Dysregulated relationship of inflammation and oxidative stress in major depression

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ABSTRACT

Chronic inflammation and oxidative stress have been implicated in the pathophysiology of Major Depressive Disorder (MDD), as well as in a number of chronic medical conditions. The aim of this study was to examine the relationship between peripheral inflammatory and oxidative stress markers in un-medicated subjects with MDD compared to non-depressed healthy controls and compared to subjects with MDD after antidepressant treatment. We examined the relationships between IL-6, IL-10, and the IL-6/IL-10 inflammatory ratio vs. F2-isoprostanes (F2-IsoP), a marker of oxidative stress, in un-medicated MDD patients ($n = 20$) before and after 8 weeks of open-label sertraline treatment ($n = 17$), compared to healthy non-depressed controls ($n = 20$). Among the un-medicated MDD subjects, F2-IsoP concentrations were positively correlated with IL-6 concentrations ($p < 0.05$) and were negatively correlated with IL-10 concentrations ($p < 0.01$). Accordingly, F2-IsoP concentrations were positively correlated with the ratio of IL-6/IL-10 ($p < 0.01$). In contrast, in the control group, there were no significant correlations between F2-IsoPs and either cytokine or their ratio. After MDD subjects were treated with sertraline for 8 weeks, F2-IsoPs were no longer significantly correlated with IL-6, IL-10 or the IL-6/IL-10 ratio. These data suggest oxidative stress and inflammatory processes are positively associated in untreated MDD. Our findings are consistent with the hypothesis that the homeostatic buffering mechanisms regulating oxidation and inflammation in healthy individuals become dysregulated in untreated MDD, and may be improved with antidepressant treatment. These findings may help explain the increased risk of comorbid medical illnesses in MDD.

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1. Introduction

Chronic inflammation and oxidative stress have been implicated in the pathophysiology of Major Depressive Disorder (MDD) (Berk et al., 2011; Capuron and Miller, 2011; Chauhan and Chauhan, 2006; Croonenberghs et al., 2002; Forlenza et al., 2007; Khanzode et al., 2003; Maes, 2011a,b, 1999; Miller, 2010; Raison and Miller, 2011; Wolkowitz et al., 2008) as well as a number of serious medical conditions (Maes et al., 2011b), including cardiovascular disease and atherosclerosis (Krishnan, 2010; Lakshmi et al., 2009; Tousoulis et al., 2008; Uno and Nicholls, 2010), chronic renal disease (Cottone et al., 2008), pulmonary disease (Jelic and Le Jemtel, 2008), rheumatoid arthritis (Stamp et al., 2012), certain cancers (Khansari et al., 2009; Maes et al., 2011b; Reuter et al., 2010), metabolic syndrome, obesity and diabetes (Agrawal et al., 2007; Assumpcao et al., 2008; Ferder et al., 2006; Guerrero-Romero and Rodriguez-Moran, 2006), and in the normal physiology of cellular aging and immuno-senescence (Cannizzo et al., 2011; De la Fuente and Miquel, 2009). While inflammation and oxidation have generally been studied separately in these conditions, the interplay between them has been less well-studied, despite mounting evidence that their interaction plays a major role in the pathogenesis of many diseases (Ambade and Mandrekar, 2012; Forlenza and Miller, 2006; Maes et al., 2011e, 2007; Rahman, 2003; Sarandol et al., 2007a). Examining the interplay between chronic inflammatory states and oxidative stress is likely to deepen our understanding of the pathophysiology of MDD and other diseases, offer greater insight into the associations between MDD and co-morbid medical illnesses with inflammatory or oxidative associations (such as diabetes, arthritis, dementia, metabolic syndrome and cardiovascular disease), and potentially guide novel approaches to the treatment of depression and its comorbid systemic diseases (Maes et al., 2011c; Nemeroff and Goldschmidt-Clermont, 2012; Wolkowitz et al., 2011b).

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A large body of evidence suggests that MDD is accompanied by activation of inflammatory pathways, reflected by an increased levels of inflammatory cytokines, such as Interleukin (IL)-1β, IL-2, IL-6, interferon-gamma (IFN-γ), and tumor necrosis factor-alpha (TNF-α) (Anisman et al., 1999a,b; Maes, 1999; Nassberger and Traskman-Bendz, 1993). Two recent meta-analyses showed significantly higher IL-6 concentrations among depressed versus non-depressed patients but no significant difference in IL-10 concentrations (Dowlati et al., 2010; Hiles et al., 2012). A causal relationship between inflammation and depression is suggested by observational studies of patients undergoing immunotherapy for hepatitis C or cancer who developed depressed mood and neurovegetative accompanying these treatments (Capuron et al., 2001; Dieperink et al., 2000; Kelley et al., 2003; Valentine and Meyers, 1995; Zdilar et al., 2000). Other small-scale studies also suggest antidepressant effects of anti-inflammatory medications (Muller et al., 2006; Nery et al., 2008) as well as anti-inflammatory effects of antidepressants (Abbasi et al., 2012; Felger et al., 2012; Hannestad et al., 2011).

A smaller but growing body of evidence also points to increased oxidative stress in MDD. Elevated plasma and/or urine oxidative stress markers (e.g., increased F2-isoprostanes [IsoPs] or 8-hydroxydeoxyguanosine [8-OHdG], along with decreased anti-oxidant compounds, such as Vitamins C and E) have been reported in individuals with MDD and in those with chronic psychological stress (Forlenza and Miller, 2006; Irie et al., 2001a,b, 2005; Maes et al., 2000), and the concentration of peripheral oxidative stress markers is positively correlated with the severity and chronicity of depression (Forlenza and Miller, 2006; Irie et al., 2003, 2002; Maes et al., 2011a; Miyaoka et al., 2005; Tsuboi et al., 2004; Yager et al., 2010). Antioxidant compounds are also being considered as possible antidepressant treatments (Gibson et al., 2012; Khanzode et al., 2003; Scapagnini et al., 2012; Wolkowitz et al., 2011b).

