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Association of Major Depressive Disorder with Serum Myeloperoxidase and other Markers of Inflammation: A Twin Study

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Abstract

Background—Major depressive disorder (MDD) has been linked to inflammation, but this association may be due to common precursors to both depression and inflammation.

Myeloperoxidase (MPO) is an inflammatory enzyme produced by activated leukocytes which predicts risk of coronary heart disease. We sought to examine whether MPO and other markers of inflammation are associated with MDD, and whether the association is confounded by genetic or other shared familial factors.

Methods—We examined 178 monozygotic and dizygotic middle-aged male twin pairs. We assessed MDD with the Structured Clinical Interview for Psychiatry Disorders. Blood markers of inflammation included MPO, interleukin-6, white blood cell count, C-reactive protein, tumor necrosis factor (TNF)- α , the TNF- α soluble receptor II, and fibrinogen. Analyses were conducted in the overall sample and among 67 twin pairs discordant for MDD using mixed effects regression.

Results—Twins with a history of MDD had 32% higher levels of MPO ($p < 0.0001$); this difference persisted after adjusting for other risk factors. Among dizygotic MDD-discordant twin pairs, twins

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with MDD had 77% higher MPO than their brothers without MDD after adjusting for other factors ($p < 0.0001$). In contrast, no significant association was found in monozygotic twins ($p = 0.13$). Similar, but weaker, associations were found between MDD and other inflammatory biomarkers.

Conclusion—MPO is a useful biomarker of immune activation in MDD. However, the association between inflammation and MDD is largely due to common genetic liability. Our results are consistent with the hypothesis that genes promoting inflammation are involved in the pathogenesis of MDD.

Keywords

Biomarkers; depression; cardiovascular disease; genetic factors; inflammation; twins

Inflammation is a key pathway in the initiation and progression of coronary heart disease (CHD), and inflammatory biomarkers such as C-reactive protein (CRP) and interleukin-6 (IL-6) have shown consistent associations with incident CHD events (1). In recent years, myeloperoxidase (MPO) has drawn growing attention as a new inflammatory biomarker of CHD risk (2–4). MPO is an enzyme produced by activated leukocytes during the innate immune response which catalyzes the formation of reactive oxidant species. It is present in human atherosclerotic plaques and exhibits a variety of proatherogenic properties (5).

Increased inflammation is a key mechanism through which several risk factors increase CHD risk (6). Depression is a risk factor for CHD (7), and whether increased inflammation is involved has attracted considerable interest (8). A role of inflammation in depression was first proposed by Smith in 1991 (9). Since then, several studies have reported a link between major depressive disorder (MDD), or depressive symptoms, and a variety of inflammatory and immune biomarkers (10–15). However, others have found no independent association (16) or mixed results (17–19), and one study even found lower levels of inflammatory biomarkers in depressed cardiac outpatients (20).

It is increasingly recognized that the relationship between depression and inflammation is more complex than initially conceived (21). Depression may cause inflammation through altered neuroendocrine function and central adiposity (22). However, depression may also be a consequence of inflammation, since a pathogenic role of inflammatory cytokines in the etiology of depression has been described (23). Although given less consideration, a third possibility is that depression is a marker of some other underlying dimension that is separately linked to depression and inflammation. Recently, it has been proposed that such underlying factor could be a specific genetic makeup (24;25). Evidence for a common genetic substrate for depression and inflammation would be of substantial scientific and clinical interest, because it would suggest that a common biological pathway links these two conditions.

In a sample of middle-aged male twins we sought to determine, first, whether plasma MPO and other markers of inflammation are associated with MDD, and, second, whether the association is confounded by shared genetic or environmental (familial) factors. Twins provide unique opportunities to study associations while controlling for shared environmental or genetic factors, because twin siblings share genes (50%, on average, if dizygotic, and all if monozygotic), maternal factors, and early familial environment.

Methods

Subjects

The Twins Heart Study (THS) is an investigation of psychological, behavioral and biological risk factors for subclinical cardiovascular disease using twins. Twins were selected from the Vietnam Era Twin (VET) Registry (26), which includes 7,369 middle-aged male-male twin pairs both of whom served in the United States military during the time of the Vietnam War.

