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Depression and oxidative damage to lipids

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Summary Depression is associated with increased morbidity and mortality from cardiovascular and cerebrovascular diseases. Oxidative damage to lipids is one of the key early events in the etiology of atherosclerosis, the pathologic condition that underlies these diseases. The current study examines the pathophysiological consequences of depression by comparing serum levels of $F_{2\alpha}$-isoprostanes (8-iso-$PGF_{2\alpha}$), a biomarker of oxidative damage to lipids, in a group of depressed individuals ($n = 73$) and a matched comparison group ($n = 72$). The depressed group had significantly higher levels of serum 8-iso-$PGF_{2\alpha}$, while controlling for age, gender, race, years of education, daily smoking, number of alcoholic drinks per week, average amount of physical activity per week, and body mass index. Analyses using interviewer ratings on the Hamilton Scale revealed that, within the depressed cohort, there was no significant association between the severity of symptoms and levels of 8-iso-$PGF_{2\alpha}$, suggesting this is a threshold rather than a dose—response relationship. Results extend on our knowledge of depression and oxidative damage to lipids. In conclusion, oxidative damage to lipid molecules may represent a common pathophysiological mechanism by which depressed individuals become more vulnerable to atherosclerosis and its clinical sequelae.

Depression is a significant public health problem that reduces patients' quality of life, impairs their productivity at work, and leads to poor medical outcomes (Kessler et al., 2005). Patients who are depressed following a myocardial infarction, for example, have a 2.2-fold increased risk of cardiac morbidity and mortality (Barth et al., 2004; VanMelle et al., 2004), and individuals who are depressed as young adults show a 1.6-fold increase in rates of cardiac morbidity and mortality when they reach middle age (Rugulies, 2002). Because this excess morbidity and mortality is independent of traditional risk factors for cardiovascular disease (Rugulies, 2002; Barth et al., 2004; VanMelle et al., 2004), there has been much interest in identifying biological mechanisms that might be responsible for it (see Musselman et al., 1998; Carney et al., 2002; Glassman, 2007).

One of the key early events in the etiology of atherosclerosis involves oxidative damage to lipids (Bhattacharyya and Libby, 1998). Unlike unmodified lipids whose cellular uptake is regulated by negative feedback inhibition, oxidized lipids are easily taken up by "scavenger" receptors on macrophages in unregulated amounts. Therefore, large quantities of modified lipids can be ingested by the cell, leading to the formation of the "foam cells" that comprise fatty streaks in arteries (Bhattacharyya and Libby, 1998). These fatty streaks are the first pathologic indication of cardiovascular disease. Lipids

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that have been oxidatively modified are also thought to promote leukocyte adhesion, platelet activation, endothelial dysfunction, and other processes that accelerate the progression of atherosclerosis (Davi et al., 2004).

Depression is associated with the activation of innate immune responses and a mild systemic inflammation (Raison et al., 2006). Activated phagocytes are significant sources of reactive oxygen species (ROS) and produce superoxide, hydrogen peroxide, nitric oxide, and peroxynitrite as part of the cytotoxic host response against invading pathogens (Babior, 2000). *In vivo* animal models of inflammation and infection suggest that the systematic oxidation of lipid molecules is part of the host response to infection and inflammation (Memon et al., 2000). Thus, it is possible that depression may lead to increased levels of oxidized lipids by promoting inflammatory pathways. Alternatively, individuals that are clinically depressed might be more likely to engage in behaviors that bring about oxidative damage, such as smoking or alcohol use.

There is significant evidence that psychological stress is associated with increased oxidative stress, in both animal and human models (see review by Gidron et al., 2006). Further, depression has been linked to oxidative stress in general (Irie et al., 2001, 2005; Forlenza and Miller, 2006) and lipid oxidation more specifically (Bilici et al., 2001; Sarandol et al., 2006; Dimopoulos et al., 2008). In humans, oxidative damage to lipids is best assessed via levels of F2-isoprostanes (Halliwell, 2000; Patrignani and Tacconelli, 2005; Roberts and Morrow, 2000). F2-isoprostanes are specific products of lipid peroxidation that are stable compounds present in detectable volumes in all normal biological fluids and tissues (Halliwell, 2000; Roberts and Morrow, 2000). Levels of 8-iso-$PGF_{2\alpha}$, a specific F2-isoprostane compound formed by free-radical induced peroxidation of arachidonic acid, are elevated in patients with coronary heart disease (Schwedhelm et al., 2004; Elesbar et al., 2006) as well as healthy adults with cardiovascular risk factors, such as smoking, hypercholesterolemia, chronic infections, obesity, and diabetes (Milne et al., 2005; Morrow, 2005). Research has also shown 8-iso-$PGF_{2\alpha}$ acts as a vasoconstrictor, mediates smooth muscle growth, and activates platelets (Davi et al., 2004).

