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Adherence to the Mediterranean Diet Attenuates Inflammation and Coagulation Process in Healthy Adults

The ATTICA Study

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OBJECTIVES We studied the effect of the Mediterranean diet on plasma levels of C-reactive protein (CRP), white blood cell counts, interleukin (IL)-6, tumor necrosis factor (TNF)-alpha, amyloid A, fibrinogen, and homocysteine.

BACKGROUND To the best of our knowledge, the mechanism(s) by which the Mediterranean diet reduces cardiovascular risk are not well understood.

METHODS During the 2001 to 2002 period, we randomly enrolled 1,514 men (18 to 87 years old) and 1,528 women (18 to 89 years old) from the Attica area of Greece (of these, 5% of men and 3% of women were excluded because of a history of cardiovascular disease). Among several factors, adherence to the Mediterranean diet was assessed by a diet score that incorporated the inherent characteristics of this diet. Higher values of the score meant closer adherence to the Mediterranean diet.

RESULTS Participants who were in the highest tertile of the diet score had, on average, 20% lower CRP levels (p = 0.015), 17% lower IL-6 levels (p = 0.025), 15% lower homocysteine levels (p = 0.031), 14% lower white blood cell counts (p = 0.001), and 6% lower fibrinogen levels (p = 0.025), as compared with those in the lowest tertile. The findings remained significant even after various adjustments were made. Borderline associations were found regarding TNF-alpha (p = 0.076), amyloid A levels (p = 0.19), and diet score.

CONCLUSIONS Adherence to the traditional Mediterranean diet was associated with a reduction in the concentrations of inflammation and coagulation markers. This may partly explain the beneficial actions of this diet on the cardiovascular system. (J Am Coll Cardiol 2004;44: 152–8) © 2004 by the American College of Cardiology Foundation

An extensive body of scientific evidence relates diet to the incidence of various types of cardiovascular diseases (CVD) (1–4). Dietary factors exert their influence largely through their effects on blood pressure (BP), lipids, and lipoproteins (2,3). A diet rich in fruits, vegetables, legumes, whole grains, fish, nuts, and low-fat dairy products protects against the development and progression of CVD (3). The traditional Mediterranean diet, whose principal source of fat is olive oil, encompasses these dietary characteristics. Several studies (1–5) have established the beneficial role of this diet in CVD, metabolic disorders, and several types of cancer (2,3,5–7). Recently, Trichopoulou et al. (8) showed that this traditional diet affects BP, body mass index, platelet aggregation, and other hemorheologic factors and decreases all-cause mortality (2,8).

Low-grade systemic inflammation seems to play a role in the pathobiology of obesity, insulin resistance, coronary heart disease, metabolic syndrome, and abnormal coagulation process (9–15). This implies that measures designed to reduce the inflammatory process could be of benefit in reducing the risk of CVD. Because it has been suggested that the Mediterranean diet protects against the development and progression of CVD, we hypothesized that the benefits of adherence to the Mediterranean diet on heart disease could be due to its ability to modulate low-grade systemic inflammation and coagulation mechanisms.

METHODS

The Attica epidemiologic study (5) was carried out in the province of Attica (including 78% urban and 22% rural areas), where Athens is a major metropolis. From May 2001 to December 2002, 4,056 inhabitants from this area were randomly selected to be enrolled in the study. Of them, 3,042 agreed to participate (75% participation rate). All participants were interviewed by trained personnel (cardiologists, general practitioners, dieticians, and nurses), who used a standard questionnaire. Five percent of men and 3% of women were excluded from this study because they reported a history of cardiovascular or any other atherosclerotic disease, as well as chronic viral infections. Moreover, participants did not have a cold or flu, acute respiratory infection, dental problems, or any type of surgery in the past

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Abbreviations and Acronyms

BP = blood pressure
CRP = C-reactive protein
CVD = cardiovascular disease
HDL = high-density lipoprotein
IL = interleukin
TNF = tumor necrosis factor

week. Also, all people living in institutions were excluded from the sampling. Power analysis showed that the number of enrolled participants was adequate to evaluate two-sided standardized differences between subgroups of the study and the investigated parameters >0.5, achieving statistical power >0.90 at a 5% probability level (p value).