THS included 93 monozygotic and 87 dizygotic twin pairs who were born between 1946 and 1956. Zygosity information was determined by DNA typing in all but 11 twin pairs. The zygosity of these 11 pairs was assessed using questionnaires supplemented with blood group typing data abstracted from military records, which in our sample had an accuracy of 94%. The twins were free of symptomatic cardiovascular disease based on survey data collected in 1990 (27). Two groups of twin pairs were randomly sampled from the VET Registry: one group included depression-discordant twins, where one member of the pair had a lifetime history of MDD previously assessed with the Diagnostic Interview Schedule (28) and the other did not; the second group of twins included pairs where neither had a history of MDD. Twin pairs were examined together at the Emory University General Clinical Research Center between March 2002 and March 2006, and maintained an identical schedule during examination. This protocol was approved by the Institutional Review Board at Emory University and informed consent was obtained from all subjects.

Assessment of Depression and Other Psychiatric Diagnoses

Lifetime history of MDD was assessed using the Structured Clinical Interview for DSM IV (SCID), which yields a clinical diagnosis of major depression based on a lifetime history of major depressive episodes (29). The SCID also provided a diagnosis of other psychiatric disorders, including a lifetime history of posttraumatic stress disorder (PTSD), and a lifetime history of alcohol and of drug abuse or dependence. Current substance abuse and other psychiatric diagnoses were infrequent. We also collected information on current level of depressive symptoms using the Beck Depression Inventory-II (BDI-II) (30), a standardized scale providing a continuous measure of depressive symptoms. This self-report instrument includes 21 symptom items and the twins rated the severity of each symptom from 0 to 3. The BDI is used extensively in community samples and has satisfactory test-retest reliability and internal consistency (31).

Assessment of Inflammatory Biomarkers

MPO was measured in serum with a commercially available ELISA (CardioMPO Test, Prognostix, Cleveland, Ohio). The inter- and intra-assay variabilities were 2% and 6%. In addition to MPO, we measured a battery of other biomarkers that were previously associated with either MDD or CHD or both. These included interleukin-6 (IL-6), white blood cell count (WBC), C-reactive protein (CRP), tumor necrosis factor α (TNF- α), the TNF- α soluble receptor II (sTNF RII), and fibrinogen. IL-6, TNF- α , and sTNF RII were assessed using commercially available ELISA kits from R and D Systems. Inter- and intra-assay variability for these assays is <10%. CRP was measured with the Beckman Coulter High Sensitivity C-Reactive Protein assay on the Synchron LX-20 analyzer. WBC count was measured with the Beckman Coulter LH 750 hematology analyzer (Brea, CA). Fibrinogen was measured by using the Dade Behring BCS coagulation analyzer. All biochemical assays for each twin pair were processed in the same analytical run.

Other Measurements

A medical history and a physical exam were obtained from all twins. Weight and height were used to calculate the body mass index. Systolic and diastolic blood pressure was measured by standard procedures; the average of two measurements 5-minutes apart was used in the analysis. Venous blood samples were drawn for the measurements of glucose and lipid profile after an overnight fast. Total triglycerides were determined by enzymatic methods (Beckman Coulter Diagnostics, Fullerton, CA). Direct high-density lipoprotein (HDL) and low density lipoprotein (LDL) cholesterol were measured with homogeneous assays (Equal Diagnostics, Exton, PA). Glucose was measured on the Beckman CX7 chemistry autoanalyzer. Physical activity was assessed with a modified version of the Baecke Questionnaire of Habitual Physical

Activity used in the Atherosclerosis Risk in Communities (ARIC) Study (32); this is a 16-question instrument documenting level of physical activity at work, during sports and non-sports activities. The global physical activity score was used in the analysis. Cigarette smoking was classified into current versus never or past smoker. A history of CHD was defined as a previous diagnosis of myocardial infarction or angina pectoris, or previous coronary revascularization procedures. Diabetes mellitus was defined as having a fasting glucose level > 126 mg/dl or being treated with anti-diabetic medications. Information on current use of other medications was also collected.

