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A Mediterranean dietary style influences TNF-alpha and VCAM-1 coronary blood levels in unstable angina patients

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Summary *Background* A Mediterranean dietary pattern has been associated with a reduced risk of coronary heart disease, as well as a reduction of oxidative stress, but studies indicating possible interactions between food intake and inflammatory mediators production at specific sites are lacking. *Aim of the study* To assess the relationship between Mediterranean diet consumption and inflammatory related molecules production in coronary vessels. *Methods* A previously reported Mediterranean-diet score was computed summing-up the quintiles of eight dietary components from a validated food frequency questionnaire in 24 patients with unstable angina. Tumor necrosis factor (TNF-α) and vascular cell adhesion molecule (VCAM-1) concentrations were measured in coronary sinus blood. *Results* Both biomarkers showed an inverse association with the Mediterranean-diet score. The association between VCAM-1 and the Mediterranean-diet score had an adjusted β coefficient of –35.1 ng/ml (95 % coefficient interval, CI: –63.5 to –6.7). The adjusted β coefficient using TNF-α as the dependent variable was –41.6 pg/ml (95 % CI: –76.2 to –7.1). The consumption of olive oil as a single item showed a significant inverse association, and a Mediterranean-diet score excluding olive oil was also inversely associated with TNF-α and VCAM-1 serum levels in coronary venous blood. *Conclusions* Adherence to a Mediterranean dietary pattern may protect against coronary artery wall production of inflammatory mediators. This finding could provide a novel mechanistic explanation for the recognized lower coronary risk associated with a Mediterranean diet.

Key words coronary disease – atherosclerosis – diet – angina – cardiovascular risk

Introduction

Atherosclerosis is currently recognized as a dynamic inflammatory process of the vessel wall, which is responsible for the clinical onset of a number of cardiovascular syndromes [1–5]. The risk of atherosclerotic coronary syndromes may be determined, at least in part, by dietary patterns, which is evidenced by the fact that the Mediterranean diet is associated with a lower risk of myocardial infarction [6–8]. Furthermore, a higher consumption of specific foods of the Mediterranean dietary style, such as olive oil, fiber, fruit or vegetables, has been associated with a lower risk of myocardial infarction [9–11]. Dietary fat influences not only LDL and HDL plasma concentrations [12], but also the proinflammatory properties of oxidized LDL, which is lower in persons fed diets rich in monounsaturated fatty acids

(MUFA) [13]. Indeed, within the concept of a Mediterranean-type model of healthy eating, a high-MUFA diet has been proposed as a healthier, cheaper and more palatable alternative to prevent cardiovascular disease [8], whereas a majority of nutrition experts have recommended that people should follow diets low in any kind of fat. Moreover, investigations about the nutritional involvement in improving the endothelial function has been almost exclusively centered on the low-fat diet approach [14, 15]. The effect of a Mediterranean dietary pattern on inflammatory molecules influencing the endothelial function has not been sufficiently investigated in patients with coronary heart disease.

Cell surface molecules, namely, vascular cell adhesion molecule (VCAM-1), intercellular adhesion molecule (ICAM-1) and selectins E and P, are involved in the adhesion and transmigration of monocytes into the vascular wall, whose occurrence on endothelial cell membranes is a prelude to the development of atherosclerosis [16]. Studies in rabbits have shown that as early as one week after initiating an unfavorable hypercholesterolemic state, the surface of the endothelial cells induces VCAM-1 [17]. Also, oxidized LDL modifies endothelial cell function promoting the expression of adhesion molecules [13], while addition of oleic acid into cultured endothelial cells diminishes surface VCAM-1 and its messenger RNA cell expression, interfering with the activation of nuclear factor κB (NF-κB) [18, 19]. Diet also can modify the activation of the gene transcription activator NF-κB, which may be increased by an atherogenic diet in rodents [20]. Interestingly, NF-κB enhanced expression has been demonstrated in peripheral monocytes from healthy subjects after a fatty meal, and this increase was prevented by red wine, which was attributed to the antioxidants contained in wine [21].

On the other hand, TNF-α is a proinflammatory molecule produced in the atheroma by macrophages, activated endothelial cells and smooth muscle cells [22, 23]. TNF-α is a powerful inductor of adhesion molecules [3, 24], induces a boosting of endothelial production of NF-κB [25], and produces endothelial and monocyte Tissue Factor necessary for thrombus formation [26]. In addition, it has been suggested that TNF-α induces apoptotic cell death in endothelial cells, thus contributing to endothelial dysfunction in coronary heart disease and heart failure [27].

