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Adherence to the Mediterranean Diet Is Inversely Associated With Circulating Interleukin-6 Among Middle-Aged Men:

A Twin Study

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Abstract

Background—The Mediterranean diet is protective against cardiovascular disease; a proposed mechanism is through a reduction in systemic inflammation. It is unknown to what extent the association between the Mediterranean diet and inflammation is due to genetic or other familial factors.

Methods and Results—We administered the Willett food frequency questionnaire to 345 middle-aged male twins and assessed adherence to the Mediterranean diet using a published adherence score. Fasting plasma levels of interleukin-6, C-reactive protein, and known cardiovascular risk factors were measured. Mixed-effect regression analyses were used to examine the relationship between diet score and inflammatory biomarkers after accounting for known cardiovascular risk factors. Adherence to the Mediterranean diet was associated with reduced levels of interleukin-6 ($P<0.001$) but not C-reactive protein ($P=0.10$) after adjustment for total energy intake, other nutritional factors, known cardiovascular risk factors, and use of supplements and medications. When the overall association of adherence to the diet with interleukin-6 levels was partitioned into between- and within-pair effects, the between-pair effect was not significant ($P=0.9$) and the within-pair effect was highly significant ($P<0.0001$). A 1-unit within-pair absolute difference in the diet score was associated with a 9% (95% CI, 4.5 to 13.6) lower interleukin-6 level.

Conclusions—Shared environmental and genetic factors are unlikely to play a major role in the association between adherence to the Mediterranean diet and systemic inflammation. These results support the hypothesis that reduced inflammation is an important mechanism linking Mediterranean diet to reduced cardiovascular risk.

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Disclosures
None.

Keywords

diet, Mediterranean; inflammation; twins, dizygotic; twins, monozygotic

Adherence to the Mediterranean diet appears to benefit the cardiovascular system,[1,2] but the underlying mechanisms are unclear. An inverse relationship between adherence to the Mediterranean diet and biomarkers of systemic inflammation such as interleukin-6 (IL-6) and C-reactive protein (CRP)[3,4] has been reported, suggesting that inflammatory processes may be implicated. However, such relations were not consistently found in randomized controlled trials.[5–7] A possible reason for the inconsistent findings may be the genetic differences in the response to diet because each of the trials studied a different European population: Italian,[5] Spanish,[6] and German.[7] In addition, because dietary habits are acquired while growing up, they are likely to be associated with other environmental conditions shared by members of the same family such as unmeasured socioeconomic and lifestyle factors, which also can influence inflammation and thus act as confounders. However, no prior study has controlled for the influence of familial factors in the association between the Mediterranean diet and inflammation.

Twins are a powerful resource for dissecting complex associations because they allow us to control for unmeasured and unknown confounding such as genetic factors and socioeconomic, behavioral, and lifestyle characteristics acquired by twins growing up in the same family. Using a sample of monozygotic (MZ) and dizygotic (DZ) middle-aged male twins raised in the same family, we examined the relationship between adherence to the Mediterranean diet and circulating biomarkers of inflammation. We sought to determine whether the association persisted after accounting for common genetic and environmental factors by comparing each twin with his co-twin.

Methods

Subjects

The Twins Heart Study (THS) is an investigation of psychological, behavioral, and biological risk factors for subclinical cardiovascular disease using twins. It included 180 pairs of MZ and DZ male twins from the Vietnam Era Twin Registry[8] who were born between 1946 and 1956 and were free of symptomatic cardiovascular diseases on the basis of survey data collected in 1990.[9] The Vietnam Era Twin Registry includes 7369 middle-aged male-male twin pairs, both of whom served in the US military during the time of the Vietnam War. Zygosity was determined using similarity questionnaires supplemented with blood group typing data abstracted from military records.[10] For the THS, random samples of twins in 2 strata were selected from the registry; 1 stratum included twins discordant for a lifetime history of major depression, and in a second stratum, neither twin had a history of depression. All twins were examined at the Emory University General Clinical Research Center between March 2002 and March 2006; their medical history was updated at the examination. We excluded twins with missing dietary data, implausible energy intake (≥6000 or <500 kcal/d),[11] or circulating IL-6 levels >10 pg/mL, defined as a cutoff for low-grade systemic inflammation. The study protocol was approved by the Institutional Review Board at Emory University, and informed consent was obtained from all subjects.