The study was approved by the Medical Research Ethics Committee of our institution and was carried out in accordance with the Declaration of Helsinki (1989) of the World Medical Association.

Dietary assessment. The evaluation of the nutritional habits was based on a validated food-frequency questionnaire provided by the Nutrition Unit of our institution. We asked all participants to report their daily or weekly average intake of several food items that they consumed during the last year. Then, the frequency of consumption was quantified approximately in terms of the number of times a month this food was consumed. Thus, daily consumption was multiplied by 30 and weekly consumption was multiplied by 4, and a value of 0 was assigned to food items rarely or never consumed. Alcohol consumption was measured in wine glasses (100 ml) and quantified by ethanol intake (g/drink). One wine glass was equal to 12 g ethanol concentration. According to a Harvard-led group with substantial input from Greek scientists (16), a dietary pyramid has been developed to describe the Mediterranean dietary pattern. This pattern consist of: 1) daily consumption of nonrefined cereals and products (e.g., whole-grain bread, pasta, brown rice, and the like), fruits (4 to 6 servings/day), vegetables (2 to 3 servings/day), olive oil (as the main added lipid), and nonfat or low-fat dairy products (1 to 2 servings/day); 2) weekly consumption of fish, poultry, potatoes, olives, pulses, and nuts (4 to 6 servings/week), as well as more rarely eggs and sweets (1 to 3 servings/week), and monthly consumption of red meat and meat products (4 to 5 servings/month). It is also characterized by moderate consumption of wine (1 to 2 wine glasses/day), moderate consumption of fat, and a high monounsaturated to saturated fat ratio.

According to the previous dietary pattern and the reported monthly frequency consumption of these food groups, we calculated each participant's special diet score, which assessed adherence to the Mediterranean diet (range 0 to 55). In particular, for the consumption of items presumed to be close to this pattern (i.e., those suggested on a daily basis or more than 4 servings/week), we assigned a score of 0 when a participant reported no consumption, a score of 1 for 1 to 4 times/month, 2 for 5 to 8 times/month, 3 for 9 to 12 times/month, 4 for 13 to 18 times/month, and 5 for more than 18 times/month. On the other hand, for the consumption of foods presumed to be away from this diet (like meat and meat products), we assigned the opposite scores (i.e., 0 when a participant reported almost daily consumption to 5 for rare or no consumption). For alcohol in particular, we assigned a score of 5 for consumption of <3 wine glasses/day, a score of 0 for consumption of more than 7 wine glasses/day, and scores of 1 to 4 for consumption of 3, 4, 5, 6, and 7 wine glasses/day. Higher values of this diet score indicate greater adherence to the Mediterranean diet, whereas lower values indicate adherence to the "Westernized" diet (17). We also calculated the tertiles of this score.

Sociodemographic and life-style variables. We recorded the mean annual income during the past three years and the educational level (years of school) of the participants (to indicate social status). Current smokers were defined as those who smoked at least one cigarette/day; never smokers as those who had never tried a cigarette in their life; and former smokers as those who had stopped smoking for at least one year. Occasional smokers (<7 cigarettes/week) were recorded and combined with current smokers because of their small sample size. For a more accurate evaluation of smoking habits, we calculated the pack-years (cigarette packs/day × years of smoking), adjusted for a nicotine content of 0.8 mg/cigarette.

For the ascertainment of physical activity status, we developed an index of weekly energy expenditure using frequency (times/week), duration (minutes/time), and intensity of sports-related physical activity. Intensity was gradated in qualitative terms such as light (expended calories <4 kcal/min [i.e., walking slowly, cycling stationary, light stretching, and so forth]), moderate (expended calories 4 to 7 kcal/min [i.e., walking briskly, cycling outdoor, swimming with moderate effort, and so forth]), and high (expended calories >7 kcal/min [i.e., walking briskly uphill, long-distance running, fast cycling or racing, swimming fast crawl, and the like]). Participants who did not report any physical activities were defined as sedentary. For the rest of the participants, we calculated a combined score by multiplying the weekly frequency, duration, and intensity of physical activity. Then, we calculated the tertiles of this score. Thus, physically active participants were classified into three groups: low physical activity (first tertile), medium physical activity (second tertile), and high physical activity (third tertile). Height and weight were recorded, and body mass index (kg/m^2) was calculated.

