.3%)	70 (17.8%)	<0.0001
Odds ratio** (95% CI)	1	0.94 (0.76 to 1.16)	0.87 (0.71 to 1.06)	0.86 (0.70 to 1.04)	0.81 (0.66 to 0.99)	0.68 (0.55 to 0.84)	0.63 (0.50 to 0.79)	0.55 (0.40 to 0.76)	

CI: confidence interval. *Adjusted for age, sex, body mass index, total calories, hypertension, dyslipidemia, diabetes, smoking habit, education, income, total physical activity. **Odds of having D-dimer 221 ng/mL or over in comparison to D-dimer less than 221 ng/mL.

for CRP or for a composite score of low-grade inflammation (including CRP, white blood cells, platelets, and granulocytes to lymphocytes ratio). Multivariable logistic regression analysis was used to test the odds of having D-dimer values of 221 ng/mL or over (in comparison with D-dimer values <221 ng/mL) according to levels of adherence to a Mediterranean dietary pattern. Odds ratios of having D-dimer values of 221 ng/mL or over decrease according to the increased adherence to a Mediterranean diet (Table 2). Table 3 shows the odds of having D-dimer of 221 ng/mL or over associated with a 2-point increase in the Mediterranean Diet Score in specific subgroups at high CVD risk. Data clearly show that the association of MD score with D-dimer levels was homogenous across strata (Table 3) and no *P*-value for difference of effect was statistically significant (*data not shown*). Data also indicate that each food item of the Mediterranean diet contributed equally to the association of the Mediterranean Diet Score with D-dimer levels, because when each component of the score was removed, the odds ratio remained practically unchanged (*data not shown*).

Our results show that a greater adherence to the Mediterranean diet is significantly associated with a reduction in D-dimer levels in multivariable models controlling for a number of possible confounders. The association was independent of a large panel of covariates, was not driven by a specific food item, and was homogenous across strata at different CVD risk. The antithrombogenic effect of a Mediterranean diet has been widely documented and partly ascribed to its high content of antioxidants, fiber, and monounsaturated and polyunsaturated fats.[7] In particular, antioxidants and polyphenols have been shown to exert a positive role against ischemic vascular disease.[11] To the best of our knowledge, this is the first study to find that high adherence to a Mediterranean diet is associated with lower D-dimer levels in a large apparently healthy adult population. The differences in D-dimer levels according to adherence to a Mediterranean diet are much higher (in percentage of population variability) than those observed for CRP levels. The observed association was independent of a large panel of potential covariates, including CRP and a composite score of low-grade inflammation, and was not driven by a specific food item. D-dimer levels correlate with inflammatory markers, and adherence to a Mediterranean diet is associated with a reduced inflammatory condition. This evidence constitutes a possible explanation of the findings of the present manuscript; however, D-dimer levels remain lower in subjects with higher adherence to a Mediterranean diet also after further controlling for CRP or for a composite score of low-grade inflammation (including CRP, white blood cell, platelets, and granulocytes to lymphocytes ratio).

Table 3. Odds of having D-dimer 221 ng/mL or over associated with 2-point increase in the Mediterranean Diet Score in subgroups.

	Risk of D-dimer ≥221 ng/mL (95%CI)
All (n=17,403)	0.86 (0.82-0.90)
Age >60 years (n=5183)	0.86 (0.80-0.93)
Men (n=8135)	0.78 (0.72-0.84)
Smokers (n=4150)	0.84 (0.76-0.93)
BMI >30 Kg/m^2 (n=4911)	0.79 (0.73-0.86)
Hypertension (n=9195)	0.83 (0.78-0.88)
Hypercholesterolemia (n=5203)	0.85 (0.78-0.92)
Diabetes (n=1402)	0.77 (0.66-0.91)
Low income (n=5222)	0.83 (0.76-0.90)
Low education (n=9028)	0.84 (0.79-0.90)
Low physical activity (n=4000)	0.84 (0.77-0.92)
C-reactive protein >3 mg/dL (n=3624)	0.87 (0.79-0.95)

CI: confidence interval; BMI: Body Mass Index; Odds ratios adjusted for age, sex, body mass index, total calories, hypertension, dyslipidemia, diabetes, smoking habit, education level, income, total physical activity.

Our finding is in agreement with results from the International MONICA study of associations of D-dimer with a European gradient of coronary heart disease,[12] which reported lower D-dimer levels in Italy and other Mediterranean countries, consistent with a possible effect of a Mediterranean diet on D-dimer levels. D-dimer levels also reportedly correlate with factor VII and tissue factor levels,[13] and a Mediterranean diet has been associated with reduced factor VII and tissue factor levels.[14] These observations suggest another possible explanation for our findings. Unfortunately, lack of data on factor VII or tissue factor in our population sample meant that we were unable to test the hypothesis that the association of D-dimer with a Mediterranean diet is modulated by these hemostatic parameters.

A major limitation of this study is its cross-sectional nature that does not permit the inference of possible causality. Caution is also needed in extending the results presented here to larger population contexts, since data were collected in a region located between Central and Southern Italy, with Mediterranean traditions and culture. Yet the main characteristics of our population sample are comparable to those of the Italian Cardiovascular Epidemiological Observatory. Our sample could, therefore, be considered representative of at least the Italian

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population. 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 in the risk of major clinical outcomes that could be ascribed to the relatively small difference in mean D-dimer levels observed between the lowest and highest group of adherence to the MD.

To our knowledge, this is the first large epidemiological study considering traditional Mediterranean diet as a possible determinant of D-dimer levels in a healthy adult population. Our data suggest that higher adherence to a Mediterranean diet is associated not only with a lower inflammatory status, but also with a reduced tendency to develop a hypercoagulable state.

Since a Mediterranean diet has been related to reduced mortality (similarly to low D-dimer levels), we might speculate that this effect could be partly explained by the association of this dietary pattern with lower D-dimer levels. However, we cannot exclude the possibility that lower D-dimer levels could simply be a marker of adherence to a Mediterranean diet without any clinical significance.

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