Statistical Analysis

Initial descriptive analyses compared means and percents of study factors according to MDD status. P values were corrected for the correlation between co-twins using generalized estimating equations for categorical variables and mixed-effects models for continuous variables with a random intercept for each pair.

The association between MDD and inflammatory markers was first examined in the entire sample of twins, treating twins as separate individuals in mixed-effects models. Analyses were done before and after adjusting for factors that were significantly different according to MDD status, or that were a priori considered potentially important confounders. These included marital status, body mass index, habitual physical activity (Baecke score), antidepressant medications, previous history of CHD, and zygosity; we also adjusted for the Framingham risk score, which incorporates age, low-density-lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, blood pressure, diabetes mellitus, and current smoking (33). Because smoking status is potentially a major confounder in our analysis, we repeated our models by including current smoking as a stand-alone variable in place of the FRS, in addition to the other variables. In order to assess if current level of depressive symptoms explained the association between MDD and inflammation, we also ran models which also adjusted for the BDI score.

The association between MDD and inflammatory biomarkers, controlling for shared genetic and environmental factors, was examined in a co-twin control analysis of the MDD-discordant twin pairs using mixed-effects models. This was a matched-pair analysis in which healthy co-twins (without MDD) were used as controls for their brothers with MDD. Twins share maternal factors, familial and childhood/adolescent environment if they are raised together as in our sample. The matched nature of the co-twin control design minimizes confounding by these factors. If the paired effects are smaller than the effects seen when twins are analyzed as separate individuals, this is evidence that there is confounding by factors shared by co-twins.

In a co-twin control study, confounding by genetic factors can be inferred by stratifying the analysis according to zygosity and testing the interaction with zygosity. Monozygotic twins share 100% of their segregating genes; therefore genetic factors are fully controlled for when monozygotic twins are compared with each other. Dizygotic twins, however, only share on average 50% of genes, and therefore genetic factors are only partially accounted for when comparing dizygotic twins within a pair. Thus, if the paired effect is smaller in monozygotic than in dizygotic twins, this is evidence that the association is confounded by genetic factors. All analyses were conducted using SAS software version 9.1 (SAS Institute). Significance level was set at 0.05, two-sided.

Results

Of the 180 THS twin pairs we excluded one pair because of missing MPO data, and another pair because of implausible TNF- α values. Of the remaining 178 pairs, 67 pairs were discordant for lifetime history of MDD and 111 were not. The majority of twins with MDD were in

remission, with only 8 subjects meeting DMS-IV criteria for a current major depressive episode and 77% having had the last depressive episode >1 year before examination.

Both in the entire population of twins and within the MDD-discordant twin pairs, twins with MDD were less likely to be married, more likely to smoke and to have lower levels of physical activity (Table 1). They were also more likely to have PTSD, although the number of twins with PTSD was small (N=23), and to have a higher level of depressive symptoms. There were no substantial differences in other characteristics according to MDD status, including medication use except for antidepressants. There were no significant differences in study variables between monozygotic and dizygotic twins, except that dizygotic twins were slightly older. (Supplemental Table 1)

Most of the inflammatory biomarkers were mildly correlated with each other, both in the entire population and in the MDD-discordant co-twins: 90% of the correlation coefficients were <0.40 (Supplemental Table 2). MPO and TNF- α were the least correlated with other markers.

In the entire sample of twins (twins analyzed as separate individuals), MPO showed a highly significant association with MDD (Table 2), with values that were 32% higher in twins with MDD than those without MDD ($p<0.0001$). IL-6, WBC count, fibrinogen and sTNF RII also showed significant (or borderline significant) associations, although the magnitude of the difference was smaller than for MPO. All the biomarkers considered, except TNF- α , had numerically higher values in twins with MDD. Multivariable analysis did not materially alter the association for MPO while it attenuated the association for most of the other biomarkers.