The goal of our research was to investigate possible links between the Mediterranean dietary pattern and the coronary wall production of inflammatory molecules involved in the pathogenesis of the atherosclerotic process. Our *a priori* hypotheses were that not only olive oil intake but also an overall Mediterranean dietary pattern would negatively influence the production of inflammatory molecules in the coronary artery wall.

Methods

We examined all consecutive coronary patients admitted to the cardiology ward during the first three weeks of February 2003, who had been scheduled for coronary angiogram. The inclusion criterion was a recent history of unstable coronary syndrome, progressive or at rest, independent of any previous history of coronary heart disease. We excluded 21 patients presenting any of the following conditions: dilated cardiomyopathy, systolic dysfunction (ejection fraction lower than 50 %) or clinical myocarditis, minimal elevations of troponin during any angina episode during their hospital admission, evidence or clinical suspicion of a myocardial infarction during the previous three months, coronary angioplasty in the previous three months, aortocoronary by-pass during the last year, or systemic inflammatory processes. Finally, 24 patients were included in the analyses with no missing data in any of them.

Signed informed consent was obtained from each patient. The project was approved by the Institutional Review Board of the Medical School. Blood was extracted from the coronary sinus previous to any manipulation of the arterial lumen during the invasive coronary angiogram. Soluble vascular cell adhesion molecule (VCAM-1) and tumor necrosis factor (TNF-α) concentrations were determined in duplicate in the serum from coronary sinus blood using specific enzymoimmunoassays obtained from Chemicon International, Inc. (Temecula, CA). The intra-assay coefficients of variation were 4.4 % and 8.3 %, respectively, and the inter-assay coefficients of variation were 6.3 % and 10.8 %, respectively.

The day before the coronary angiogram was performed patients were interviewed in a standard way with the same protocol. It included a previously validated semi-quantitative food-frequency questionnaire (118 food items) [28]. For each food item, a commonly used portion size was specified, and participants were asked how often they had consumed that unit on average over the previous year. A dietitian updated the nutrient databank using the latest available information included in the food composition tables. Total energy-adjusted intakes were computed using the residuals method [29].

The detailed methods for the calculation of the Mediterranean diet score (diet score-1) for each patient have been previously reported [7]. Briefly, the average daily consumption (adjusted for total energy intake) of olive oil, fruit, vegetables, fiber and fish was categorized in quintiles. Total alcohol intake in grams was also categorized in quintiles for each patient. The score was obtained by totaling the quintile values (1 for the first quintile, 2 for the second, etc.) of the protective food items, but for the intake of meat products and for carbohydrate-rich foods we inversely ranked the score, with 1 representing the highest and 5 representing the lowest quintile. Thus, the score had a possible range from 8 to 40. To assess the independent specific contribution of olive oil we also computed the combined score using only the quintiles for the other seven food items and excluding the quintiles of olive oil intake (diet score-2).

On the day prior to the invasive procedure, the interviewer also collected information about the classical coronary risk factors in each patient (current or past smoking, high blood pressure, diabetes), and current drug use (antiplatelet drugs, statins, and ACE inhibitors or β-blockers), and venous blood was drawn for determining plasma cholesterol fractions and triglycerides. For each participant the body mass index (BMI) was calculated as the weight in kilograms divided by the squared height in meters (Kg/m^2).

No data were included from the review of previous medical record of patients, except for the confirmation of previous coronary diseases.

Statistical analyses were based on multiple regression models using TNF-α or VCAM-1 as outcome, and the Mediterranean dietary scores or the energy-adjusted olive oil daily intake as the independent variables. We adjusted the estimates for potential confounding introducing those variables showing variations of the estimates in previous analyses: gender, body mass index and the accumulated number of classical cardiovascular factors in each patient (current smoking, arterial hypertension, hypercholesterolemia and diabetes mellitus).

To test the influence of the asymmetric distribution of TNF-α and VCAM-1 values on the results, several transformations of the variables were made, with the square roots resulting in greater accuracy in the indices of normal distribution in both variables. Furthermore, to study influences from the previously diagnosed coronary diseases, or invasive treatments, dummy variables were constructed to adjust the regression models. Additional adjustments were also made for current treatments with antiplatelet drugs, statins, and β-blockers or ACE inhibitors, and also for social class (4 categories) and occupation (5 categories).