Inflammation and oxidative stress are inextricably connected in physiologic as well as disease states; they have even been termed "essential partners" in certain diseases (Ambade and Mandrekar, 2012). Under normal physiologic conditions, oxidative stress and activation of the immune system are generally short-lived due to intrinsic negative feedback mechanisms, such as increased production of anti-oxidant compounds or of anti-inflammatory cytokines. In certain chronic disease states, however, both of these systems remain activated and may, indeed, form a positive self-sustaining feedback loop, or a "co-activation" state (Jesmin et al., 2010). Over time, such co-activation may lead to a higher risk of disease and to more serious disease (Ambade and Mandrekar, 2012; Il'yasova et al., 2008; Jesmin et al., 2010; Khansari et al., 2009; Kotani and Taniguchi, 2012; Kregel and Zhang, 2007; Martinon, 2010; Rahman, 2003; Skalicky et al., 2008; Terlecky et al., 2012; Tschopp and Schroder, 2010).

The aim of the current study was to examine the relationship between inflammation and oxidative stress in the plasma of un-medicated subjects with MDD before and after antidepressant treatment, and in un-medicated subjects with MDD compared to non-depressed healthy controls. We hypothesized that un-medicated subjects with MDD would show stronger positive correlations between oxidative stress and inflammatory markers than the same subjects after antidepressant treatment and than healthy non-depressed controls.

2. Methods

2.1. Subjects

Twenty subjects with MDD, diagnosed with the Structured Clinical Interview for DSM-IV-TR (SCID) (First et al., 2002), and 20 matched healthy controls (matched by sex, ethnicity and age ±3 yr) were enrolled. Subjects were recruited by fliers, craigslist postings (http://sfbay.craigslist.org), and newspaper advertisements and, in the case of depressed subjects, clinical referrals. Subjects gave written informed consent to participate in this study, which was approved by the University of California, San Francisco (UCSF) Committee on Human Research and were paid for their participation. SCID interviews were conducted by an experienced clinical psychologist and were clinically verified by a separate psychiatric interview with a Board-certified psychiatrist. Depressed subjects with psychosis, post-traumatic stress disorder or bipolar histories were excluded, although other co-morbid anxiety disorders were allowed when the depressive diagnosis was determined to be the primary diagnosis. Seven of the depressed subjects had one or more secondary co-morbid psychiatric diagnoses as follows: three with generalized anxiety disorder, two with obsessive compulsive disorder, two with binge eating disorder (one of whom was in remission) and one with social anxiety disorder. Healthy controls were also screened with the SCID, and were required to have no present or past history of any DSM-IV Axis I or Axis II diagnosis. Potential subjects were excluded if they met SCID criteria for alcohol or substance abuse within 6 months of entering the study. Subjects in both groups were medically healthy (assessed by physical examination, review of systems and screening routine laboratory tests), had no acute illnesses or infections, and had not had any vaccinations within 6 weeks of entering the study. All subjects (depressed and control) were free of any psychotropic medications, including antidepressants, antipsychotics and mood stabilizers, as well as any hormone supplements, steroid-containing birth control or other interfering medications or Vitamin supplements above the U.S. Recommended Daily Allowances (e.g. Vitamin C, 90 mg/day), for a minimum of 6 weeks before entry into the study (with the exception of short-acting sedative-hypnotics, as needed, up to a maximum of 3 times per week, but none within 1 week prior to testing). The sample described in the present report incorporates the smaller sample previously reported on, which was used to test a different set of hypotheses (Dhabhar et al., 2009).

2.2. Procedures

Subjects were admitted as outpatients to the UCSF Clinical and Translational Science Institute's Clinical Research Center at 8:00 am, having fasted (except water) since 10:00 pm the night before. Before proceeding with testing, all subjects were required to test negative on a urine toxicology screen (measuring the presence of abused drugs) and, in women of childbearing capacity, a urine pregnancy test. After the subjects had sat quietly for 45 min, blood samples were obtained for the assay of serum IL-6, IL-10 and for the oxidative metabolite, plasma F2-IsoP. Severity of depression in the depressed subjects was ascertained with the observer-rated 17-item Hamilton Depression Rating Scale (HDRS-17) (Hamilton, 1967). Depressed and control subjects were also rated with the Inventory of Depressive Symptomatology, 30-item self-rated version (IDS) for purposes of comparing the two groups, since the HDRS-17 is not intended for use in non-depressed populations, and since the IDS shows greater sensitivity at lower levels of depressive severity (Rush et al., 1996). The IDS was also used in analyses in which depressive severity was utilized as an independent variable across groups. Scores on the IDS range from 0 to 84 and are highly correlated with scores on the HDRS-17 (Inventory of Depressive Symptomatology (IDS) and Quick Inventory of Depressive Symptomatology (QIDS), 2012). Ratings and blood assays were performed blind to each other.

Following these baseline behavioral and biochemical tests, the MDD subjects were treated in an open-label manner with sertraline for 8 weeks, after which blood was re-drawn for F2-IsoP and cytokine assays, and depression ratings repeated. Sertraline dosing

was based on clinicians' judgment, based on efficacy and tolerability. Sertraline dosing began with 50 mg per day, increasing to a maximum of 200 mg per day, as tolerated and as warranted by clinical response. In two cases, the beginning dose was initially lowered to 25 mg per day because of transient side effects. Medication compliance was monitored by pill counts and by plasma antidepressant levels at week 4 and week 8 of treatment. The mean plasma concentration of (sertraline + *N*-desmethylsertraline) at week 4 was 46 ± 23 ng ml^{-1}; range: $10–97$ ng ml^{-1}, and at week 8 was 67 ± 37 ng ml^{-1}; range: $10–146$ ng ml^{-1}. All individuals had plasma concentrations within the range of published steady state concentrations for sertraline at therapeutic doses (Mauri et al., 2002), indicating good compliance with medication treatment. One depressed subject prematurely dropped out of the study due to clinical worsening while on sertraline, and two were excluded due to developing new exclusionary criteria (periodontitis and elevated fasting blood sugar) during the 8 week follow-up period, leaving 17 treated MDD subjects. Control subjects were not re-evaluated after their initial baseline visit.