Table 3 and Figure 1 show the results of the association between MDD and inflammation from the co-twin control analysis among twin pairs discordant for MDD, stratified by zygosity. This analysis controlled by design for all factors shared by the twins. Consistent with the hypothesis of genetic confounding, larger associations between MDD and inflammatory markers were found among dizygotic twins than among monozygotic twins. Among dizygotic twins, those with MDD had significantly higher MPO, IL-6, WBC count, sTNF RII and fibrinogen levels than their brothers without MDD. Again, the difference was most marked for MPO, with 62% higher levels in MDD twins ($p<0.0001$). As in the analysis of the entire sample, adjustment for risk factors did not weaken the association between MPO and MDD, with 77% higher MPO levels in MDD twins in multivariable analysis. However, adjustment did attenuate the relationship of the other biomarkers, with only WBC count and (borderline) sTNF RII remaining significantly associated with MDD in multivariable analysis. In contrast, there was no evidence for an association of MDD and any of the inflammatory biomarkers in monozygotic twins. The interaction between MDD and zygosity was significant for MPO, IL-6, and sTNF RII. Although not statistically significant, the interaction p value approached significance for all the remaining biomarkers except TNF- α . These results confirm a confounding effect due to genetic factors. On the contrary, shared environment did not confound in the association between MDD and inflammation, since the paired differences in biomarker levels between dizygotic twin brothers were no less, and actually numerically larger, than in the overall analysis of twins as separate individuals.

When the analyses were repeated by including current smoking as a stand-alone variable in place of the FRS, the results were virtually identical and are not reported. In addition, we examined whether current level of depressive symptoms, measured with the BDI, would explain the relationship between MDD and inflammatory biomarkers among the dizygotic twins. The BDI score was not significantly correlated with MPO ($r=0.02$, $p=0.69$), but it was significantly associated with IL-6, CRP, WBC count and sTNF RII. However, adjustment for BDI score did not substantially affect the relationship between MDD and any of the biomarkers, suggesting that the relationship was not due to current level of depressive symptoms.

Discussion

We found that MDD is associated with higher levels of inflammation, and that this association is particularly robust for MPO, an inflammatory biomarker that was never studied before in relation to depression. However, we also found evidence for genetic confounding in this association. Our results are consistent with the hypothesis that there is a common genetic substrate linking MDD and inflammation, suggesting that these two phenotypes share a common pathophysiological mechanism.

MPO, Other Inflammatory Markers, and Depression

MPO is an enzyme of the innate immune system which exhibits a wide array of pro-atherogenic features (5;34). MPO is secreted upon leukocyte activation, contributing to innate host defenses. However, it also increases oxidative stress thereby contributing to tissue damage during inflammation and atherogenesis. MPO generates numerous reactive oxidants that cause lipid peroxidation, post-translational modifications to target proteins and decrease of nitric oxide bioavailability resulting into oxidation of LDL and apolipoprotein A1, protein carbamylation and endothelial dysfunction (5;35;36). Transgenic mice containing the human MPO gene show significantly larger atherosclerosis build up than the wild type (34;37). In humans, individuals with total or subtotal MPO deficiency, a defect with a frequency of 1 in every 2000 to 4000 whites, are less likely to develop cardiovascular diseases, and those harboring a promoter polymorphism associated with a 2-fold reduction in MPO expression appear cardioprotected (5;38–40). Consistent with these pro-atherogenic properties, MPO has received growing attention as a novel risk marker for future cardiovascular events (2–4).

Oxidative stress has also been linked to neuronal degeneration in the central nervous system (41;42). MPO is both expressed and enzymatically active in the human brain (43;44) and is associated with Alzheimer's disease (44). Previous studies have described abnormalities of oxidant-antioxidant systems in MDD suggestive of higher oxidative stress. For example, elevated levels of antioxidant enzymes, particularly superoxide dismutase (SOD), and biomarkers of oxidation, such as malondialdehyde, were found in plasma, red blood cells or other peripheral tissues of acutely depressed MDD patients compared with controls (45–47). In some cases (46;47), but not others (45), these abnormalities were reduced with antidepressant treatment. SOD coenzyme concentrations are also higher in postmortem brain tissue (prefrontal cortex) of MDD patients than in control brains (48).