Clinical and biochemical characteristics. Resting arterial BP was measured three times in the right arm, at the end of the physical examination, with the subject in the sitting position. Fasting blood samples were collected from 8:00 to 10:00 AM. The biochemical evaluation was carried out in the same laboratory, following the criteria of the World Health Organization Reference Laboratories. C-reactive protein (CRP) and serum amyloid A were assayed by particle-

Table 1. Lifestyle, Clinical, and Biochemical Characteristics of the Participants, According to the Mediterranean Diet Score

	Tertile of Diet Score						
	Men (n = 1,524)			Women (n = 1,518)			
	1st (0–20)	2nd (21–35)	3rd (36–55)	1st (0–20)	2nd (21–35)	3rd (36–55)	p Value*
Physical inactivity (%)	70	56	53	77	57	64	0.001
Body mass index (kg/m^2)	27 ± 5	26 ± 4	26 ± 5	26 ± 3	24 ± 4	24 ± 3	0.125
Systolic blood pressure (mm Hg)	129 ± 17	125 ± 18	125 ± 17	129 ± 18	120 ± 18	120 ± 19	0.001
Diastolic blood pressure (mm Hg)	83 ± 11	81 ± 14	80 ± 11	80 ± 11	75 ± 12	75 ± 10	0.22
Hypertension (%)	51	27	20	50	36	10	0.001
Total cholesterol (mg/dl)	197 ± 43	194 ± 41	194 ± 43	196 ± 40	190 ± 42	188 ± 47	0.10
Hypercholesterolemia (%)	45	39	36	51	47	25	0.09
HDL cholesterol (mg/dl)	43 ± 12	43 ± 12	46 ± 13	49 ± 12	52 ± 15	54 ± 11	0.03
Triglycerides (mg/dl)	139 ± 65	133 ± 70	132 ± 77	121 ± 65	120 ± 69	113 ± 57	0.02
LDL cholesterol (mg/dl)	134 ± 43	124 ± 38	124 ± 42	126 ± 39	120 ± 37	120 ± 41	0.08
Blood glucose (mg/dl)	98 ± 25	95 ± 25	95 ± 30	98 ± 25	95 ± 25	95 ± 30	0.30
Diabetes (%)	10	8	6	11	6	2	0.12

*Probability values are from multiple linear regression models that evaluated the associations between the diet score and clinical and biochemical variables, after adjusting for age, gender, smoking, physical activity, and body mass index of the participants. Data are presented as the percentage of patients and the mean value ± SD.
HDL = high-density lipoprotein; LDL = low-density lipoprotein.

enhanced immunonephelometry. Interleukin-6 (IL-6) was measured with high-sensitivity enzyme-linked immunoassay. The intra- and interassay coefficients of variation were <5% for CRP and serum amyloid A and <10% for IL-6. Plasma fibrinogen levels were measured using automatic nephelometry. Homocysteine was measured using the pulsar fluorescence method. The intra- and interassay coefficients of variation for fibrinogen did not exceed 9%. White blood cell counts were measured using a special analyzer. We used the ELISA method for the quantitative determination of human tumor necrosis factor (TNF)-alpha (in duplicate) in serum samples of the participants by the Quantikine HS/human TNF-alpha immunoassay kit (R & D Systems Inc., Minneapolis, Minnesota).

Blood lipids (serum total cholesterol, high-density lipoprotein [HDL] cholesterol, and triglycerides) were measured using the chromatographic enzymic method in an automatic analyzer RA-1000 (GMI Inc., Beckmann Array Protein System Model RA-100, Albertville, Minnesota). Low-density lipoprotein cholesterol was calculated using the Friedewald formula: total cholesterol − HDL cholesterol − 1/5 triglycerides. The intra- and interassay coefficients of variation for cholesterol levels did not exceed 3%, triglycerides 4%, and HDL cholesterol 4%.