2.3. Assays

2.3.1. Blood collection

Blood for the plasma F2-IsoP assay was collected into EDTA tubes with no vacuum, and blood for the serum IL-6 and IL-10 assays was collected into serum separator tubes. Plasma and sera were frozen and stored at -80 C until assay.

2.3.2. Oxidative stress assay

F2-IsoPs are prostaglandin-like compounds formed *in vivo* via a non-enzymatic mechanism involving the free radical-initiated peroxidation of arachidonic acid. F2-IsoPs are very accurate measures of lipid peroxidation and are important markers of oxidative stress (Milne et al., 2005). F2-IsoPs were measured by the Eicosanoid Laboratory at Vanderbilt University. F2-IsoPs were extracted and purified with solid phase extraction and thin layer liquid chromatography and then converted to trimethylsilyl ether derivatives and analyzed by gas chromatography–mass spectrometry (GC–MS) as described previously (Milne et al., 2007; Morrow and Roberts, 2002). F2-IsoP values were not available for one MDD subject at baseline and for three MDD subjects at Week 8 due to poor chromatography, and for one MDD subject at Week 8 due to a value below the level of detection.

2.3.3. Inflammatory cytokine assays

IL-6 is a prototypic pro-inflammatory cytokine (although it also has some anti-inflammatory effects, especially during acute inflammation) (Scheller et al., 2011; Xing et al., 1998). It exerts stimulatory effects on T- and B-cells, and thus generally favors chronic inflammatory responses. IL-10 is an anti-inflammatory/immuno-modulating cytokine, and the IL-6/IL-10 ratio is considered an index of net pro-inflammatory activity (Dhabhar et al., 2009; Fredericks et al., 2010). A high sensitivity enzyme-linked immunosorbent assay was used to quantify IL-6 and IL-10 concentrations (R&D Systems, Minneapolis, MN). The IL-6 assay intra- and inter-assay coefficients of variation are 7% and 8% respectively. For IL-10, intra- and inter-assay coefficients of variation are 8% and 11% respectively. Each sample was analyzed in duplicate according to manufacturer protocol. Cytokine assays were performed in the lab of Dr. Firdaus Dhabhar at Stanford University. One healthy control was an extreme outlier with serum IL-6 and IL-10 concentrations > 16 and > 10 SD's (respectively) above the mean, which were not normalized by standard transformation methods; this subject's cytokine data were excluded from analysis. In several cases (7 controls and 7 MDD subjects at baseline and 7 MDD subjects at Week 8), IL-10 concentrations were below the level of

detection of the assay; in such cases, IL-10 values were conservatively set to just below the minimum observed value in either group, specifically 0.031 pg/ml.

Due to lack of assay data or the lack of Week 8 treatment visits for certain subjects, the final samples sizes for F2-IsoPs were: 20 controls, 19 MDD subjects at baseline and 13 MDD subjects at Week 8; for IL-6 were: 19 controls, 20 MDD subjects at baseline and 17 MDD subjects at Week 8; and for IL-10 were: 19 controls, 19 MDD subjects at baseline and 17 MDD subjects at Week 8.

2.4. Statistics

Distributions were examined for normality, and non-normal distributions were natural log transformed. Accordingly, each of the major dependent variables, IL-6, IL-10, IL-6/IL-10 ratio and F2-IsoPs, was transformed to its natural log prior to analysis. As mentioned, one healthy control subject had extremely elevated cytokine levels (10–16 SD's above the mean), which could not be normalized by log or square root transformation, and that subject's cytokine data were not included. We assessed the impact of age, sex, body mass index, and current tobacco use as potential confounds of these biochemical markers. Between-group comparison of the demographic variables was by independent sample *t*-tests, Chi-square tests, and univariate analysis of covariance (ANCOVA), controlling for the significant demographic confounders where indicated. Within-group changes with treatment were assessed with paired *t*-tests. Correlations between the biochemical measures (e.g., IL-6 vs. F2-IsoPs) were assessed by partial correlations, controlling for significant covariates: IL-6 and F2-IsoPs were significantly associated with BMI ($r = 0.32$, $p = 0.05$; $p = 0.38$, $p = 0.02$, respectively), and IL-10 and IL-6/IL-10 with current tobacco use ($t = -3.43$, $p < 0.01$; $t = 3.32$, $p < 0.01$, respectively). For these analyses, independent sample tests were utilized rather than paired tests, since the total sample sizes for most analyses were unequal after taking missing data into account. Next, we tested whether the relationship between oxidative (F2-IsoPs) and immunologic markers were significantly different in subjects depending on presence and degree of depressive symptoms with linear regression modeling and interaction analyses. First, we tested the dichotomous group affiliation (MDD vs. controls) interaction with F2-IsoPs predicting IL-6, IL-10, and IL-6/IL-10. Then, we tested an interaction between the IDS and F2-IsoPs predicting IL-6, IL-10, and IL-6/IL-10. We followed moderation techniques as outlined by Cohen et al. (2003), Hayes and Matthes (2009), and Preacher et al. (2006), and used the *modprobe* macro for SPSS at http://www.afhayes.com/spss-sas-and-mplus-macros-and-code.html. All tests were two-tailed with an alpha = 0.05.

3. Results

3.1. Demographics

As shown in Table 1, age, sex distribution, ethnicity distribution, BMI and current tobacco use did not significantly differ between the MDD and control groups. The mean IDS ratings in the control and MDD groups at baseline were 4.95 ± 4.89 versus 37.21 ± 9.59, respectively ($p < 0.003$) (Table 1).