Diet Assessment

We used the Willett self-administered semiquantitative food frequency questionnaire,[12] which collected dietary data over the past 12 months. The questionnaire classifies average food intake according to 9 frequency categories ranging from “almost never or less than

once per month" to "≥6 times/d" using standardized portion sizes for each dietary item, including beverages and nutritional supplements. Questionnaires were scored by the Nutrition Questionnaire Service Center, Channing Laboratory, Harvard University, and nutrient intake data were derived following the nutrient database of the US Department of Agriculture. Daily food intake in grams was calculated from food intake frequency and portion sizes.

Mediterranean Diet Score

The term Mediterranean diet refers to a dietary pattern typical of many regions in Greece and southern Italy in the early 1960s, including a high intake of fruits, vegetables, bread, other forms of cereals, potatoes, beans, nuts, and seeds; low to moderate amounts of dairy products, fish, poultry, and wine; low amounts of red meat; eggs consumed no more than 4 times weekly; and olive oil as an important fat source.[13] We measured adherence to the Mediterranean diet using the Mediterranean Diet Score (MDS) described by Trichopoulou et al[1] based on a priori assumptions about 9 desirable or undesirable dietary components (Table I in the online Data Supplement): (1) 7 desirable dietary components for health, including cereals (excluding potatoes), vegetables, fruits and nuts, legumes, fish, dietary ratio of monounsaturated to saturated fatty acids (mainly a result of the use of olive oil as the main cooking oil in the Mediterranean diet), and moderate alcohol consumption, and (2) 2 undesirable dietary components for health, including meat and dairy food products. The score was constructed using zygosity-specific, rather than gender-specific, median of food intake (adjusted to 2500 kcal)[14] to conduct analyses stratified by zygosity in our all-male sample. We assigned a value of 1 to a high intake (greater than or equal to the median) of each desirable component, a value of 1 to a low intake (<median) of each undesirable food, and a value of 0 to all other intakes.[14] For alcohol, a value of 1 was assigned to moderate consumption, ie, intake above the zygosity-specific median (2.38 g/d for MZ and 1.72 g/d for DZ) and ≤33 g/d. The latter, the daily upper limit of alcohol intake considered "moderate" among American men,[15] equals ≈2 alcoholic drinks per day.[15,16] The MDS was the sum of all values from the 9 components, ranging from 0 to 9; the higher the score was, the greater the adherence to the Mediterranean diet was.

We also devised 3 slight variations of the MDS to evaluate the robustness of our findings. First, we followed an earlier method published by Trichopoulou et al[14] to calculate a score, MDS_1, ranging from 0 to 8 in which fish was included in the meat group[17] and potatoes and eggs were included among cereals and meats, respectively.[14] A second variant, MDS_2, was similar to the MDS_1 except fish was excluded from meat.[17] Fish intake as a covariate was forced into the models. In a third variation of the score, MDS_3, we excluded fish from the meat group and included fish as a desirable component.

Assessment of Known Cardiovascular Risk Factors

We assessed smoking (never smoked and current and past smoker), education, and marital status using standardized questionnaires. Physical activity was evaluated with the Atherosclerosis Risk in Communities study Baecke questionnaire.[18] Waist and hip circumference were measured and used to calculate a waist-to-hip ratio. Systolic and diastolic blood pressures were measured with a mercury sphygmomanometer according to a standard protocol.[19] Hypertension was defined as systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg or current use of antihypertensive medicines. Diabetes was defined as a fasting plasma glucose concentration ≥126 mg/dL[20] or current treatment with insulin or oral hypoglycemic agents. Depressive symptoms were measured with the Beck Depression Inventory, which yielded a continuous score.[21] Current uses of aspirin and statins also were recorded.