Overall, twins with MDD had 32% higher levels of MPO than those without MDD, an association that was not explained by other risk factors. Other inflammatory biomarkers, except TNF- α , tended to be elevated in twins with MDD; in contrast to MPO, however, these associations became weaker and mostly non-significant after adjusting for behavioral and CHD risk factors. Depressive symptoms were not correlated with MPO, and adjustment for BDI score in the analysis did not alter the results for any of the biomarkers. It is possible that MPO is a more specific indicator of brain immune activation than other inflammatory biomarkers. MPO is a myeloid-specific enzyme produced by activated phagocytic cells, including brain microglia (43). In contrast, inflammatory cytokines are produced by a variety of cell types, while acute-phase proteins such as CRP and fibrinogen are mostly secondary products in response to cytokine signaling (49). Thus, MPO is potentially a more specific marker of microglial immune activation and therefore more relevant for MDD and other brain disorders.

Genetic Influences

We found that the paired association between MDD and MPO was significantly larger in the dizygotic than in the monozygotic MDD-discordant twin pairs, a finding that was paralleled by other inflammatory biomarkers. These results suggest that the link between inflammation

and depression is, at least in part, due to shared genes, perhaps genes regulating immune function and inflammatory response. Our data indicate that inflammation and depression are both expression of a common pathophysiological process linked to immune dysregulation. Our work substantiates the suspicion that there may be shared etiological mechanisms across chronic conditions, and that a key link may be a shared genetic vulnerability to inflammation (24;25). This might be true for other chronic conditions, such as CHD, diabetes, cancer and neurodegenerative diseases (50). Our results also provide a clue for the inconsistencies in previous literature concerning the association between MDD and inflammation (21), since results may vary depending on genetic admixture and population stratification.

Previous twin and family studies have demonstrated a role of genetic factors in the liability to depression (51) and to inflammation (52;53); however, no study has examined whether a common genetic substrate underlies vulnerability to both. Potential candidates, of course, are gene variations influencing the encoding or expression or inflammatory cytokines (25). Given our results, genetic polymorphisms influencing MPO levels could be implicated, but these variants have never been studied in relation to depression. A functional G-463A polymorphism in the promoter region of the MPO gene, which increases MPO production (54), has been associated with a variety of diseases with inflammatory components, including CHD (39) and Alzheimer's disease (43). Data, however, are not entirely consistent, and in some studies the A variation was the risk allele (55). Other common genes that may influence both depression and inflammation include those involved in the hypothalamus-pituitary-adrenal (HPA) axis, or in the sympathetic, parasympathetic and serotonin pathways, which have been related to stress and depression (51) and may be relevant to immune regulation secondary to the stress response (53;56).

Limitations

Our study is cross-sectional, thus limited in the ability to discern the temporal order between MDD and inflammation. However, based on our results, the covariation of these two phenotypes may mostly be due to a common genetic precursor rather than a cause-effect relationship. Another limitation is that few twins met the criteria for a current major depressive episode, preventing us from examining the data in relation to current versus past depression. However, there was no relationship between MPO and current depressive symptoms measured with the BDI, and although other inflammatory markers were related to the BDI score, adjustment for BDI did not alter the results. These data suggest that the link between depression and inflammation is due to common genetic liability between MDD, a heritable trait, and inflammation, and that current depressive symptoms do not play a role. Furthermore, the finding that a relationship between MDD and inflammation is seen in our sample of persons mostly in remission indicates that such a relationship may be of significance for chronic consequences of MDD such as CHD. Lastly, because our twins were all middle-aged male military veterans, caution should be used in generalizing our results to women or older individuals. Our study provides the foundation for future investigations where our findings can be confirmed in diverse sociodemographic groups.