As far as we are aware only 1 study has assessed whether depression is related to F2-isoprostanes. Dimopoulos et al. (2008) found that serum levels of 8-iso-$PGF_{2\alpha}$ were increased in a population of depressed elderly adults as compared to controls. While this study provides initial evidence of a relationship between depression and 8-iso-$PGF_{2\alpha}$, it was based on a small geriatric population, some of whom had comorbid medical conditions that may have contributed to this association. It also did not assess the role of lifestyle variables such as physical activity and alcohol use as confounds or mediators. Thus, the goal of the current study was to build on this work by determining whether 8-iso-PG $F_{2\alpha}$ levels are increased in a younger and presumably healthier sample of depressed patients, and also to evaluate the role of lifestyle factors in this association.

1. Methods

1.1. Participants

All volunteer participants were recruited from January 2001 to May 2003 by advertisements in mass transit stations and newspapers, as well as posted flyers in the St. Louis, Missouri metropolitan area. Seventy-three participants met criteria for clinical depression, and constituted our depressed group. The control group was made up of 72 individuals that were free of psychiatric illness and matched case-by-case to patients in the depressed group on age, gender, and ethnicity. Participants were fully informed of the study procedures and gave written consent.

To be eligible for the study, participants had to report being without a lifetime history of chronic medical illnesses, including cardiovascular disease, diabetes, cancer, arthritis, and chronic infection. They also had to be without prescribed medications, other than oral contraceptives, for the past 6 months, and be free of acute infectious disease at the time of participation, as indicated by self-report and complete blood count with differential (Beckman Coulter, Fullerton, CA). None of the depressed patients were using anti-depressant medications. Serum albumin was determined by spectrophotometry (Kyowa Medes, Shizuoka, Japan) and used as a measure of gross nutritional status to exclude subjects with protein-store deficiencies (there were none). The same participant sample was used in a previous article by our group on depression and DNA oxidation (Forlenza and Miller, 2006).

1.2. Depression measures

All participants in the depressed group met diagnostic criteria for a current major depressive disorder or minor depressive disorder as defined in the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) (American Psychiatric Association, 1994). Diagnoses were made by trained interviewers using the Depression Interview and Structured Hamilton Interview, a semi-structured interview which provides DSM-IV major and minor depressive disorder diagnoses and a severity score based on the Structured Interview Guide for the Hamilton Depression Scale (Freedland et al., 2002). Determination of comorbid psychiatric disease was made using modified versions of the Diagnostic Interview Schedule and the Primary Care Evaluation of Mental Disorders (Robins et al., 1981; Spitzer et al., 1994). Participants were excluded from the study if they had comorbid psychotic disorders, eating disorders, substance abuse disorders, or anxiety disorders other than generalized anxiety disorder. Diagnostic agreement among the project interviewers was excellent. On 20 interviewers that were co-rated, kappa averaged 0.79, a value indicating *good*-to-*excellent* diagnostic agreement.

Blood was drawn by antecubital venipuncture and centrifuged at 1000 *g* for 25 min. The serum was then aspirated, divided into aliquots, and stored at $-70\,^{\circ}C$ until assay. All blood draws were taken between 9 and 11 in the morning to control for diurnal rhythms.

1.3. Measures of 8-iso-prostaglandin $F_{2\alpha}$

Serum levels of 8-iso-$PGF_{2\alpha}$ were determined by an Enzyme-linked ImmunoSorbent Assay (ELISA) kit (Stressgen Bioreagents Corp., Product # EKS-210); a competitive immunoassay for the quantification of total (both free and esterified) 8-iso-$PGF_{2\alpha}$. This kit is specific for the measurement of 8-iso-$PGF_{2\alpha}$ in serum or plasma. Serum samples were thawed and pre-

pared according to the manufacturer's instructions. All samples were run in a single batch. Standards were added to the pre-coated Goat Anti-rabbit IgG microtiter plates ($n = 7$) along with serum samples in triplicate. Next, the 8-iso-$PGF_{2\alpha}$ Direct Conjugate and the 8-iso-$PGF_{2\alpha}$ ELISA antibody were added to the appropriate wells and incubated at 4 °C overnight. Assays were conducted using the overnight method as sensitivity is considerably improved (our validation study showed a significant correlation between the 2 h method and the overnight method, $r = 0.997$). The following day, wells were emptied, washed 3 times, and incubated with p-Npp Substrate solution for 45 min. At this time the reaction was stopped and absorbance was measured at 405 nm on a microplate reader.