Biochemical Analysis

Plasma samples were separated from 9-hour overnight fasting blood samples and stored at −80°C until analysis. Concentrations of glucose, triglycerides, and total, low-, and high-density lipoprotein cholesterol were measured with standardized methods. Plasma IL-6 concentrations were determined with commercial enzyme-linked, high-sensitivity immunosorbent kits (R&D Systems, Minneapolis, Minn). High-sensitivity CRP (hsCRP) concentrations were tested with the high-sensitivity Beckman Coulter assay. The interassay and intra-assay variabilities for all assays were <10%; the samples were analyzed blindly; and twin pairs were assessed in the same analytical run.

Statistical Analysis

We ranked twins in each pair on the basis of their MDS scores and calculated the within-pair absolute difference in the MDS as the difference between a twin with a higher MDS score and his twin brother with a lower MDS score. Inflammatory biomarkers were log-transformed because of skewed distributions. Intraclass correlation coefficients were calculated for the MDS and log-transformed inflammatory biomarkers in MZ and DZ twins. The association between the MDS and inflammatory markers was assessed by fitting linear regression models adapted for twin studies.[22] We first used the entire sample by treating twins as individuals and accounting for the twin pair clustering. The MDS was analyzed primarily as a continuous variable and secondarily as a 4-level variable categorized as 0 to 3, 4, 5, and 6 to 9. Category midpoints were used for analyses.[23] Because the dependent variables were log-transformed and the association was inverse, we expressed the results as percent difference of the nontransformed values by using the following formula: $[1-(\exp^{\beta})] \times 100$ (%), where β is the regression coefficient and $\exp^{\beta}$ returns the exponential value of the parameter. The “base model” adjusted only for total energy intake and nutritional factors that were not part of the MDS, including potato and egg consumption.[1] To this model, we subsequently added sociodemographic factors, including age, years of education, and current marital status; lifestyle factors such as smoking, waist-to-hip ratio, and physical activity; comorbidity and cardiovascular risk factors, including previous coronary heart diseases, depressive symptoms (Beck Depression Inventory score), fasting glucose, systolic blood pressure, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol; supplements and medications such as fish oil supplements; and use of aspirin and statins. Potential multicollinearity was investigated using condition indexes and variance decomposition proportions by means of a SAS macro (SAS Institute, Inc, Cary, NC) using criteria including both a condition index of ≥20 and at least 2 nonintercept variables with variance decomposition proportions values of ≥0.5.[24]

Next, we performed within-pair analyses to examine differences in inflammatory biomarkers between co-twins in each pair. The within-pair effects are inherently controlled for demographic, shared familial, and environmental influences; in addition, environmental factors during the day of testing are controlled because co-twins were examined at the same time and under the same conditions. We fitted mixed-effects models for twins,[22] which allow partitioning within- and between-pair differences in the dependent variable as a function of the independent variables. In these models, the within-pair β coefficient describes the individual twin variation from the twin pair average; this formulation has the advantage of being independent of twin ordering. The between-pair regression coefficient is just for the pair average. The within-pair coefficient is identical to the β coefficient from a model that fits the absolute difference between the co-twins.[22] Thus, the percent difference calculated from the within-pair coefficient can be interpreted as the difference in inflammatory biomarker concentrations per 1-unit absolute difference in the MDS between co-twins in a pair. Because twin pairs were raised together and MZ pairs share 100% of their genetic material, any association between the MDS and inflammatory markers within MZ

pairs cannot be ascribed to genes or family environment. DZ pairs also share familial factors but on average share only 50% of their genetic material and as such are less tightly matched. These models included separated variance components for twin type to accommodate the different residual correlation in MZ and DZ pairs. MZ and DZ twins, however, also were examined separately. Obesity is tightly associated with systemic inflammation[25]; thus, we further examined whether the diet-inflammation association was different among obese versus nonobese (body mass index ≥30 versus <30 kg/m^2) twins. All analyses were conducted with mixed models to control for the pair clustering using SAS software version 9.1 (SAS Institute). Significance levels were set at P=0.05 (2 sided).