3.2. Cytokine and isoprostane concentrations in depressed and control subjects

At study entry, the depressed and control groups did not significantly differ in concentrations of IL-6, IL-10, IL-6/IL-10, or F2-IsoPs, (Table 1), although this study was not intended to test differences in baseline cytokine levels, since that was tested previously

Table 1
Characteristics of the control and MDD groups.

	Controls	n	MDD	n	t, F, Chi	p
Age (yr)	36.42 ± 12.13	19	37.00 ± 10.77	20	−1.58	0.88
Sex (% male)	37.00%	19	35.00%	20	0.12	0.91
BMI	24.48 ± 3.73	19	24.78 ± 4.29	20	-0.23	0.82
Ethnicity		19		20	0.84	0.84
Caucasian	73.68%		70.00%			
African-American	15.79%		10.00%			
Asian	5.26%		10.00%			
Other or mixed	5.26%		10.00%			
Current tobacco use (%)		19		20	2.30	0.32
None	89.47%		75.00%			
Some days	10.53%		15.00%			
Daily	0.00%		10.00%			
Lifetime tobacco use (%)	42.11%	19	50.00%	20	0.48	0.63
IDS	4.95 ± 4.89	19	37.21 ± 9.59	14	−11.53	0.00
F2-IsoP (ng/ml)	0.044 ± 0.015	19	0.043 ± 0.016	19	0.11	0.75
IL-6 (pg/ml)	0.73 ± 0.37	18	0.84 ± 0.823	20	0.75	0.39
IL-10 (pg/ml)	0.55 ± 0.61	18	0.40 ± 0.66	19	0.03	0.87
IL-6/IL-10	8.63 ± 12.21	18	11.68 ± 14.20	19	0.00	0.95

Data are means ± SD of raw data. p values for IL-6, IL-10, IL-6/IL-10, and F2-IsoP were derived from natural log-transformed data. Between-group means comparisons were assessed by univariate ANCOVA for F2-isoP's and IL-6, adjusted for BMI, and IL-10 and IL-6/IL-10, adjusted for current tobacco use. The sample sizes above were not identical across all variables where data were missing.

in a subset of the current sample (Dhabhar et al. 2009). Notably, anxiety levels as measured by the Hamilton Anxiety Rating Scale were also not found to correlate significantly with cytokine levels or F2-IsoPs.

3.3. Effects of sertraline treatment

With sertraline treatment, depressed subjects showed highly significant declines in depressive symptoms. HDRS-17 ratings decreased from 18.71 ± 3.22 to 10.24 ± 6.32 ($p < 0.001$). Mean IL-6 concentration increased from 0.96 to 1.31 pg/mL ($p = 0.03$). However, F2-IsoPs, IL-10, and the IL-6/IL-10 ratio did not significantly change with treatment (Table 2).

3.4. Correlation between inflammation and oxidation markers as a function of MDD diagnosis

Among the depressed subjects, F2-IsoP concentrations were significantly positively correlated with IL-6 concentrations ($r = 0.47$, $p < 0.05$) (Fig. 1b) and significantly negatively correlated with IL-10 concentrations ($r = -.69$, $p = 0.002$) (Fig. 1e). Accordingly, F2-IsoP concentrations were strongly positively correlated with the ratio of IL-6/IL-10 ($r = 0.70$, $p = 0.005$) (Fig. 1h). No significant correlations between F2-IsoPs and both cytokines and their ratio were seen in the control group (Fig. 1a, d and g). Following 8 weeks of sertraline treatment in the MDD group, the correlations between F2-IsoPs and IL-6 ($r = 0.44$, $p = 0.13$), IL-10 ($r = 0.12$, $p = 0.72$), and the IL-6/IL-10 ratio ($r = -0.14$, $p = 0.67$) were no longer statistically significant (Fig 1c, f and i).

Table 2
Baseline measurements vs. Week 8 of sertraline treatment for MDD group.

	Baseline	Week 8	n	Paired-t	p
HDRS	18.71 ± 3.22	10.24 ± 6.32	17	5.23	<.001
F2-IsoP (ng/ml)	0.047 ± 0.016	0.048 ± 0.019	13	0.07	0.94
IL-6 (pg/ml)	0.96 ± 0.84	1.31 ± 0.98	17	−2.37	0.03
IL-10 (pg/ml)	0.27 ± 0.35	0.37 ± 0.62	17	−0.19	0.85
IL-6/IL-10	12.92 ± 14.52	16.06 ± 16.19	17	−1.62	0.13

Data are means ± SD of raw data. p values were calculated using paired-sample t-tests of log-transformed F2-IsoP and cytokine values. Certain sample sizes and baseline values for the MDD's in this table differ from those in Table 1, because only MDD's with both baseline and Week 8 data are included in Table 2.

3.5. Differential correlation between inflammation and oxidation markers as a function of depressive symptom severity

In a final set of analyses, we tested whether subjects with greater or lesser severity ratings of depression (across diagnostic groups) would show different degrees of association between cytokines (IL-6, IL-10, and IL-6/IL-10) and F2-IsoPs, as described in the Methods section above. Results indicated that group affiliation (MDD vs. control) did not significantly interact with F2-IsoPs to predict IL-6. On the other hand, IDS did moderate the relationship between F2-IsoPs and IL-6 ($\beta = 0.059$, SE = 0.028, $p < 0.05$). These results indicate that, although the relationship between oxidative and inflammation markers did not significantly differ by group affiliation, they did differ along the continuum of depressive symptom severity across groups. Specifically, oxidative and inflammatory markers were significantly positively correlated as depressive symptom severity increased. We employed the Johnson–Neyman statistical approach to examine the significant intervals at which depression significantly moderated the relationship between oxidative stress and inflammation (Preacher et al., 2006). At IDS ratings at or above 17.5 (which corresponds to a HDRS-17 score of 22, indicating "severe" depression), F2-IsoPs were significantly correlated with IL-6. However, at values below 17.5, F2-IsoPs were not significantly correlated with IL-6 (see Fig. 2). There were no significant interaction effects between IDS ratings and F2-IsoPs in predicting IL-10 or the IL-6/IL-10 ratio.