Results

The mean age was 61.4 years (SD: 12.6 years) and 42% of the patients were women. The mean time from admission to the hospital to the invasive procedure (including blood extraction from the coronary sinus) was 1.4 days (SD: 0.3 days). Table 1 describes the patients' characteristics, including the presence of risk factors, drug treatments and their clinical status. As expected, a high frequency of classical risk factors and of protective drug use was observed, since more than half of the patients had been previously diagnosed for coronary heart disease. The observed dietary scores, olive oil intake and cytokines values are described in Tables 2 and 3.

To assess the relationship between diet and coronary inflammation markers, linear regression models were fitted using TNF-α concentration as the outcome (dependent variable) and the dietary scores as the independent variables. When diet score-1 was assessed, a β coefficient of −41.5 pg/ml was observed with a 95% confidence interval (95% CI) from −76.2 to −6.9 pg/ml ($p = 0.021$). This coefficient was −41.6 pg/ml (95% CI: −76.2 to −7.1; $p = 0.021$) when we adjusted for potential

Table 1 Descriptive data of the patients (14 men, 10 women)

	Mean (SD)	n (%)
Age (years)	61.4 (12.6)	
Body mass index (kg/m^2)	26.1 (4.3)	
Total cholesterol to HDL cholesterol ratio	4.5 (1.7)	
Arterial hypertension		17 (70.8)
Hypercholesterolemia		13 (54.2)
Current smoking		8 (33.3)
Diabetes mellitus		9 (37.5)
Statin current use		15 (62.5)
Antiplatelet drug current use		23 (95.8)
ACE inhibitor or β-blocker current use		16 (66.7)
Previous myocardial infarction		12 (50)
Previous coronary stent		14 (58.3)
Previous aortocoronary by-pass		2 (8.3)

Table 2 Values for the Mediterranean diet scores, olive oil intake

	mean	SD	Observed Range
Diet score-1*	24.3	5.0	14–32
Diet score-2** (without olive oil)	21.4	4.6	13–31
Olive oil daily intake (g/day)	21.9	17.2	0.2–54.4

* We assessed six food items previously found to be protective: 1) olive oil, 2) fiber, 3) fruit, 4) vegetables, 5) fish and 6) alcohol. For each of these six dietary factors, we calculated the distribution according to quintiles within the study and assigned each participant a score of 1 to 5 corresponding to the intake quintile, with 1 representing the lowest and 5 representing the highest quintile. We estimated also the quintiles for two other elements found to be associated with a higher risk: 7) meat/meat products and 8) some items with a high glycemic load (white bread, pasta and rice) . For these two elements we inversely ranked the score, with 1 representing the highest and 5 representing the lowest quintile. Finally, we totaled the eight quintiles values for each participant.
** We excluded olive oil and included the rest of food items as above

Table 3 Serum VCAM-1 and TNF-α concentrations in coronary sinus blood

	VCAM-1 (ng/ml)	TNF-α (pg/ml)
Median	415.0	342.9
25th percentile	168.7	241.2
75th percentile	557.5	722.4

VCAM-1 Vascular cell adhesion molecule; *TNF-α* Tumor necrosis factor alpha confounding factors (gender, BMI and the number of classical coronary risk factors).

We also fitted a multiple regression model using the energy-adjusted intake of olive oil instead of the Mediterranean diet score as the independent variable. The adjusted β coefficient was –18.3 pg/ml (95% CI: –29.0 to –7.7; p = 0.002). The multiple regression model that used diet score-2 as the independent variable (excluding olive oil) also showed a negative but non-significant association with an adjusted β value of –31.6 (95% CI: –71.2 to 7.9 pg/ml; p = 0.111). The adjusted R^2 had a value of 0.23, showing that 23% of the variability in TNF-α concentration was explained by the model including the Mediterranean diet score. However, the adjusted R^2 was higher when the olive oil intake was examined, explaining the 39% of the TNF-α serum concentration variability in the coronary sinus (Table 4).

When serum VCAM-1 concentration in the coronary sinus was used as the outcome, the observed effects paralleled those of TNF-α. The overall Mediterranean diet score (diet score-1) showed a significant inverse association with the VCAM-1 coronary concentration. The β coefficient of the univariate linear regression was –30.9 ng/ml (95% CI: –59.5 to –2.5; p = 0.034) and, after adjusting as above, it increased to –35.1 ng/ml (95% CI: –63.5 to –6.7 ng/ml; p = 0.018). The β coefficients for olive oil and for the diet score-2 were –12.0 ng/ml (95% CI: –21.9 to –2.2; p = 0.019) and –30.3 ng/ml (95% CI: –62.2 to 1.5; p = 0.061) respectively, in the adjusted multivariable model (Table 5).