The authors had full access to and take responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Sample Characteristics

From the initial sample of 360 twins, we excluded 15 subjects (1 with no dietary data, 6 with implausible energy intake, and 8 with plasma IL-6 levels >10 pg/mL). Therefore, our analyses were based on 345 twins (88 MZ and 77 DZ twin pairs, 5 MZ and 10 DZ unpaired twins). The sample was 94% non-Hispanic white, 3% black, and 3% other race/ethnic groups. This distribution reflected the racial distribution of the Vietnam Era Twin Registry from which it was sampled. The median biomarker concentration was 1.7 pg/mL (interquartile range, 1.1 to 2.7 pg/mL) for IL-6 and 1.3 mg/L (interquartile range, 0.5 to 2.7 mg/L) for hsCRP. Twins with higher MDS were older, more educated, less likely to be smokers, and more likely to use fish oil supplements (Table 1). The mean within-pair absolute difference in the MDS was 1.6 (range, 5) in MZ and 1.8 (range, 7) in DZ twins. Intraclass correlation coefficients for the MDS, IL-6, and hsCRP were larger in MZ than DZ twin pairs (Table 2), suggesting that genetic factors contribute to these traits.

Overall Associations

Increasing MDS was associated with decreasing levels of IL-6 and hsCRP in a dose-response fashion (Table 3). When the MDS was treated as a continuous variable, for each 1-unit increase in the MDS, IL-6 and hsCRP levels decreased by 6.6% (P=0.001) and 5.6% (P=0.064), respectively, after adjustment for nutritional factors (Table 3). After simultaneous adjustment for known cardiovascular risk factors, the association between the MDS and hsCRP was no longer significant. However, even after full adjustment, the relationship between the MDS and IL-6 remained strong (model 4 in Table 3): A 1-unit increment in the MDS was associated with a 5.1% decrease in IL-6 concentration (P=0.005). Similar results were observed with the MDS as an ordinal variable, confirming the presence of a dose-response association (Table 3). In the fully adjusted model, twins in the highest MDS category had an IL-6 level that was 21.3% (95% CI, 6.9 to 33.8) lower than those in the lowest category, and there seemed to be a threshold effect: Subjects with a score ≥6 had lower IL-6 levels than those with a score <6.

Zygosity-Specific Within-Pair Results

For MZ pairs, a 1-unit within-pair absolute difference in the MDS was associated with an 8.6% (95% CI, 1 to 15) and a 7.7% (95% CI, 1 to 14) lower IL-6 level before and after adjustment for the other risk factors, respectively (Table 4). The IL-6 results for DZ twins were similar. Neither MZ nor DZ twin pairs showed significant within-pair associations for hsCRP. The interaction between within-pair differences in the MDS and zygosity was not significant (Table 4). The between-pair associations of the MDS with inflammatory markers

were not significant for either IL-6 or hsCRP (P=0.9 for IL-6, P=0.5 for hsCRP in the fully adjusted model), suggesting that shared familial factors were not important.

Because inflammation is a strong correlate of obesity, we also examined whether the within-pair association between the MDS and inflammatory markers differed by obesity. The interaction between within-pair differences in the MDS and obesity was not significant for either IL-6 or hsCRP in the fully adjusted model for the entire sample and by zygosity status (all P>0.25).

Finally, we repeated the analyses after excluding subjects with a previous history of coronary heart disease and by using published variations of the MDS (MDS_1, MDS_2, and MDS_3). The results were very similar and are not shown.

Discussion

We found an inverse association between adherence to the Mediterranean diet and inflammation as measured by IL-6 that was independent of a wide range of known cardiovascular risk factors. This finding persisted when co-twins within pairs, either MZ or DZ, were compared, suggesting that shared familial factors do not confound the association between adherence to the Mediterranean diet and systemic inflammation as measured by IL-6. Results were robust and persisted after exclusion of persons with a previous history of coronary heart disease and when slight variations of the MDS were used.

Prior studies on the association between the Mediterranean diet and systemic IL-6 levels are limited. Inverse associations between adherence to the Mediterranean diet and IL-6 were reported in observational studies among healthy subjects[3,4] and intermediate-[6] or long-term[5] randomized controlled trials among subjects at high risk for cardiovascular disease[5,6] but not in a short-term trial among healthy individuals,[26] perhaps as a result of a lack of sufficient time to observe potential effects.