4. Discussion

The major aim of this study was to assess the relationship between oxidative stress and inflammatory markers in un-medicated MDD. As hypothesized, oxidation and inflammation were significantly positively correlated in un-medicated MDD subjects but not in healthy controls. Interaction analyses of baseline data demonstrated that this differential interplay between inflammation and oxidative stress was observed with increased levels of depression (IDS ratings ⩾17.5). Oxidative stress in un-medicated MDD subjects was positively correlated with the pro-inflammatory cytokine IL-6, negatively with the anti-inflammatory cytokine IL-10, and hence positively correlated with the inflammatory index IL-6/IL-10. After MDDs were treated with sertraline for 8 weeks, the partial correlation coefficient between IL-6 and F2-IsoPs dropped
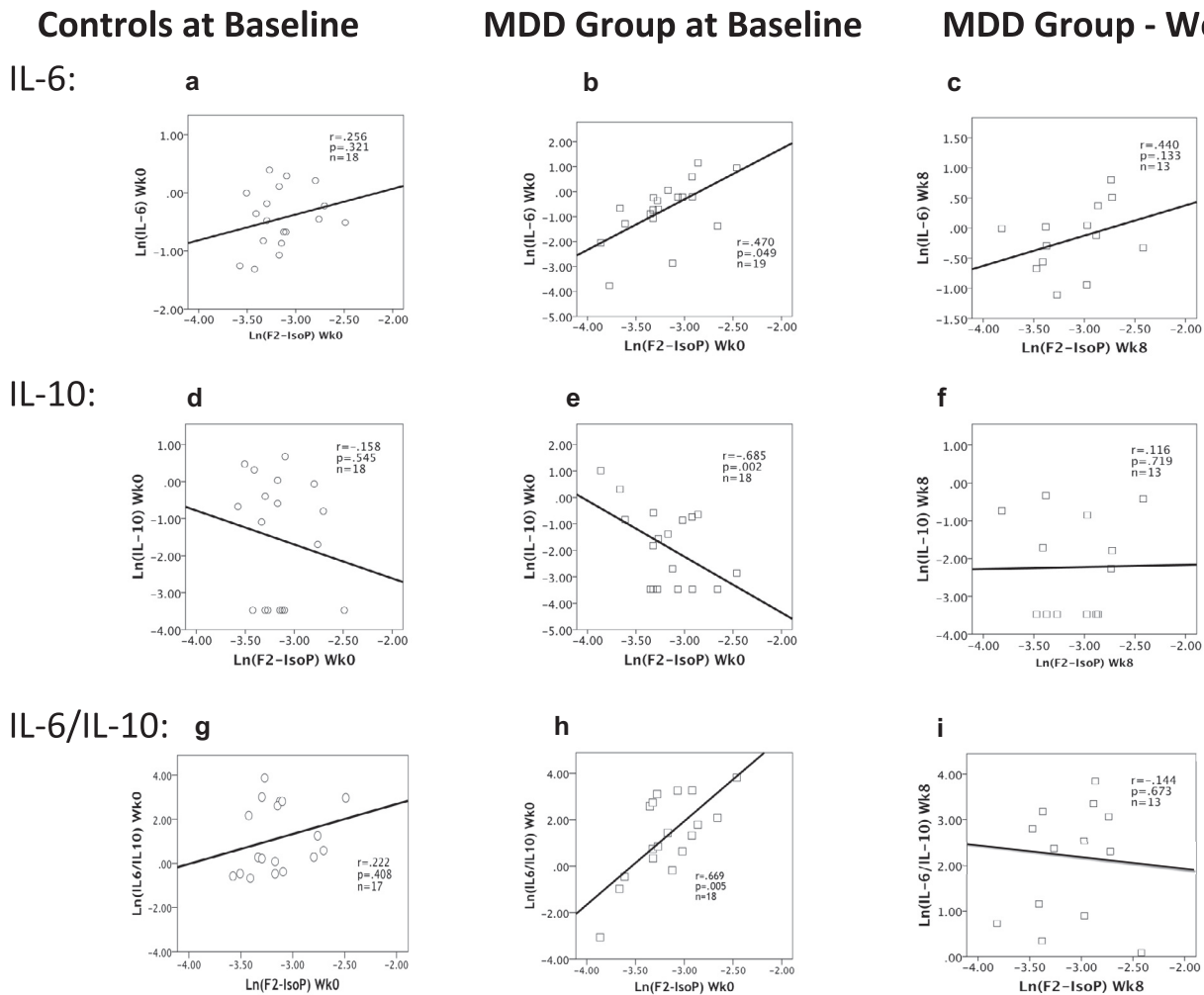
Fig. 1. Correlations between F2-IsoPs and IL-6, IL-10 and the IL-6/ IL-10 ratio among the control group at baseline (1a, d and g, respectively), and among the MDD group at baseline (1b, e and h, respectively) and after 8 weeks treatment with sertraline (1c, f and i, respectively). Figures portray raw data; correlation coefficients and *p* values are determined by partial correlation tests, adjusted for covariates BMI and tobacco when significantly correlated.

from 0.47 to 0.44 and became non-significant, likely due to the reduced sample size at week 8. However, F2-IsoP's strong negative association with IL-10 and strong positive association with IL-6/IL-10 observed at baseline were no longer observed after 8 weeks treatment with sertraline. To our knowledge, this study is the first to show in un-medicated depressed subjects that there is a significant positive association between inflammatory and oxidative stress markers, which is not present in healthy non-depressed controls, suggesting that an important biological characteristic of major depression may be the absence of a counter-regulatory mechanism buffering the relationship between these two systems.