Patients whose average BP levels were ≥140/90 mm Hg or who were taking antihypertensive medication were classified as hypertensives. Hypercholesterolemia was defined as total serum cholesterol levels >200 mg/dl or the use of lipid-lowering agents. Diabetes mellitus was defined as a fasting blood glucose level >125 mg/dl or the use of antidiabetic medication.

Statistical analysis. Continuous variables are presented as the mean value ± SD. Qualitative variables are presented as absolute and relative frequencies. Associations between categorical variables were examined by the chi-square test, whereas differences between categorical and several biochemical, clinical, and nutritional variables were tested by the Student *t* test and Mann-Whitney criterion (for normally distributed and skewed variables, respectively). Comparisons between inflammation and coagulation variables and tertiles of the diet score were performed using analysis of variance. However, due to multiple comparisons, we used the Bonferroni correction in order to account for the increase in type I error. Multiple linear regression models were applied to test the association between the diet score and the investigated biomarkers, after controlling for several potential confounders. Finally, generalized linear regression models, through the evaluation of Hotelling's trace, were applied to test the inflammatory markers by food components of the diet score. Because of skewed distribution, CRP levels were log-transformed.

All reported p values are based on two-sided tests and compared to a significance level of 5%. SPSS version 11.0.5 (SPSS Inc., Chicago, Illinois) software was used for all the statistical calculations.

RESULTS

The distribution of several characteristics of the participants according to the Mediterranean score is shown in Table 1. As we can see, participants in the highest tertile of the diet score had lower systolic BP and triglyceride levels and higher HDL levels and they were more educated (13 ± 4 vs. 11 ± 4; p < 0.001), as compared with those in the lowest tertile. These associations were confirmed even after adjusting for age, smoking, physical activity, and body mass index of men and women. No association was found between the diet score and the other blood lipids measured, glucose, diastolic BP levels (Table 1), age (p = 0.215), financial status (p = 0.125), and daily number of cigarettes smoked (p = 0.205).

Table 2 illustrates the weekly frequency consumption of 10 major food groups or nutritional variables. Moreover, 86% of men and 89% of women reported that they

Table 2. Weekly Frequency Consumption (in Servings) of Several Food Groups, According to the Mediterranean Diet Score

	Tertile of Diet Score						
	Men (n = 1,524)			Women (n = 1,518)			
	1st (0–20)	2nd (21–35)	3rd (36–55)	1st (0–20)	2nd (21–35)	3rd (36–55)	p Value*
Dairy products (high fat)	6.1 ± 2.1	5.9 ± 2.2	5.2 ± 2.1	7.0 ± 2.3	6.8 ± 2.0	6.3 ± 1.9	0.001
Fish	1.0 ± 1.1	1.3 ± 2.1	1.6 ± 2.2	1.0 ± 1.3	1.3 ± 2.0	1.9 ± 1.4	0.876
Fruits and nuts	5.0 ± 3.1	5.3 ± 2.9	7.1 ± 3.8	5.0 ± 3.2	5.7 ± 3.9	7.0 ± 3.2	0.778
Meat (red) and meat products	3.0 ± 1.1	1.6 ± 1.9	1.1 ± 1.8	2.5 ± 1.1	1.7 ± 1.2	0.9 ± 1.1	0.021
Nonrefined cereals	1.2 ± 1.5	1.8 ± 1.3	2.4 ± 1.4	1.2 ± 1.4	1.7 ± 1.5	2.2 ± 1.2	0.121
Potatoes	1.7 ± 1.4	1.9 ± 1.6	2.1 ± 1.3	1.9 ± 1.5	2.7 ± 1.4	2.9 ± 1.1	0.034
Poultry	1.7 ± 1.1	1.4 ± 0.6	0.8 ± 0.9	1.3 ± 0.5	1.3 ± 0.7	1.0 ± 0.4	0.443
Pulses	1.3 ± 1.1	1.8 ± 1.2	2.1 ± 1.1	0.9 ± 1.5	1.3 ± 1.4	1.7 ± 0.7	0.033
Vegetables	1.4 ± 1.4	1.9 ± 1.2	2.4 ± 1.5	1.2 ± 1.1	2.3 ± 1.2	3.1 ± 1.2	0.018
Sweets	1.0 ± 1.4	1.1 ± 1.3	1.2 ± 1.2	1.1 ± 1.9	1.2 ± 1.6	1.3 ± 1.9	0.335
Alcohol (g)	160 ± 44	115 ± 35	95 ± 25	85 ± 30	80 ± 25	75 ± 45	0.001