Standard curves were calculated and plotted for each plate with the percent bound on the *y*-axis and (log) standard concentration on the *x*-axis. R^2 for each standard ranged from 0.94 to 0.97. Sample concentrations of 8-iso-$PGF_{2\alpha}$ (ng/ml) were determined by interpolation from the standard curves for each plate.

1.4. Covariates

We assessed a number of variables that could provide alternative explanations for any observed association between depression and 8-iso-$PGF_{2\alpha}$. Demographic characteristics including age, gender, ethnic/racial identity and years of education were all self-reported. Body mass index was calculated using height and weight measurements obtained on a balance-beam scale with a height rod. Smoking and alcohol were assessed with self-report measures that had been extensively validated in previous research (Miller et al., 1999). Subjects who reported smoking 10 or more cigarettes per day were classified as regular smokers. Alcohol consumption was defined as the reported average number of alcoholic drinks per week. Physical activity was measured with the Paffenbarger Physical Activity Scale (Paffenbarger et al., 1999) and was defined as the number of minutes per week that the subject participates in any regular activity long enough to work up a sweat (e.g. brisk walking, jogging, bicycling, etc.).

1.5. Statistical analyses

We conducted statistical analyses with SPSS (Version 14.0, SPSS, Inc., 2005). Linear regression was used to estimate differences in serum levels of 8-iso-PG $F_{2\alpha}$ as a function of depression, adjusting for the covariates: age, gender, ethnicity, years of education, daily smoking, average number of alcoholic drinks per week, average amount of brisk physical activity per week, and body mass index. Pearson's correlations were also computed to estimate the magnitude of the association between symptom severity and levels of 8-iso-PG $F_{2\alpha}$.

2. Results

Table 1 presents the means and standard deviations for all study variables for both groups. The groups were similar with respect to age, race, and gender, as would be expected from the case-matched design we used. Analyses of the other potential confounds revealed that depressed patients were significantly less educated ($F[1,144] = 7.497$, $p < 0.001$), and significantly more likely to be regular daily smokers ($\chi^2[1] = 21.94$, $p < 0.001$), than control subjects. The groups did not differ significantly on any other covariates.

2.1. Group differences in lipid oxidation

Analyses revealed that depression was associated with oxidative damage. In a simple linear regression comparing raw unadjusted 8-iso-PG $F_{2\alpha}$ values, depressed subjects had levels that were 2.34 times higher than controls (50.57 ± 5.95 vs. 21.57 ± 3.29, $B = 29.01$, SE = 6.82, $\beta = 0.34$, $p < 0.001$). This disparity persisted when covariates were added to the equation. Specifically, in a linear regression model adjusting for age, gender, ethnicity, education, smoking, alcohol, exercise, and adiposity, depressed subjects had 8-iso-PG $F_{2\alpha}$ values that were 2.28 times higher than control subjects (50.35 ± 4.90 vs. 21.79 ± 4.94, $B = 28.55$, SE = 7.20, $\beta = 0.33$, $p < 0.001$) (see Table 2). Fig. 1 displays the unadjusted mean comparisons for the two groups. To gauge the magnitude of this difference, we computed

Table 1 Characteristics of the sample.

	Depressed group ($n = 73$)	Control group ($n = 72$)	*p*-Value
Age (years ± SD)	28.4 ± 9.1	28.8 ± 9.2	0.850
Women, *n* (%)	61 (82.4)	59 (79.7)	0.802
White, *n* (%)	35 (47.3)	35 (47.3)	0.931
Black, *n* (%)	32 (43.2)	34 (45.9)	0.686
Other, *n* (%)	7 (9.5)	5 (6.8)	0.566
Smokers, *n* (%)	25 (33.8)	5 (6.8)	<0.001
Body mass index (mean ± SD)	28.4 ± 9.1	27.0 ± 6.7	0.327
Years of education (mean ± SD)	14.3 ± 2.3	15.2 ± 1.9	0.007
Alcoholic drinks per week (mean ± SD)	3.8 ± 7.5	2.8 ± 6.6	0.353
Brisk physical activity per week (minutes ± SD)	95.7 ± 177.5	114.0 ± 182.1	0.498
Serum 8-iso-$PGF_{2\alpha}$ (mean ± SD) (ng/mL)	50.57 ± 50.82	21.56 ± 27.94	<0.001
Serum 8-iso-$PGF_{2\alpha}$ (range) (ng/mL)	3.77—172.90	1.80—145.46	—
Serum 8-iso-$PGF_{2\alpha}$ (median) (ng/mL)	22.63	13.67	—

8-Iso-PG $F_{2\alpha}$ = 8-isoprostaglandin-$F_{2\alpha}$; SD = standard deviation.