Although previous studies adjusted for sociodemographic and cardiovascular risk factors, no study has accounted for familial or genetic influences that may be shared between adherence to the Mediterranean diet and inflammatory response. Heritable and familial determinants affect both inflammatory biomarkers[27] and dietary behavior, including food preference,[28] food consumption frequency,[29] and perception of hunger.[30] A variety of other unmeasured environmental and behavioral factors that twins share may confound the diet-inflammation association and provide spurious associations. However, by comparing twins within pairs, we found that the MDS and IL-6 association was not diminished, which points to the conclusion that genetic influences or other familial factors do not play major roles.

We found an independent association between adherence to the Mediterranean diet and plasma levels of IL-6 but not CRP. In the base model, CRP was marginally associated with the MDS (P=0.06), but after known cardiovascular risk factors were controlled for, this relationship was substantially reduced. Several studies have found that the association between CRP and cardiovascular disease is attenuated after adjustment for confounding factors.[31–35] One possible explanation is that IL-6 is a more sensitive indicator of atherosclerosis and cardiovascular risk than CRP.[36–39] IL-6 has numerous biological actions[40] that may promote atherosclerosis, including regulation of immune cells, recruitment of lymphocytes via stimulation of endothelial synthesis of cellular adhesion molecules, procoagulant effects, and stimulation of the hepatic synthesis of CRP. Thus, IL-6 may have a direct atherogenic role,[36,37,39,40] and CRP may serve mostly as a marker of IL-6 or other atherogenic risk factors.[41]

Although the within-pair effect was robust, the between-pair effect was weak and nonsignificant. This may be related to less confounding in the within-pair analyses. Because our twins were raised together, within-pair analyses naturally controlled for unmeasured confounding factors related to family environment or early development, which may act as negative confounders in this association. In general, a strong within-pair effect with a small or absent between-pair effect is consistent with a causal mechanism.[22]

There are some limitations to our study. The sample was restricted to middle-aged male Vietnam era veterans, and our results may not be generalizable to women. As in other common food frequency questionnaires used across the United States, combined food items, containing ≥2 components of the MDS, may misclassify individual MDS components. However, we carefully decomposed combined items into individual ingredients using appropriate recipes, therefore minimizing misclassification. Our results reflect only the cumulative effects of various foods in the MDS,[42] and our findings should not be extrapolated to a single food component. As in all observational studies, our results may be affected by unmeasured confounding. However, we controlled for many known lifestyle factors and compared twins raised in the same family; thus, it is unlikely that other behavioral factors substantially confound the association between the MDS and inflammation.

Conclusions

Using a twin sample, we were able to demonstrate that shared environmental and genetic factors do not confound the association between adherence to the Mediterranean diet and systemic inflammation. Our findings add weight to the biological plausibility of the cardioprotective effect of the Mediterranean diet and substantiate the protective effect of the Mediterranean diet on cardiovascular risk. Furthermore, our data support the importance of behavioral interventions that encourage consumption of a healthier diet to prevent cardiovascular disease.

CLINICAL PERSPECTIVE

The Mediterranean diet is protective against cardiovascular disease; a proposed mechanism is through reducing systemic inflammation. Dietary habits are acquired while growing up and may be confounded by other behavioral, environmental, or genetic factors shared by family members. It is unknown to what extent the association between the Mediterranean diet and inflammation is due to other familial factors. We used data on identical and fraternal twins raised together to answer this question because twins are naturally matched for measured and unmeasured common environmental factors while growing up. Monozygotic twin pairs are also 100% matched for genetic factors, whereas fraternal pairs share on average 50% of their genes. We constructed a 10-unit diet score to measure adherence to the Mediterranean diet; a higher score indicates greater adherence to the Mediterranean diet. We found that a 1-unit increase in the Mediterranean diet score was associated with a 5% decrease in systemic inflammation measured by means of plasma interleukin-6 concentration. After further controlling for shared familial and genetic factors by examining differences between twins in a pair, we found that a twin with a 1-unit-higher Mediterranean diet score than his twin brother had a 9% lower interleukin-6 concentration. In contrast, shared familial and genetic factors did not significantly contribute to the association between MDS and inflammation. Our findings substantiate the protective role of the Mediterranean diet on cardiovascular risk and support the importance of consuming a healthier diet to prevent cardiovascular disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Age, Lifestyle, and Clinical and Biochemical Characteristics of Subjects According to the MDS