*p values are for gender differences. Data are presented as the mean value ± SD.

consumed olive oil in their daily cooking, whereas 15% of men and 17% of women consumed seed oil. Furthermore, women were "closer" in adherence to the Mediterranean diet, as compared with men (diet score: 38 ± 12 vs. 36 ± 11; $p < 0.001$).

The unadjusted associations between the investigated inflammatory and coagulation markers and adherence to the Mediterranean diet are presented in Table 3. We observed that participants who were in the highest tertile of the diet score had, on average, 20% lower CRP levels, 17% lower IL-6 levels, 15% lower homocysteine levels, 14% lower white blood cell counts, and 6% lower fibrinogen levels, as compared with those who were in the lowest tertile. A decreased, but nonsignificant, trend was observed for TNF-alpha and amyloid A levels (Table 3).

However, it could be argued that the benefits of the Mediterranean diet on atherosclerotic markers are confounded by several social or lifestyle factors, like the presence of physical activity or the absence of smoking habits. Therefore, we repeated the data analysis after controlling for age, gender, smoking, physical activity, financial and education status, body mass index, presence of hypertension, diabetes, hypercholesterolemia, and family history of coronary heart disease. The multiple regression analysis confirmed the aforementioned associations (Table 4). Furthermore, an additional multivariate analysis revealed that consumption of fruits (Hotelling's trace = 3.7, $p < 0.001$), vegetables (Hotelling's trace = 3.4, p = 0.02), and moderate alcohol (Hotelling's trace = 5.4, p = 0.001) were inversely associated with the spectrum of the investigated inflammatory markers, whereas consumption of meat (Hotelling's trace = 4.1, p = 0.02) and increased alcohol intake (Hotelling's trace = 5.2, p = 0.001) had the most positive effect on the investigated biomarkers, after controlling for the previous set of covariates and factors.

Nevertheless, even after adjusting for smoking habits and physical activity status, residual confounding may still exist. Thus, we stratified the multiple regression analyses by smoking status (i.e., never/former smoker, <20 cigarettes/day, and ≥20 cigarettes/day) and physical activity level (i.e., low, medium, and high). These analyses showed results (not presented in the text or tables) similar to those observed in the multivariate models (Table 4).

Finally, the benefits of the Mediterranean diet on the concentrations of inflammation and coagulation indexes were significant even in high-risk groups of participants (Table 4). Figure 1 illustrates the associations between the investigated biomarkers by tertile of diet score and the number of cardiovascular risk factors (smoking, hypertension, hypercholesterolemia, diabetes mellitus, and obesity). A higher reduction in the concentrations of pro-inflammatory markers was observed in those who showed a higher degree of adherence to the traditional Mediterranean diet (Fig. 1).

Table 3. Inflammation and Coagulation Markers by Tertile of the Mediterranean Diet Score

	Tertile of Diet Score			
	1st (0–20)	2nd (21–35)	3rd (36–55)	p Value*
White blood cell (×1,000 counts)	7.4 ± 1.3	6.9 ± 2.7	6.2 ± 1.4	0.001
C-reactive protein (mg/l)	2.0 ± 1.8	1.8 ± 2.1	1.6 ± 1.5	0.01
Fibrinogen (mg/dl)	319 ± 79	309 ± 76	302 ± 74	0.02
Interleukin-6 (pg/ml)	2.1 ± 0.9	1.84 ± 1.1	1.45 ± 0.99	0.02
Homocysteine (μmol/l)	12.4 ± 5.8	11.7 ± 6.4	10.5 ± 6.0	0.03
Tumor necrosis factor-alpha (pg/ml)	5.8 ± 1.3	5.5 ± 1.4	5.1 ± 2.1	0.07
Amyloid A (mg/l)	5.2 ± 6.2	4.4 ± 4.6	3.6 ± 5.4	0.19

*Unadjusted p values by analysis of variance. Data are presented as the mean value ± SD.