Table 2 Results of linear regression with serum 8-iso-$PGF_{2\alpha}$ as dependent variable.

Source of variance	*B*	SE	β	*p*-Value
Age	0.464	0.435	0.096	0.288
Gender (0 = men, 1 = women)	−17.231	9.569	−0.155	0.074
Ethnicity (0 = other, 1 = white)	5.831	7.242	0.067	0.422
Daily smoker (0 = no, 1 = yes)	7.435	9.591	0.690	0.440
Years of education	0.785	1.817	0.039	0.666
Alcoholic drinks per week	−0.985	0.570	−0.146	0.087
Minutes of brisk physical activity per week	−.025	0.020	−0.104	0.216
Body mass index	0.152	0.475	0.028	0.749
Group (0 = control, 1 = depressed)	28.555	7.195	0.330	<0.001

8-Iso-PG $F_{2\alpha}$ = 8-isoprostaglandin-$F_{2\alpha}$; SE = standard error.

Cohen's *d*, a widely used measure of effect size. The value was $d = 0.71$, which means that on average, depressed patients have 8-iso-PG $F_{2\alpha}$ levels 0.71 standard deviation's higher than control subjects.

To further evaluate the potential confounding influence of cigarette use, we reran the analyses after excluding all subjects who were regular smokers. This left 49 depressed subjects and 69 controls. In this subgroup, depression remained significantly associated with levels of 8-iso-$PGF_{2\alpha}$ ($B = 32.99$, SE = 7.99, $\beta = 0.36$, $p < 0.001$), even while controlling for age, gender, ethnicity, education, alcohol, exercise, and adiposity.

Using information gathered as part of the Depression Interview and Structured Hamilton Interview, we examined whether the severity or duration of the depressive episode were associated with the extent of oxidative damage to lipids. There was no evidence of a significant relationship between 8-iso-$PGF_{2\alpha}$ and depression severity ($B = 0.28$, SE = 0.15, $\beta = 0.02$, $p = 0.584$) nor duration ($B = -0.35$, SE = 1.32, $\beta = -0.35$, $p = 0.791$).

In a previous article with this sample of patients, we reported that depression was associated with increased 8-hydroxy-2′-deoxyguanosine (8-OHdG), a biomarker of oxidative damage to DNA which has been implicated in the pathogenesis of cancer (Forlenza and Miller, 2006). We evaluated whether these findings were independent by computing Pearson's correlations between 8-iso-$PGF_{2\alpha}$ and 8-OHdG. Results indicated that there was little sample-wide correlation between these biomarkers ($r = -0.094$, $p = 0.262$). Further, adjusting for 8-OHdG values did not attenuate the reported differences in 8-iso-$PGF_{2\alpha}$ between depressed and control subjects (p remained <0.001), suggesting the two effects were statistically independent.

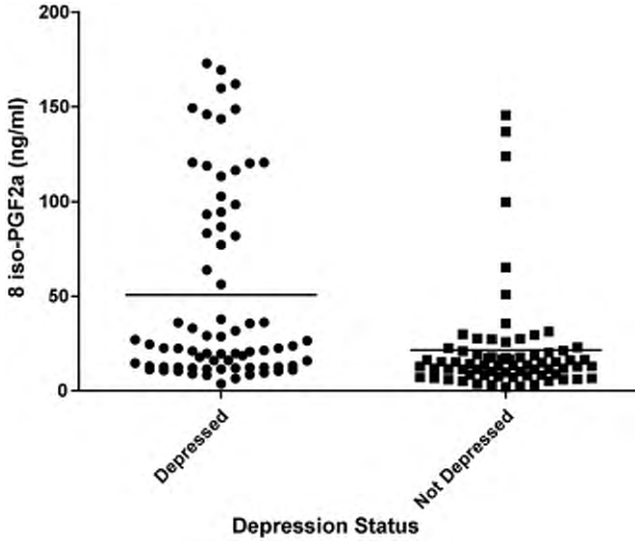

Figure 1 Comparison of raw, unadjusted 8-isoprostaglandin-$F_{2\alpha}$ (8-iso-PG $F_{2\alpha}$) levels in the depressed and control groups 8-iso-PG $F_{2\alpha}$ = 8-isoprostaglandin-$F_{2\alpha}$.