	MDS				
Variable	**0–3 (n=104)**	**4 (n=70)**	**5 (n=81)**	**6–9 (n=90)**	***P****
Age, y	53.8±0.3	54.3±0.3	54.5±0.3	54.8±0.3	0.02
Education, y	13.8±0.2	14.1±0.2	14.7±0.2	14.5±0.2	0.003
Body mass index, kg/m^2	29.6±0.5	29.5±0.6	29.5±0.5	28.5±0.5	0.13
Waist-to-hip ratio	0.94±0.01	0.95±0.01	0.95±0.01	0.94±0.01	0.75
Physical activity, unit	7.15±0.16	7.34±0.19	7.69±0.18	7.49±0.17	0.07
Smoking status, n (%)					0.001
Former	29 (27.9)	24 (34.3)	33 (40.7)	44 (48.9)	
Current	41 (39.4)	34 (48.6)	13 (16.0)	9 (10.0)	
Never	34 (32.7)	12 (17.1)	35 (43.2)	37 (41.1)	
Married, n (%)	77 (74.0)	54 (77.1)	66 (81.5)	77 (85.6)	0.07
Employed, n (%)	78 (75.0)	58 (82.9)	64 (79.0)	74 (82.2)	0.28
Depressive symptoms, BDI score	6.04±0.62	4.44±0.74	4.15±0.70	3.98±0.67	0.02
Plasma glucose, mg/dL	98.2±1.8	102.7±2.1	100.0±2.0	103.3±1.9	0.052
Systolic blood pressure, mm Hg	130.3±1.6	129.5±1.8	128.5±1.7	129.3±1.6	0.61
Diastolic blood pressure, mm Hg	80.8±1.1	80.6±1.2	79.8±1.2	82.3±1.1	0.37
Blood lipids, mg/dL					
Total triglycerides	176.6±10.0	192.9±11.6	179.2±11.6	182.5±10.5	0.75
Total cholesterol	185.3±3.7	193.2±4.4	186.0±4.2	189.1±4.0	0.57
HDL cholesterol	38.9±0.9	38.4±1.0	39.7±1.0	37.7±1.0	0.41
LDL cholesterol	124.0±3.3	125.9±3.9	120.8±3.7	125.0±3.5	0.96
Previous coronary heart disease, n (%)	13 (12.5)	2 (2.9)	12 (14.8)	6 (6.7)	0.31
Diabetes mellitus, n (%)	8 (7.7)	8 (11.6)	11 (13.6)	10 (11.1)	0.35
Hypertension, n (%)	36 (34.6)	21 (30.0)	24 (29.7)	26 (28.9)	0.40
Use of fish oil supplements, n (%)	2 (1.9)	2 (2.9)	5 (6.2)	10 (11.1)	0.01
Use of statins, n (%)	26 (25.0)	11 (15.7)	27 (33.3)	22 (24.4)	0.79
Use of aspirin, n (%)	29 (27.9)	12 (17.1)	25 (30.9)	21 (23.3)	0.67
Plasma inflammatory biomarkers					

Variable	MDS				
	0–3 (n=104)	4 (n=70)	5 (n=81)	6–9 (n=90)	*P**
IL-6, pg/mL†	2.1 (1.8–2.3)	1.9 (1.6–2.2)	1.8 (1.6–2.1)	1.5 (1.3–1.7)	<0.0001
hsCRP, mg/L†	1.4 (1.1–1.7)	1.3 (1.0–1.7)	1.1 (0.9–1.4)	1.0 (0.8–1.3)	0.02

BDI indicates Beck Depression Inventory; HDL, high-density lipoprotein; and LDL, low-density lipoprotein. Results are expressed as mean±SEM when appropriate. Variables (%) are dichotomous except smoking status.

*Test for trend across diet groups. All probability values are corrected for pair clustering. Mixed models were used for continuous variables, generalized estimating equation logistic models for dichotomous variables, and repeated proportional odds model with generalized estimating equation for the 3-level ordinal smoking variable.

†Geometric means (95% CIs).