Conclusions

Inflammation is a correlate of MDD largely because of common genetic liability. Of several biomarkers, MPO, a specific marker of leukocyte activation, shows the strongest association with MDD. Inflammatory genes involved in MPO expression or in other aspects of immune/inflammatory regulation should be investigated as potential precursors of MDD. Overall, our results are consistent with the hypothesis that inflammation and depression are both expression of a common pathophysiological process linked to immune dysregulation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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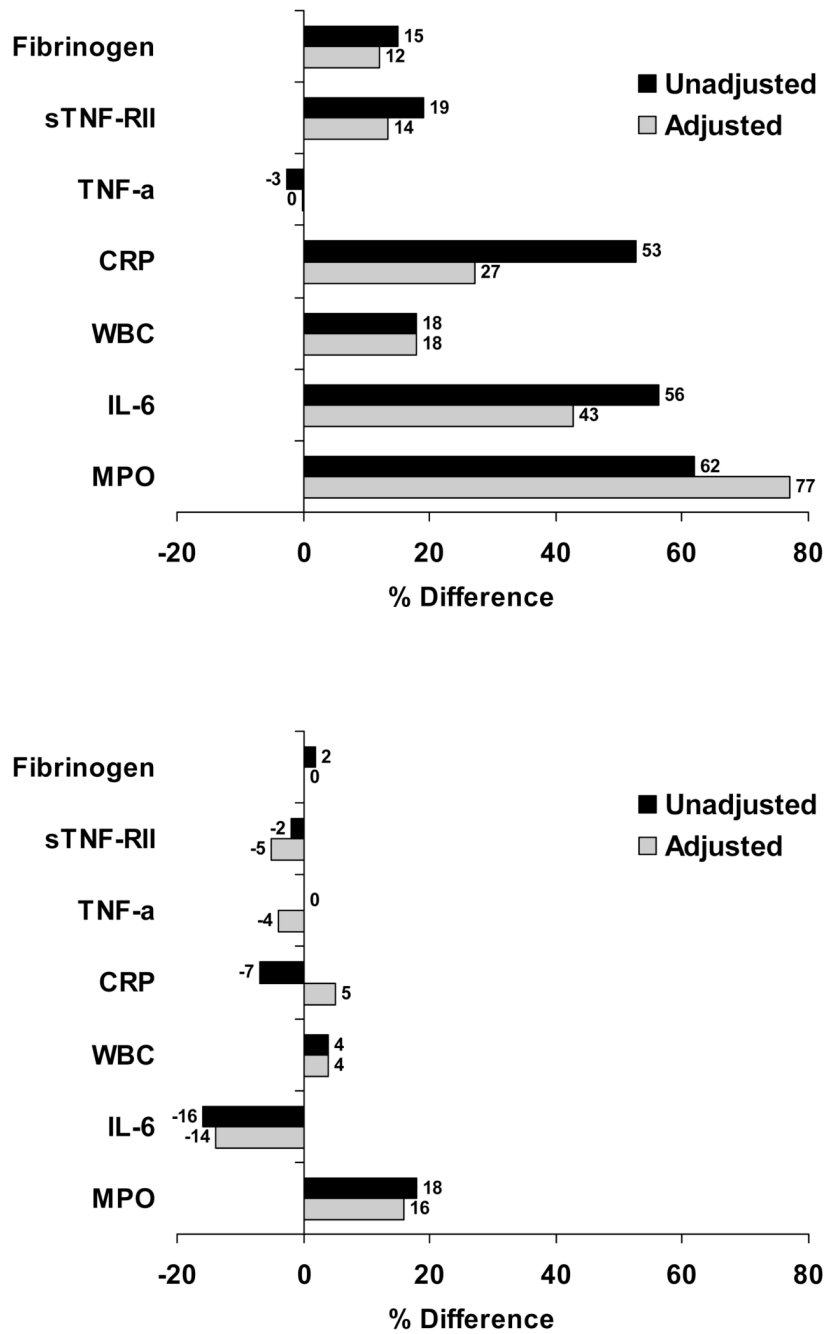


Figure 1. Unadjusted and adjusted percent differences in inflammatory markers in dizygotic (upper panel) and monozygotic (lower panel) twin pairs discordant for MDD, comparing twins with MDD to their co-twins without MDD. Based on data in Table 5 (see this table for p values).

Table 1 Distribution of demographic, behavioral and coronary risk factors in the entire sample (n=356) and in 67 twin pairs discordant for MDD.