As discussed above, theories regarding the roles of inflammation and oxidation in the pathophysiology of MDD are gaining empirical support, although few, if any, studies have previously assessed the interplay between these pathologic processes in MDD. Our results are consistent with the growing literature of positive correlations between oxidative and inflammatory processes in several serious chronic medical conditions (such as cardiovascular, renal and pulmonary diseases, diabetes and metabolic syndrome, etc., reviewed above (Agrawal et al., 2007; Cottone et al., 2006; Ferder et al., 2006; Guerrero-Romero and Rodriguez-Moran, 2006; Jelic and Le Jemtel, 2008; Krishnan, 2010; Lakshmi et al., 2009). The direct correlation between oxidation and inflammation seen in MDD might help explain the increased risk of certain serious med-

ical co-morbid illnesses that have oxidative and/or inflammatory underpinnings (Forlenza and Miller, 2006; Leuner et al., 2012; Maes et al., 2011b; Nemeroff and Goldschmidt-Clermont, 2012; Tousoulis et al., 2008; Uno and Nicholls, 2010). Of course, in the absence of prospective longitudinal data, it is impossible to gauge whether the oxidative/immune interplay we observed is causally related to depression or medical comorbidities and, if so, which antedates the other. Notably, all of the subjects in the present study were medically healthy, with no comorbid medical conditions or medications that would be expected to alter oxidative or inflammatory markers, showing that the presence of medical illness is not necessary for this relationship. Further, BMI and tobacco use, which can affect immune and oxidative markers, did not differ between groups, and analyses were controlled for BMI and tobacco use when needed, making BMI and tobacco use unlikely explanations of our findings.

In normal physiologic conditions, inflammatory and oxidative processes assist in adaptive responses to stressors and defense against pathogens. Restoration to a healthy state, however, depends upon these processes diminishing when no longer needed. Elaborate systems have evolved to keep these systems in check under physiologic conditions through negative feedback mechanisms, such as increased enzymatic and non-enzymatic antioxidant defenses (e.g. superoxide dismutase, glutathione peroxidase, catalase,
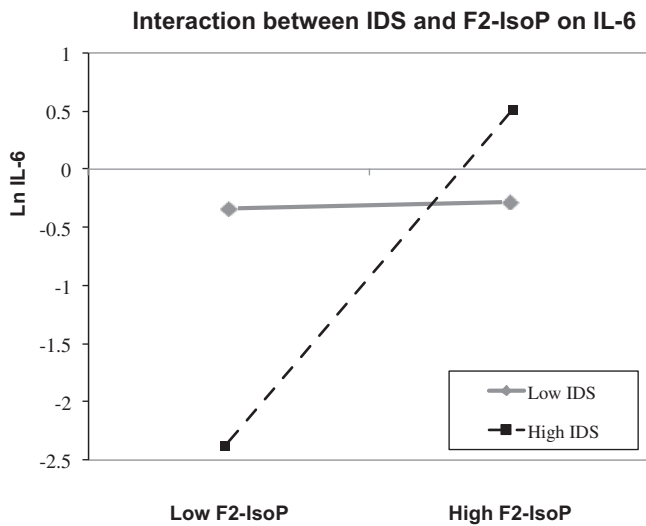
Interaction between IDS and F2-IsoP on IL-6

Fig. 2. Two-way interaction plot illustrating the differential relationship between F2-IsoPs and IL-6, as a function of high versus low depression scores on the IDS. High and low are defined as 1 SD above or below the mean value for both F2-IsoPs and the IDS, based on the approach described by Hayes and Matthes (2009) and the corresponding modprobe macro in SPSS. In subjects with low depression scores, F2-IsoP and IL-6 concentrations are not significantly related. But in subjects with high depression ratings, progressively higher F2-IsoP concentrations are associated with higher IL-6 concentrations.

GSH, vitamin C and vitamin E, etc.) (De la Fuente and Miquel, 2009) as well as anti-inflammatory cytokines (e.g. IL-10, IL-4, IL-13 (Iso-maki and Punnonen, 1997)) and negative acute phase reactants (APR's) (e.g., albumin, transferrin, transthyretin, etc.) (Gruys et al., 2005). For example, reactive oxygen species (ROS) release can stimulate production of antioxidant enzymes through the re-dox responsive transcription factor Nrf2 to counteract oxidative stress (Singh et al., 2010). In response to acute inflammation, al-pha-1 antitrypsin, an APR, suppresses TNF-α expression and mod-ulates endothelial cell activation (Subramaniyam et al., 2008), while haptoglobin and albumin, other APRs, can bind ROS (Leonard and Maes, 2012). Similarly, adenosine triphosphate (ATP) appears to act as a redox-signaling molecule that buffers oxidation and inflammation through downregulation of TNF-α and upregulation of IL-10 (Kaur et al., 2006; Swennen et al., 2006), thereby dampen-ing ROS-induced nuclear factor-kappa B (NF-κB) stimulation and inflammatory-driven ROS production. Under prolonged pathologic conditions, however, such counter-regulatory effects may be attenuated.