Table 2

Intraclass Correlation Coefficients*

	MZ	DZ
MDS, unit	0.26	0.0
IL-6, pg/mL	0.35	0.16
hsCRP, mg/L	0.70	0.26

* Calculated using linear mixed model with SAS.

Table 3

Associations Between the MDS and Inflammatory Biomarker Concentrations in the Entire Sample

Outcome	Difference Per 1-Unit Increase in MDS (n=345), (%)	*P*	Geometric Means of IL-6 or hsCRP Levels				*P**
			MDS=0–3 (n=104)	MDS=4 (n=70)	MDS=5 (n=81)	MDS=6–9 (n=90)	
Model 1: adjusted for zygosity and other nutritional factors not included in the MDS†							
IL-6, pg/mL	−6.6	0.001	2.1	1.9	1.8	1.5	<0.0001
hsCRP, mg/L	−5.6	0.064	1.4	1.3	1.2	1.0	0.06
Model 2: further adjusted for demographic and lifestyle factors‡							
IL-6, pg/mL	−4.6	0.016	2.1	2.1	2.0	1.7	0.007
hsCRP, mg/L	−3.7	0.22	1.3	1.3	1.3	1.1	0.22
Model 3: further adjusted for comorbidity and cardiovascular risk factors§							
IL-6, pg/mL	−5.1	0.007	2.1	2.1	2.1	1.7	0.005
hsCRP, mg/L	−4.0	0.18	1.4	1.3	1.3	1.1	0.17
Model 4: further adjusted for use of supplements and medications‖							
IL-6, pg/mL	−5.1	0.008	2.1	2.0	2.0	1.6	0.005
hsCRP, mg/L	−3.9	0.20	1.2	1.2	1.2	1.0	0.18

The percent difference was calculated from the β coefficient of the MDS in mixed models treating twins as separate individuals but accounting for clustering within a pair and allowed for different correlations in MZ and DZ pairs.

* Test for trend across diet groups.

† Total energy intake, egg and potato consumption.

‡ Age, education, marital status, smoking, waist-to-hip ratio, and physical activity.

§ Previous coronary heart disease, depressive symptom score, plasma glucose, systolic blood pressure, and low- and high-density lipoprotein cholesterol.

‖ Fish oil supplements, statins, and aspirin.

Table 4

Within-Pair Differences in Inflammatory Biomarker Concentrations per 1-Unit MDS Overall and by Zygosity

	MZ+DZ (n=345)		MZ (n=181)		DZ (n=164)		
Outcome	**Within-Pair Difference, %**	***P***	**Within-Pair Difference, %**	***P***	**Within-Pair Difference, %**	***P***	***P* for Interaction With Zygosity**
Model 1: adjusted for zygosity and other nutritional factors not included in the MDS*							
IL-6, pg/mL	−9.9	<0.0001	−8.6	0.022	−11.3	0.001	0.55
hsCRP, mg/L	−5.4	0.14	−3.9	0.45	−6.8	0.26	0.69
Model 2: further adjusted for demographic and lifestyle factors†							
IL-6, pg/mL	−8.8	<0.0001	−8.6	0.018	−10.4	0.003	0.92
hsCRP, mg/L	−4.4	0.21	−3.0	0.58	−5.8	0.31	0.66
Model 3: further adjusted for comorbidity and cardiovascular risk factors‡							
IL-6, pg/mL	−9.2	<0.0001	−9.5	0.009	−11.3	0.001	0.85
hsCRP, mg/L	−4.4	0.22	−3.0	0.53	−6.8	0.26	0.57
Model 4: further adjusted for use of supplements and medications§							
IL-6, pg/mL	−9.2	<0.0001	−7.7	0.029	−11.3	0.001	0.80
hsCRP, mg/L	−4.1	0.26	−0.5	0.93	−7.7	0.22	0.46

The within-pair difference (%) is calculated from the β coefficient and is expressed per 1-unit difference in the MDS between 2 twins in a pair.

*Total energy intake, egg and potato consumption.

†Age, education, marital status, smoking, waist-to-hip ratio, and physical activity.

‡Previous coronary heart disease, depressive symptom score, plasma glucose, systolic blood pressure, and low- and high-density lipoprotein cholesterol.

§Fish oil supplements, statins, and aspirin.