2.2. Ancillary analysis

To explore the possibility that depression is linked to lipid peroxidation by means of inflammation, we examined the relationship between C reactive protein (CRP), a measure of systemic inflammation, and 8-iso-$PGF_{2\alpha}$ in our data set. CRP and 8-iso-$PGF_{2\alpha}$ were only marginally correlated and adding CRP to our analysis did not alter the observed relationship between depression and 8-iso-$PGF_{2\alpha}$ ($B = 29.41$, SE = 7.13, $\beta = 0.34$, $p < 0.001$).

3. Discussion

Depression is a significant and independent risk factor for cardiac morbidity and mortality (Rugulies, 2002; Barth et al., 2004; VanMelle et al., 2004), but the biological mechanism(s) responsible for this relationship has yet to be determined. The current study examined lipid oxidation, a key early event in the course of cardiovascular disease, as one possible mechanism by which depression might become linked to poor cardiac outcomes. Using a cross-sectional study design, we measured serum concentrations of 8-iso-$PGF_{2\alpha}$, a marker of lipid oxidation, in a group of depressed individuals and a matched comparison group. Depressed participants were found to have 8-iso-$PGF_{2\alpha}$ levels that were more than 2 times higher than controls, even after controlling for demographic and lifestyle factors of both groups.

Previous research has linked depression to increased serum levels of malondialdehyde (MDA), a breakdown product of oxidized apolipoprotein B-containing lipoproteins, and thus a marker of the rate of peroxide breakdown (Bilici et al., 2001; Sarandol et al., 2006). Although this is used as a marker of lipid peroxidation, it is considered to be less stable than 8-iso-$PGF_{2\alpha}$, and more susceptible to confounding factors such as antioxidants from the diet (Halliwell, 2000). A

recent study by Dimopoulos et al. (2008) compared serum levels of 8-iso-$PGF_{2\alpha}$ in a group of elderly people with depression, as characterized by high scores on the Geriatric Depression Scale, to a group of age-matched controls. The mean serum level of 8-iso-$PGF_{2\alpha}$ was significantly higher in the depressed group. Results of this study provide initial evidence of an association between 8-iso-$PGF_{2\alpha}$ and depression in a population of individuals over 60 years of age. This study included a small sample size ($N = 66$), many of who suffered from chronic diseases such as hypertension, diabetes, and hypercholesteremia. The current study extends these findings by examining a non-elderly population free of chronic illness. Further, we statistically controlled for lifestyle variables such as body mass index, alcohol use, and physical activity, in order to control for the possible confounding effects of such behaviors on the relationship between depression and lipid oxidation.

The oxidation of lipids is particularly relevant to cardiovascular disease in that oxidized lipids contribute to the pathogenesis (Bhattacharyya and Libby, 1998) and the progression (Davi et al., 2004) of atherosclerosis. Further, both patients with coronary heart disease and healthy adults at risk for it display elevated 8-iso-$PGF_{2\alpha}$ (Schwedhelm et al., 2004; Milne et al., 2005; Elesbar et al., 2006). Thus, lipid oxidation could be a common mechanism linking depression to poor cardiac outcomes in both healthy adults and patients with established disease.

It remains unclear why depression is associated with lipid peroxidation. One potential explanation is that clinical depression is associated with behaviors that bring about oxidative damage, such as cigarette smoking, alcohol use, or sedentary lifestyle. However, in our sample, these factors were unrelated to 8-iso-$PGF_{2\alpha}$ level, and statistically controlling for them did not attenuate the observed association with depression. None of the patients had acute or chronic diseases that could account for the findings, nor were they using prescription medications other than oral contraceptives. We did not gather adequate information on participants' use of non-prescription drugs to include this as a variable in analyses. Thus, the use of non-prescription medications associated with a reduction in oxidative damage, such as aspirin or fish oils, may account for some of the observed covariation between depression and oxidative damage. Diet is another factor that may underlie depression's association with 8-iso-$PGF_{2\alpha}$; unfortunately, we do not have data on diet that would allow for an evaluation of this hypothesis. Examining the roles of non-prescription drug use and diet should be a priority in future research on lipid peroxidation.