When the transformed variables (square root of the TNF-α value or VCAM-1 value) were considered as dependent variables, multiple regression models also showed significant associations with dietary scores and olive oil intake. Previous coronary diagnosis or invasive or surgical treatments did not influence the estimates of the regression models with either TNF-α or VCAM-1 as outcomes. To adjust the multiple regression models we also used in different models the variables indicating the treatment with protective drugs (statins, antiplatelet drugs and β-blockers or ACE inhibitors), but the results did not substantially differ. Further adjustments for potential confounders associated to Mediterranean diet, such as social class based on education or occupation, did not change the estimates.

Discussion

In this research we have found that a score of adherence to a Mediterranean dietary pattern was inversely associated to TNF-α and VCAM-1 concentrations in blood emerging from the coronary vascular tree. These two important inflammatory molecules are believed to play a key role in atherogenesis-related vascular damage [3–5].

A prospective study of a Greek cohort showed a significant reduction of coronary death in people with greater adherence to the Mediterranean dietary pattern [31]. A recent case-control study by our group reported

Table 4 Multiple regression results using Tumor Necrosis Factor alpha concentration (pg/ml) as the outcome (dependent variable in a linear regression model)

	Univariate			Multivariate adjusted*			
	β	95% CI	p	β	95% CI	p	$R^{2\,a}$
Mediterranean diet score-1[b]	–41.5	–76.2 to –6.9	0.021	–41.6	–76.2 to –7.1	0.021	0.23
Energy adjusted olive oil daily intake (grams)	–16.9	–26.9 to –6.9	0.002	–18.3	–29.0 to –7.7	0.002	0.39
Mediterranean diet score-2[b] (excluding olive oil)	–31.5	–71.3 to –8.4	0.116	–31.6	–71.2 to 7.9	0.111	0.10

* Adjusted for gender, body mass index, and the accumulated number of classical coronary risk factors (current smoking, arterial hypertension, hypercholesterolemia, diabetes mellitus)
[a] Adjusted R^2 coefficient
[b] See footnotes in Table 2

Table 5 Multiple regression results using Vascular Cell Adhesion Molecule concentration (ng/ml) as the outcome (dependent variable in a linear regression model)

	Univariate			Multivariate adjusted*			
	β	95% CI	p	β	95% CI	p	$R^{2\,a}$
Mediterranean diet score-1[b]	–30.0	–59.5 to –2.5	0.034	–35.1	–63.5 to –6.7	0.018	0.20
Energy adjusted olive oil daily intake (grams)	–8.2	–17.7 to 1.2	0.083	–12.0	–21.9 to –2.2	0.019	0.20
Mediterranean diet score-2[b] (excluding olive oil)	–28.9	–60.5 to 2.7	0.071	–30.3	–62.2 to 1.5	0.061	0.11

* Adjusted for gender, body mass index, and the accumulated number of classical coronary risk factors (current smoking, arterial hypertension, hypercholesterolemia, diabetes mellitus)
[a] Adjusted R^2 coefficient
[b] See footnotes in Table 2

a strong inverse association of a first non-fatal myocardial infarction with a defined Mediterranean-diet score [7].

The use of a Mediterranean-type diet for the prevention of coronary heart disease has recently been assessed using randomized studies [11, 32], and also a number of observational studies have suggested that a diet with a moderate alcohol intake, mainly from red wine, and rich in olive oil, vegetables, fruits, fish, fiber, and reduced in meats and in foods with a high-glycemic index, can protect from a first myocardial infarction [6–10, 30, 33, 34].

Consistent with these findings, Mediterranean-type diets and oleic acid have been associated with a number of beneficial effects related to endothelial function and the immune molecular cascade [13, 18]. A MUFA-rich diet decreased the ICAM-1 membrane expression in leukocytes [35]. Oleic acid added to cultured endothelial cells induced a decrease in VCAM-1 expression and in the transcription of its messenger RNA, and a diminished adherence of a monocyte cell line [18, 19]. A randomized study showed that a MUFA-rich diet, similar to a Mediterranean-type diet, increased the endothelium dependent vasodilation with respect to a NCEP-type diet [36].