Oxidative stress occurs when the synthesis of oxygen free rad-icals exceeds the body's ability to offset them with antioxidant molecules (e.g., Vitamins C and E, glutathione, glutathione peroxi-dase, superoxide dismutase enzymes, catalase, etc.) and sets in motion multiple pathways capable of stimulating inflammatory activity (Khansari et al., 2009; Khanzode et al., 2003; Sarandol et al., 2007b; Spooner and Yilmaz, 2011). ROS activate inflamma-somes such as NLRP3, a cytoplasmic protein complex that modu-lates innate immune function by activating caspase-1, which increases pro-inflammatory cytokines such as IL-1β (Jin and Flav-ell, 2010; Martinon, 2010; Tschopp and Schroder, 2010; Zhou et al., 2010). ROS also increase inflammation by activating certain stress-activated kinases such as ERK, JNK, and p38 (Closa and Fol-ch-Puy, 2004; Perricone et al., 2009). Another major mechanism by which ROS regulate inflammatory processes involves stimulation of transcription factors, e.g. NF-κB and activator protein-1 (AP-1) (Closa and Folch-Puy, 2004; Hayley et al., 2005; Rahman, 2003; Reuter et al., 2010), which have multiple downstream effects including: (a) synthesis of pro-inflammatory cytokines, (b) activa-

tion of mitogen-activated protein (MAP) kinase pathways (which activate both pro- and anti-inflammatory gene transcription), (c) activation of stress-response protective genes (e.g., manganese superoxide dismutase [MnSOD] and glutamylcysteine synthetase), and (d) histone/chromatin modifications (viz., increased acetyla-tion and decreased deacetylation [HDAC2]) that lead to an increase in pro-inflammatory gene expression (Rahman, 2003). Increases in pro-inflammatory cytokine release provoke an inflammatory cas-cade, recruiting additional inflammatory cells and amplifying leu-kocyte infiltration (Caballero and Coto-Montes, 2012; Rahman, 2003; Tschopp and Schroder, 2010). In the CNS, IFN-γ has been implicated in stimulating ROS release from microglia (Pawate et al., 2004). Also, various pro-inflammatory cytokines in the CNS activate indoleamine 2,3-dioxygenase (IDO), a rate-limiting en-zyme involved in catabolism of tryptophan, a serotonin precursor. IDO upregulation results in a depletion of tryptophan and a con-comitant increase in metabolites, 3-hydroxy-kynurenine and quinolonic acid, which then provoke ROS production (Maes et al., 2011d; Miller et al., 2009; Wichers and Maes, 2004).

Acute inflammation may result in activation of guanosine-tri-phosphate-cyclohydrolase-1 (GTP-CH1), which is necessary for tet-rahydrobiopterin (BH4) synthesis, and subsequently for aromatic amino acid and biogenic amine (norpeinephrine, epinephrine and serotonin) synthesis. However, chronic inflammation together with increased ROS production results in shunting biopterin syn-thesis to inactive neopterin, while the cofactor tetrahydrobiopterin (BH4), necessary for aromatic amino acid hydroxylase activities, is reduced and destroyed by ROS, resulting in reduced concentrations of biogenic amines. Such a situation has been proposed and re-ported in the elderly, and may be associated with increased sus-ceptibility to depression in this population (Capuron et al., 2011). As illustrated in Fig. 3, the complex interactions between the oxi-dative and inflammatory pathways contain mechanisms for both mutual amplification (positive feedback or a "vicious cycle") and for negative feedback homeostasis.

We infer from our data and previous literature on this subject, that certain counter-regulatory processes may be disturbed in se-vere MDD and in certain chronic medical illnesses, converting a homeostatic negative feedback system into a positive feed-forward one (Dhabhar et al., 2009; Sternberg et al., 1992). Such a vicious cy-cle of inflammation and oxidation would predictably have adverse physiological consequences (Biswal et al., 2012; Khansari et al., 2009; Rahman, 2003). An analogous process of dysregulated feed-back was previously described within the inflammatory system it-self (Dhabhar et al., 2009). Other examples of defective feedback regulation in MDD, which may or may not bear any relationship to that shown here, include the hypothalamic–pituitary–adrenal (HPA) axis glucocorticoid receptor negative feedback dysregulation (Modell et al., 1997), the TNF-α-HPA axis feedback system (Himmerich et al., 2006), the relationship between IL-6 and IL-10 concentrations (Dhabhar et al., 2009) and the relationship between noradrenergic system and CRH/glucocorticoid system regulation (Wong et al., 2000).

Surprisingly, the MDD subjects showed a significant increase in IL-6 concentrations after 8 weeks of sertraline treatment. Although one study similarly found an increase in IL-6 concentrations after treatment with duloxetine, an SNRI (Fornaro et al., 2011), a recent meta-analysis of 22 studies showed that IL-6 concentrations non-significantly decreased with antidepressant treatment (Hannestad et al., 2011). In our study the IL-6/IL-10 ratio, which may be a more accurate reflection of the inflammatory milieu, did not change with treatment.