Alternatively, depression may contribute to the production of, and exposure to, reactive oxygen species, and thus lead to increased oxidation of lipid molecules. Depression is associated with the activation of the innate immune response (Raison et al., 2006) and subsequent production of pro-inflammatory mediators like tumor necrosis factor-α, interleukin-6, and C-reactive protein (e.g. Miller et al., 2002; Penninx et al., 2003; Lesperance et al., 2004; Ranjit et al., 2007). Depressed patients also show heightened vulnerability to latent infection with herpes viruses (Miller et al., 2005). *In vivo* animal models suggest that systemic oxidation of lipids occur as part of the host response to infection and inflammation (Memon et al., 2000). Activated phagocytes are significant sources of reactive oxygen species and produce superoxide, hydrogen peroxide, nitric oxide, and peroxynitrite as part of the cytotoxic host response against invading pathogens (Babior, 2000). Further, activated leukocytes secrete myeloperoxidase, a heme protein that generates reactive nitric oxide-derived oxidants that promote lipid peroxidation (Zhang et al., 2002). As such, it is possible that depression is linked to lipid oxidation by means of inflammation. We indirectly explored this possibility by examining the relationship between C reactive protein (CRP) and 8-iso-$PGF_{2\alpha}$ in our data set. However, CRP and 8-iso-$PGF_{2\alpha}$ were only marginally correlated and adding CRP to our analysis did not alter the observed relationship between depression and 8-iso-$PGF_{2\alpha}$. That said, future research should more closely examine the relationships between depression, inflammation, and lipid peroxidation by measuring myeloperoxidase and a broader panel of markers of infection and inflammation.

We considered the possibility that the observed differences in 8-iso-$PGF_{2\alpha}$ were part of a broader oxidative process occurring among depressed individuals. While the depressed patients in this sample also had elevated levels of serum 8-OHdG (Forlenza and Miller, 2006), a biomarker of oxidative damage to DNA, correlational analyses suggested these disparities were independent of each other. We are not certain why this is. One possibility is that this may be due to differences in where these oxidative reactions take place. Although we have measured biomarkers of oxidative damage to DNA and to lipids in the serum, it is likely that the damage to these different classes of biomolecules occurred in different biological compartments. Oxidative damage to DNA occurs within the cytoplasm or the nucleus of cells (i.e. intracellular) while oxidative damage to lipids occurs within the plasma itself or to lipids embedded in cell membranes (i.e. extracellular). Within these differing compartments, the sources and types of damaging reactive oxygen species (ROS) differ, as do the antioxidant defense systems.

Because unrepaired oxidative DNA damage has significant consequences for mutagenesis and carcinogenesis, there are multiple intracellular mechanisms for the repair of oxidative DNA damage such as base excision repair and nucleotide excision repair. There are no such protective repair mechanisms for damaged lipids. As previously discussed, damaged lipids are scavenged by macrophages in an unregulated fashion. Thus, it is possible that these markers of oxidative damage are not correlated within individuals because of differences in antioxidant scavenging or oxidative damage repair within the different biological compartments.

This study has several limitations that need to be considered. First, we did not measure several variables that could explain the observed association between depression and 8-iso-$PGF_{2\alpha}$. The most important among these were dietary intake of antioxidants and the use of over-the-counter supplements and medications, particularly those with known antioxidant and anti-inflammatory properties. Second, the cross-sectional design of our study does not allow inferences regarding the direction of causality. It is possible that oxidative damage contributes to mood problems or that both are the result of an underlying factor we did not identify (e.g., a common genetic liability or a latent infection). Rigorous prospective studies with more thorough assessment of potential confounds are needed to resolve these issues.

Finally, this study did not include pre-clinical markers of cardiovascular disease or disease endpoints, so it remains unclear whether the observed differences in 8-iso-$PGF_{2\alpha}$ will explain the differential coronary morbidity and mortality seen among depressed individuals.

Despite these limitations, this study extends the body of literature suggesting that clinical depression is accompanied by oxidative damage to lipids, a process that is important to both the pathogenesis and the progression of atherosclerosis. It also rules out the possibility that this association is attributable to a host of potential demographic or biobehavioral confounders. In future research it will be important to extend these findings by pinpointing the mechanism underlying their association, and evaluating their clinical implications for coronary heart disease outcomes.

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Conflicts of interest statement

All authors declare that they have no conflicts of interest.

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