Table 4. Results From Multivariate Regression Analysis (Beta-Coefficient ± SE) Regarding the Association Between the Mediterranean Diet Score (per 10-U Increase) and Inflammatory and Coagulation Markers in Subgroups of Participants

	All Subjects	Hypertensive Subjects (n = 860)	Diabetic Subjects (n = 194)	Hypercholesterolemic Subjects (n = 1,142)	Smokers (n = 1,704)
Amyloid A (per 1 mg/dl)	−0.09 ± 0.24	−0.14 ± 0.75	−0.14 ± 0.32	−0.08 ± 0.5	−0.1 ± 0.11
C-reactive protein (per 1 mg/l)	−0.21 ± 0.12*	−0.20 ± 0.31*	−0.15 ± 0.15*	−0.17 ± 0.17*	−0.18 ± 0.24*
Fibrinogen (per 1 mg/dl)	−12.7 ± 1.15*	−11.5 ± 1.53*	−10.6 ± 1.0*	−11.6 ± 1.21*	−12.2 ± 1.23*
Homocysteine (per 1 μmol/l)	−0.83 ± 0.25*	−0.84 ± 0.26*	−0.77 ± 0.83	−0.67 ± 0.35*	−0.75 ± 0.24*
Interleukin-6 (per 1 pg/ml)	−0.25 ± 0.34*	−0.15 ± 0.55*	−0.17 ± 0.31*	−0.18 ± 0.81	−0.17 ± 0.26*
TNF-alpha (per 1 pg/ml)	−0.25 ± 1.23	−0.17 ± 1.68	−0.21 ± 1.32	−0.17 ± 0.90	−0.17 ± 0.3
White blood cell (×1,000 counts)	−0.26 ± 0.11*	−0.21 ± 0.10*	−0.16 ± 0.17*	−0.17 ± 0.19*	−0.16 ± 0.18*

*p values <0.05 after adjusting for age, gender, smoking, physical activity, education status, presence of hypertension, diabetes, hypercholesterolemia, family history of coronary heart disease, and body mass index. Data are presented as the mean value ± SD.
TNF = tumor necrosis factor.

DISCUSSION

We revealed that greater adherence to the Mediterranean diet was independently associated with a reduction in various inflammatory and coagulation markers related to CVD. Our findings widened the current scientific knowledge about the benefits of this traditional diet on atherosclerotic disease, as they provided pathobiologic evidence for the ability of this dietary pattern to modulate low-grade systemic inflammation and coagulation mechanisms.

Mediterranean diet and atherosclerosis. Several studies have documented that people in the Mediterranean countries have a longer and healthier life than do those in other industrialized countries, despite the high prevalence of smoking and gaps in health services available to them (2,5,8,18–20), which has been attributed to several cultural and behavioral differences between the populations, as well as special dietary characteristics (1,19,20). In this context, the Mediterranean diet has received much attention. The protective effect of this traditional diet against atherosclerosis has been attributed to its ability to reduce BP levels, body mass index, platelet aggregation, and other hemorheologic factors (2,4,8). To the best of our knowledge, the influence of the Mediterranean diet on newer cardiovascular risk factors, like inflammatory markers, has never been investigated. Some previous studies have illustrated the effect of omega-3 fatty acids on various anti-inflammatory markers, including inhibition of TNF and IL production (21,22). The results of the present study revealed that participants who were closer to the Mediterranean diet had lower CRP, IL-6, homocysteine, and fibrinogen levels, as well as white blood cell counts, as compared with those who were "away" from this dietary pattern. A borderline decrease was also observed with regard to TNF-alpha and amyloid A levels.