Among the strengths of this study were our use of medically healthy, rigorously screened and diagnosed subjects who were free of psychotropic and other medications, our use of closely matched samples (by age, sex, and ethnicity), our ascertainment of possible
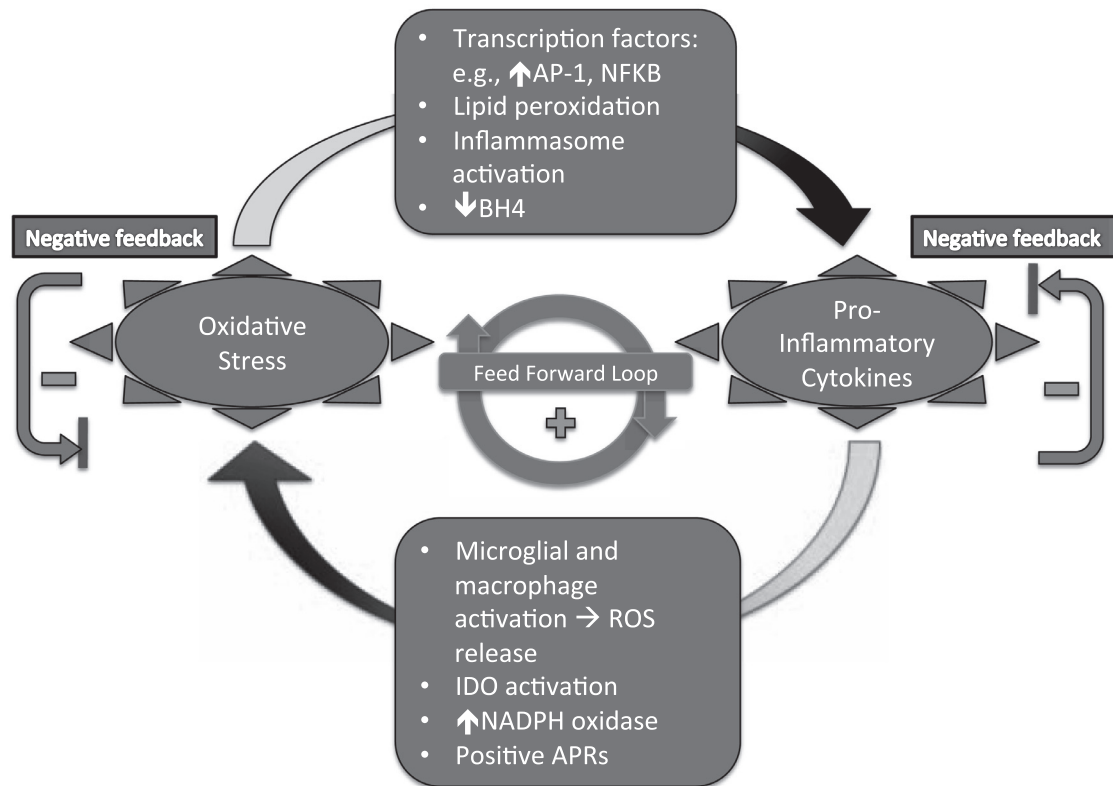
Fig. 3. Complex interactions between the oxidative and inflammatory pathways contain mechanisms for both mutual amplification (positive feedback or a "vicious cycle") and for negative feedback homeostasis. In this Figure, some of the specific mediators of positive feedback are portrayed. For example, reactive oxygen species (ROS) activate inflammasomes such as NLRP3, which increase pro-inflammatory cytokines such as IL-1β. ROS also increase inflammation by activating certain stress-activated kinases such as ERK, JNK, and p38. Also, ROS can stimulate transcription factors, e.g. NF-κB and activator protein-1 (AP-1), to stimulate pro-inflammatory cytokine expression. Conversely, pro-inflammatory cytokines can indirectly provoke oxidative stress by activating microglia and macrophages, which release ROS; activating indoleamine 2,3-dioxygenase (IDO), which produces byproducts, 3-hydroxy-kynurenine and quinolonic acid; and stimulating nicotinamide adenine dinucleotide phosphate-oxidase (NADPH oxidase), which generates free radicals. Just as negative acute phase reactants (APRs) can have antioxidant effects, some positive APRs, such as C-reactive protein (CRP), have been linked to increased oxidative stress (Il'yasova et al., 2008).

covariates such as BMI and tobacco use, and a naturalistic 8 week antidepressant treatment arm, verified with plasma antidepressant concentration assessments. Among the study's weaknesses, our sample size was small, especially with several subjects' assay results not available. Due to low circulating levels of IL-10, several subjects' values were below the level of detection. Hence imputed values (set conservatively just below the lowest observed value in both groups) were used, although this would tend to bias towards the null hypotheses. It is possible that the lack of a significant inflammation-oxidation correlation in the MDD subjects after 8 weeks of treatment is secondary to the smaller sample size that that time point. This would be especially concerning if the subjects who failed to complete the treatment trial were less severely depressed at baseline, as severity predicted positive inter-correlations between inflammation and oxidation. We assessed this possibility and found no significant difference in baseline depression ratings in the subjects who dropped out compared to the remainder of the MDD subjects. Also, our biochemical data are based on single time-point blood samplings, and, since blood samples were all obtained in a rested state in the morning, it is unknown whether similar relationships between oxidation and inflammation markers would be seen at other times in the day or under states of acute or chronic stress. We also were not able to control for extraneous variables that may have impacted our findings, such as differences in diet, exercise, sleep, etc. Although age was not significantly correlated with any of our variables, our sub-

jects' age range was rather limited (our subjects had an average age of 37 yr old, and only two were over the age of 60, when cellular senescence and decreased buffering capacity for oxidative stress and inflammation are more likely to become apparent (Caballero and Coto-Montes, 2012). In addition, several of our subjects had comorbid anxiety diagnoses, which could have influenced the biochemical data, although in each case the anxiety diagnosis was considered secondary to the MDD diagnosis, and we found no significant correlations between anxiety ratings and cytokines or F2-IsoPs. Another limitation is that control subjects were not re-assessed at a Week 8 time point, so changes observed with 8 weeks of sertraline treatment in the MDD subjects cannot definitively exclude effects of time. Finally, our blood-based measurements of oxidative/ inflammatory markers were taken peripherally, from which we cannot infer local tissue or CNS levels.

Our understanding of the intricacies of how inflammation and oxidation are co-regulated in depression is in its infancy; however, evidence is growing that inflammation and oxidation may form part of a vicious and self-reinforcing signaling cycle in a number of illnesses, including depression. Oxidative stress and inflammation may be among the clues we have to understanding the increased co-morbidity between depression and numerous physical illnesses that are also characterized by inflammation and oxidation. For example, we previously reported in subjects with MDD that peripheral blood mononuclear cell telomere length (an index of cell aging and a predictor of poor health outcomes and early

mortality (Cawthon et al., 2003; Epel et al., 2004; Wolkowitz et al., 2010; Wolkowitz et al., 2011a) is inversely correlated with markers of inflammation as well as oxidation (Wolkowitz et al., 2011a). Elucidation of the complex interactions between the multiple pathways and networks linking inflammation and oxidative stress will require additional research, including identifying population subgroups and system biology approaches (Jesmin et al., 2010). Determining the mechanisms responsible for the apparent loss of counter-regulatory control between the oxidative and inflammatory systems may lead to new insights into the underlying pathophysiology of MDD and may suggest novel treatment targets for depression and its associated medical comorbidities.

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