	All Twins			MDD-Discordant Pairs		
	No MDD n=275	MDD n=81	P Value*	No MDD n=67	MDD n=67	P Value*
Age, years, mean ± SD	54.6 ± 2.9	53.6 ± 2.9	0.11	53.9 ± 2.8	53.9 ± 2.8	—
Greater than high school education, %	31.3	22.2	0.15	31.3	25.4	0.35
Married, %	83.3	64.2	0.001	85.1	64.2	0.003
Systolic blood pressure, mm Hg, mean ± SD	130 ± 16.0	127 ± 15.0	0.16	127 ± 17.1	126 ± 14.0	0.51
Diastolic blood pressure, mm Hg, mean ± SD	81.2 ± 10.8	79.3 ± 10.0	0.18	79.7 ± 10.2	78.5 ± 9.0	0.38
LDL-cholesterol, mg/dL, mean ± SD	122 ± 33.6	124 ± 35.2	0.62	122 ± 36.2	124 ± 35.8	0.72
HDL-cholesterol, mg/dL, mean ± SD	38.4 ± 8.9	39.1 ± 12.0	0.87	37.6 ± 8.4	36.8 ± 9.5	0.49
Diabetes, %	7.7	14.8	0.12	12.1	13.4	0.72
Body mass index, mean ± SD	29.2 ± 4.8	29.2 ± 5.2	0.69	29.5 ± 5.3	29.0 ± 4.7	0.47
Current smoker, %	16.7	32.1	0.001	16.4	34.3	0.01
Framingham Risk Score	5.7 ± 2.2	5.6 ± 2.1	0.95	5.4 ± 2.4	5.5 ± 2.1	0.64
Physical activity (Baecke) score, mean ± SD	7.4 ± 1.6	6.9 ± 2.2	0.02	7.5 ± 1.8	7.0 ± 2.2	0.12
Number of alcoholic beverages in typical week, mean ± SD	4.5 ± 7.2	6.3 ± 13.7	0.14	4.1 ± 5.6	5.1 ± 9.1	0.41
Prior coronary heart disease, %	9.4	11.1	0.89	13.4	10.5	0.52
Lifetime history of PTSD, %	4.4	13.6	0.002	4.5	14.9	0.03
Lifetime history of alcohol abuse or dependence, %	41.1	55.5	0.02	41.8	55.2	0.80
Lifetime history of drug abuse or dependence, %	16.0	14.8	0.89	14.9	16.4	0.11
BDI score	3.8 ± 4.9	8.8 ± 9.6	<0.0001	3.9 ± 4.7	8.4 ± 9.7	<0.0001
Taking antidepressants, %	9.1	32.1	<0.0001	8.9	28.3	0.01
Taking ace-inhibitors, %	13.4	16.0	0.73	19.4	13.4	0.35
Taking statins, %	24.4	24.7	0.80	34.3	28.3	0.39
Taking beta-blocker medications, %	7.3	12.3	0.25	10.4	13.4	0.56
Taking aspirin, %	26.5	22.2	0.44	23.9	20.9	0.65

BDI: Beck Depression Inventory; MDD: Major Depression Disorder. SD: Standard Deviation.

* P values are obtained from mixed models for continuous variables or generalized estimating equations (GEE) for categorical variables.

Unadjusted and adjusted differences in inflammatory biomarkers in the entire sample (n=356), considering twins as separate individuals.

Table 2

	Unadjusted						
	MPO (pmol/L)	IL-6 (pg/mL)	WBC Count (1000/ μ L)	CRP (mg/L)	TNF α (pg/mL)	sTNF R-II (pg/mL)	Fibrinogen (mg/dL)
No MDD, mean* (95% CI)	603 (556, 654)	1.80 (1.65, 1.97)	6.08 (5.86, 6.31)	1.21 (1.03, 1.42)	1.29 (1.20, 1.39)	2.00 (1.93, 2.07)	327 (319, 336)
MDD, mean* (95% CI)	794 (701, 899)	2.16 (1.85, 2.52)	6.70 (6.31, 7.11)	1.42 (1.10, 1.83)	1.30 (1.16, 1.46)	2.10 (1.99, 2.22)	346 (330, 362)
% Difference	31.7	20.0	10.2	17.4	1.0	5.0	5.8
P	<0.0001	0.04	0.002	0.22	0.93	0.07	0.03
	Adjusted [†]						
	MPO (pmol/L)	IL-6 (pg/mL)	WBC Count (1000/ μ L)	CRP (mg/L)	TNF α (pg/mL)	sTNF R-II (pg/mL)	Fibrinogen (mg/dL)
No MDD, mean* (95% CI)	606 (559, 656)	1.84 (1.69, 2.00)	6.09 (5.88, 6.30)	1.25 (1.07, 1.44)	1.28 (1.19, 1.38)	2.01 (1.94, 2.08)	329 (321, 338)
MDD, mean* (95% CI)	791 (696, 899)	2.04 (1.74, 2.38)	6.65 (6.27, 7.06)	1.35 (1.05, 1.73)	1.29 (1.14, 1.45)	2.04 (1.93, 2.16)	343 (327, 359)
% Difference	30.6	10.6	9.3	8.3	1.0	1.6	4.2
P	0.0001	0.26	0.008	0.56	0.92	0.57	0.12