Role of the Mediterranean diet on inflammatory and thrombogenic indexes. The Mediterranean diet is low in saturated and high in monounsaturated fat (oleic acid, 18:1 omega-9), mainly from olive oil; high in complex carbohydrates, from legumes; and high in fiber, mostly from vegetables and fruits. Total fat may be high (>40% of total energy intake), but the monounsaturated to saturated fat ratio is around 2. Daily foods and dishes include large quantities of bread, pasta, legumes, and vegetables, cooked meals with olive oil, and soups and salads rich in olive oil. Intake of milk is rather low, but the consumption of low-fat feta cheese and yogurt is high. Also, people prefer to consume fish instead of meat, especially in the rural areas (16). The high content of vegetables, fresh fruits, cereals, and olive oil guarantees a high intake of beta-carotene, vitamins B_6, B_{12}, C, and E, polyphenols, and various minerals. Finally, wine is consumed in moderation and almost always during meals (8,16).

Recent evidence shows low-grade systemic inflammation to be a strong predictor of cardiovascular events (18–20). The source of inflammation is uncertain; various inflammatory factors have been proposed as stimulators of this inflammatory process, such as infectious agents and any factor leading to endothelial injury (11). Prospective epidemiologic studies done in healthy subjects showed an association between elevated CRP levels and the risk of cardiovascular events (11,23) and peripheral vascular disease (24), independent of traditional risk factors. It has also been suggested that higher concentrations of plasma IL-6 and TNF-alpha could cause low-grade systemic inflammation, which might increase coronary risk (11). The association between elevated plasma fibrinogen and coronary risk may also partly reflect an ongoing inflammatory process (25). However, the exact association between these pro-inflammatory markers and diet is not clear. Raised plasma homocysteine has been associated with low dietary intake of vitamin B_{12} and folic acid, smoking, alcohol consumption, hypertension, hyperlipidemia (6,26), and, in some studies, an increased risk of coronary disease (27). A significant inverse relationship observed between plasma folate and B_{12} concentrations and homocysteine implies that the type and quality of diet may have a significant impact on plasma homocysteine and BP, as well as their perceived relationship (27). Moreover, alcohol consumption has received considerable attention in the past years as a factor with properties that tend to reduce the risk of developing coronary heart disease events (28). Its protective effect has been illustrated in low-quantity intake and has been mainly attributed to a mild vasodilator effect, an increase in