Recently, the monocyte chemoattractant protein (MCP-1) concentration in peripheral blood, a cytokine directly related to monocyte recruitment, has been found to be directly related with the progression of coronary disease [37]. Furthermore, MCP-1 levels in coronary sinus blood exhibit a direct relationship with the extension of vascular lesions into the coronary tree [38]. These findings illustrate the parallel significance of local and systemic concentrations of some inflammatory markers. To date, most of the evidence supporting the relationship of inflammatory molecules with a higher coronary disease risk have been derived from studies using specimen extracted from peripheral blood and their values might be partly distorted by their expression in distant vascular areas [1, 4, 39, 40]. However, in order to measure the production of molecular vascular inflammation markers and endothelial activation of an arterial segment, it is worth collecting emerging blood from that same segment. In a recent report, a widespread activation of leukocytes has been reported in the coronary circulation of unstable angina patients [41]. The findings we report in this article, with TNF-α and VCAM-1 measured in the coronary venous blood, add new data to improve the knowledge about the way in which diet could modify cardiovascular risk.

The higher content of leukocytes in the coronary intimal neovasculature correlates with the expression of VCAM-1 in non-endothelial vascular cells [42], and peripheral blood VCAM-1 concentration has been reported as a prognostic marker of coronary death, showing a two-fold fatal risk in patients in the top quartile [39]. On the other hand, cardiovascular risk after a myocardial infarction is higher in those patients with TNF-α persistently elevated in serum [4].

The negative multiple regression of TNF-α concentrations found in coronary blood using a Mediterranean diet score as the independent variable indicates a decrease in that inflammatory marker as the adherence to the Mediterranean dietary pattern becomes progressively higher. Energy-adjusted olive oil intake alone also negatively modifies the TNF-α concentration in coronary sinus, suggesting that the main protective effect would depend on olive oil. Also, the alternative Mediterranean diet score excluding the olive oil intake also showed an inverse association in the multivariate regression model. The soluble VCAM-1 concentration in sinus blood was also inversely and significantly related to the Mediterranean diet score. It also showed a significant inverse association with energy-adjusted olive oil consumption. The lack of statistical significance in the analyses in the regression models with the diet score-2 can be explained by a lower statistical power due to the reduced sample size.

Taken together, these findings suggest that the prevention of the coronary wall inflammation may be greater when adherence to an overall healthy Mediterranean dietary pattern is the basis of dietary habits [43]. However, olive oil seems to be one of the most important protective foods among those included in that pattern, since the multiple regression models constructed with olive oil exhibited the higher adjusted R^2, explaining 39 % of the TNF-α and 20 % of the VCAM-1 variabilities.

The effect of diet on inflammatory molecules reported in this paper suggests a protection on the endothelium, and a decrease in the infiltration of vascular wall by monocyte-producing cytokines. It can be speculated that these effects might be mediated through a diminished NF-κB activation. This gene-activator of multiple inflammatory mediators of the coronary artery disease has been previously demonstrated to be sensitive to dietary changes [21].

This study is a first attempt to describe an inverse link between a Mediterranean diet and the expression of inflammatory molecules in the heart. We have selected patients with unstable angina because of certainty of vascular disease without myocardial necrosis, which is a well known cause of myocardial production of cytokines. We did not include any patient with a suspicion of recent processes that could produce myocardial or peripheral inflammation, or with heart failure [44], to avoid biases from any cause other than the coronary disease causing unstable angina. Indeed, the results of multiple regression analysis showed a well-defined inverse relationship between the studied inflammatory cytokines and the dietary scores.

It can be argued that all patients included in this study had an acute coronary syndrome, and those with higher adherence to a Mediterranean dietary pattern did not seem to be protected. It is well known, however, that other risk factors may directly induce coronary wall dysfunction and inflammation, and consequently be responsible for the ischemic heart disease even in the presence of an overall protective lifestyle. In unstable angina patients with definite lesions in the coronary angiogram, the higher the Mediterranean adherence score is, the lower the inflammatory cytokine levels in coronary blood, as shown by lineal multiple regression models. This inverse relationship in coronary patients suggests that lower inflammatory injury of the vessel wall is associated with higher adherence to a Mediterranean diet also in cases with overt coronary disease. Even though coronary blood of patients with normal hearts could be examined in the same way to investigate diet-cytokines associations, the absence of vascular damage in them would explain very low levels of inflammation mediators released from the vascular wall and subsequently no apparent effects of diet on coronary arterial tree.

These data suggest that a higher adherence to a traditional Mediterranean diet might be associated with decreased levels of inflammatory biomarkers in the coronary vascular tree of patients with vascular damage, and this inverse association is a likely mechanism to explain the lower observed cardiovascular mortality found in the countries where this healthy traditional diet is consumed. The actual meaning of our finding should be confirmed with longitudinal studies analyzing the associated risks of dietary habits on inflammatory cytokines in cardiovascular disease.

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