MPO: myeloperoxidase. IL-6: interleukin-6. WBC: white blood cell. CRP: C-reactive protein. TNF α : tumor-necrosis factor α . sTNF R-II: soluble TNF α receptor II. MDD: major depressive disorder. SE: standard error.

* Geometric means calculated from means of log-transformed values.

[†] Adjusted for marital status, body mass index, habitual physical activity (Baecke score), antidepressant medications, the Framingham risk score, zygosity and previous history of coronary heart disease.

s in 67 twin pairs discordant for MDD. Matched co-twin control analysis.

	Adjusted									
	Dizygotic (32 Twin Pairs)					Monozygotic (35 Twin Pairs)				
	TNF α (pg/mL)	sTNF R-II (pg/mL)	Fibrinogen (mg/dL)	MPO (pmol/L)	IL-6 (pg/mL)	WBC Count (1000/ μ L)	CRP (mg/L)	TNF α (pg/mL)	sTNF R-II (pg/mL)	Fibrinogen (mg/dL)
	1.16 (0.94, 1.42)	1.80 (1.65, 1.98)	317 (289, 348)	518 (427, 628)	1.80 (1.31, 2.47)	5.60 (5.08, 6.17)	1.00 (0.60, 1.65)	1.14 (0.93, 1.41)	1.85 (1.68, 2.03)	323 (294, 354)
	1.13 (0.92, 1.39)	2.15 (1.96, 2.35)	365 (333, 400)	917 (753, 1115)	2.57 (1.86, 3.54)	6.58 (5.95, 7.26)	1.27 (0.76, 2.12)	1.13 (0.92, 1.39)	2.10 (1.91, 2.31)	360 (328, 396)
	0.79	0.005	0.002	<0.0001	0.13	0.01	0.43	0.90	0.051	0.11
	TNF α (pg/mL)	sTNF R-II (pg/mL)	Fibrinogen (mg/dL)	MPO (pmol/L)	IL-6 (pg/mL)	WBC Count (1000/ μ L)	CRP (mg/L)	TNF α (pg/mL)	sTNF R-II (pg/mL)	Fibrinogen (mg/dL)
	1.49 (1.27, 1.75)	2.15 (1.96, 2.37)	320 (301, 341)	628 (508, 776)	2.01 (1.65, 2.50)	6.78 (6.16, 7.46)	1.24 (0.84, 1.83)	1.52 (1.29, 1.79)	2.19 (2.00, 2.41)	325 (305, 346)
	1.49 (1.27, 1.75)	2.11 (1.92, 2.32)	327 (306, 349)	727 (588, 899)	1.74 (1.40, 2.16)	7.07 (6.42, 7.80)	1.30 (0.89, 1.92)	1.46 (1.24, 1.72)	2.08 (1.89, 2.28)	322 (301, 344)
	0.99	0.57	0.64	0.18	0.28	0.37	0.75	0.64	0.14	0.85
	0.84	0.005	0.10	0.02	0.02	0.14	0.10	0.57	0.009	0.10

umor-necrosis factor α , sTNF R-II: soluble TNF α receptor II, MDD: major depressive disorder.

medications, the Framingham risk score, and previous history of coronary heart disease.