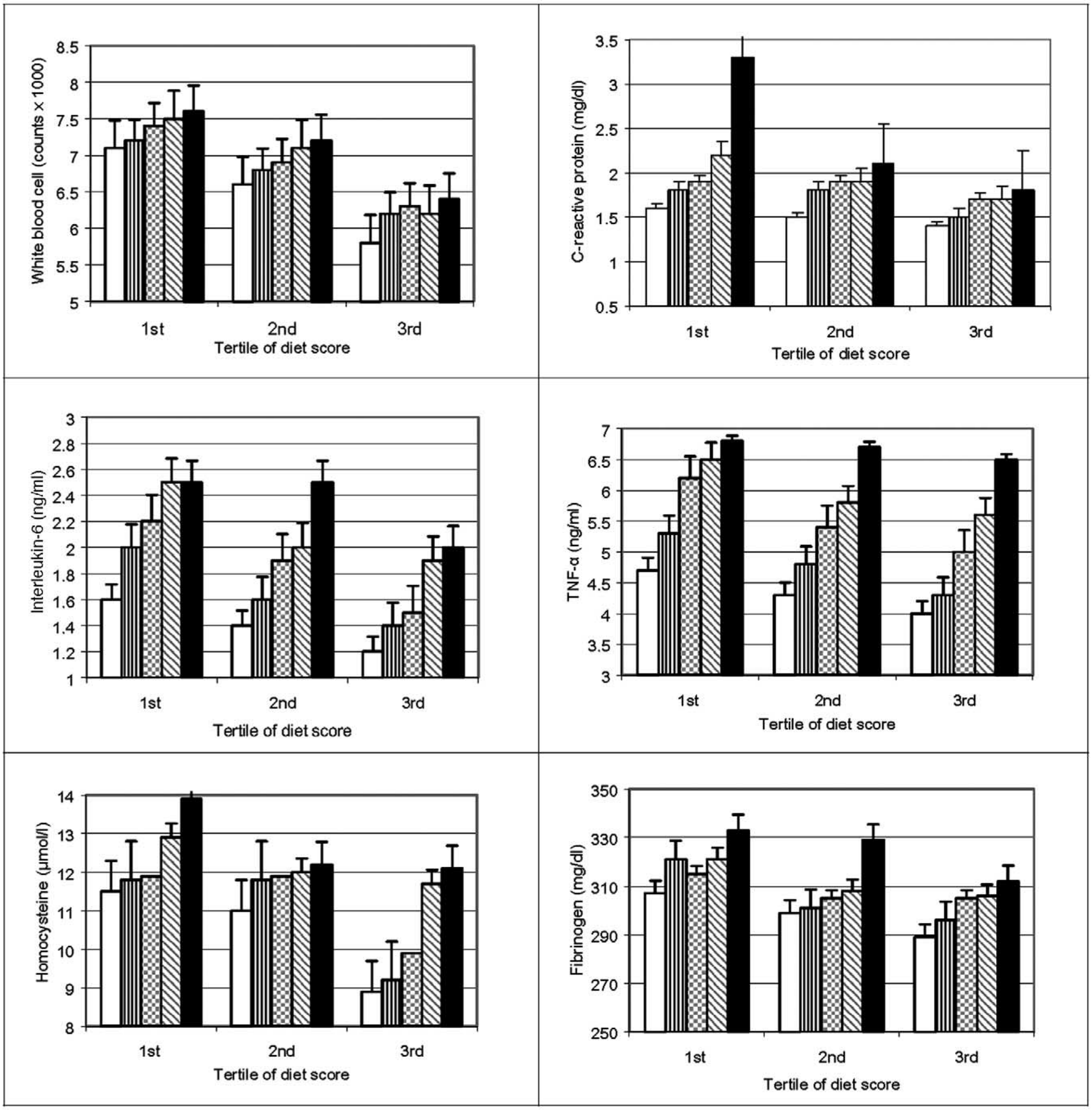

Figure 1. Levels of inflammatory markers by tertile of diet score and number of cardiovascular risk factors (smoking, hypertension, hypercholesterolemia, diabetes mellitus, and obesity). **White bars** = no risk factor; **bars with vertical lines** = one risk factor; **checkered bars** = two risk factors; **bars with diagonal lines** = three risk factors; **solid bars** = four risk factors. TNF = tumor necrosis factor.

HDL cholesterol levels, the anti-inflammatory characteristics of flavonoids and other anti-oxidative nutrients (29). It is of interest that among the various food groups evaluated in this study, fruits, vegetables, and moderate alcohol intake (negative), as well as meat and increased alcohol (positive), showed the highest degree of association with the investigated inflammatory and coagulation markers. However, further studies need to be performed to determine the component(s) of the Mediterranean diet which suppress these pro-inflammatory and coagulation indexes.

This study, as a cross-sectional one, cannot establish causal relationships, but can only generate a hypothesis. Misreporting of food items consumed and especially alcohol consumption, due to social class, can be a potential confounder. Another limitation is that we have not completed the dietary analysis for nutrient components (including electrolytes), so the role of dietary sodium and saturated and monosaturated fatty acids was not evaluated. It is of interest that the diet score was positively associated with education status, but not with annual income. This could be explained by several psychosocial factors that were not evaluated in the present work. Nevertheless, the previous "paradox" could not alter our findings.

Conclusions. The present study provides a pathophysiologic explanation to the growing scientific evidence for the beneficial effect of the Mediterranean diet on human health and, especially, atherosclerotic disease. We found that greater adherence to this traditional diet was independently associated with a reduction in the inflammation and coagulation indexes that are believed to have an important role in CVD. The World Health Organization (7) reports that the three major components involved in preventing atherosclerotic disease are smoking, physical inactivity, and an unhealthy diet, as they are factors that can be changed. Our findings emphasize the need for actions from public health care professionals in order to prevent the development and progression of atherosclerotic diseases through the adoption of low animal fat diets, like the Mediterranean diet.

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