



## Oxidative stress, inflammation and treatment response in major depression



Daniel Lindqvist<sup>a,b,\*</sup>, Firdaus S. Dhabhar<sup>c</sup>, S. Jill James<sup>d</sup>, Christina M. Hough<sup>a</sup>, Felipe A. Jain<sup>a</sup>, F. Saverio Bersani<sup>a,e</sup>, Victor I. Reus<sup>a</sup>, Josine E. Verhoeven<sup>a,f</sup>, Elissa S. Epel<sup>a</sup>, Laura Mahan<sup>a</sup>, Rebecca Rosser<sup>a</sup>, Owen M. Wolkowitz<sup>a,1</sup>, Synthia H. Mellon<sup>g,1</sup>

<sup>a</sup> Department of Psychiatry, UCSF Weill Institute for Neurosciences, University of California San Francisco (UCSF) School of Medicine, San Francisco, CA, United States

<sup>b</sup> Lund University, Faculty of Medicine, Department of Clinical Sciences, Psychiatry, Lund, Sweden

<sup>c</sup> Department of Psychiatry & Behavioral Sciences, Sylvester Comprehensive Cancer Center, University of Miami, FL, United States

<sup>d</sup> Arkansas Children's Research Institute, Department of Pediatrics, University of Arkansas for Medical Sciences, Little Rock, AR, United States

<sup>e</sup> Department of Neurology and Psychiatry, Sapienza University of Rome, Rome, Italy

<sup>f</sup> Department of Psychiatry and EMGO Institute for Health and Care Research, VU University Medical Centre, Amsterdam, The Netherlands

<sup>g</sup> Department of OB/GYN and Reproductive Sciences, University of California San Francisco (UCSF) School of Medicine, San Francisco, CA, United States

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### ABSTRACT

**Objective:** Increased inflammation and oxidative stress have been shown in Major Depressive Disorder (MDD), although there is significant heterogeneity across studies. Whether markers of inflammation and oxidative stress are associated with antidepressant treatment response in MDD is currently unclear. The goals of the present study are to investigate markers of inflammation and oxidative stress in unmedicated MDD subjects and controls and test the relationship between these markers and antidepressant response in MDD subjects.

**Methods:** Interleukin (IL)-6, tumor necrosis factor (TNF)- $\alpha$ , C-reactive protein, F2-isoprostanes, 8-OH-2-deoxyguanosine (8-OHdG), glutathione peroxidase, glutathione, and vitamin C were quantified in blood samples from 50 unmedicated MDD subjects and 55 healthy controls. Depression symptom severity was rated with the 17-item Hamilton Depression Rating Scale (HDRS). All subjects were somatically healthy and free from medications that could interfere with inflammation and oxidative stress markers. A subgroup of 22 MDD subjects underwent open-label selective serotonin reuptake inhibitor (SSRI) antidepressant treatment for eight weeks, after which blood sampling and the HDRS were repeated. Antidepressant treatment "response" was defined as  $\geq 50\%$  decrease in HDRS ratings over 8 weeks of treatment.

**Results:** After controlling for the effects of age, sex, body mass index and smoking, MDD subjects had significantly higher levels of IL-6 ( $p < 0.001$ ), TNF- $\alpha$  ( $p < 0.001$ ), 8-OHdG ( $p = 0.018$ ), and F2-isoprostanes ( $p = 0.012$ ). Compared to Responders, Non-responders to SSRI antidepressant treatment had higher levels of F2-isoprostanes at baseline ( $p = 0.006$ ), and after eight weeks of treatment ( $p = 0.031$ ). Non-responders showed a significant increase in 8-OHdG over the course of treatment ( $p = 0.021$ ), whereas Responders showed a significant decrease in IL-6 over the course of treatment ( $p = 0.019$ ).

**Conclusion:** Our results are in line with previous reports of increased levels of markers of inflammation and oxidative stress in MDD. Moreover, poorer antidepressant treatment response was related to higher baseline levels of the major oxidative stress marker, F2-isoprostanes, *in vivo*. Further, antidepressant response was associated with changes in oxidative (8-OHdG) and inflammatory (IL-6) markers.

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\* Corresponding author at: Department of Psychiatry, University of California, San Francisco (UCSF), School of Medicine, 401 Parnassus Avenue, San Francisco, CA 94143, United States.

E-mail addresses: [daniel.lindqvist@ucsf.edu](mailto:daniel.lindqvist@ucsf.edu), [daniel.lindqvist@med.lu.se](mailto:daniel.lindqvist@med.lu.se) (D. Lindqvist).

<sup>1</sup> Joint senior authors.

## 1. Introduction

Major Depressive Disorder (MDD) is a debilitating condition with a lifetime prevalence of more than 15% (Kessler et al., 2003). Despite scientific advances during the last decades, the biological underpinnings of MDD and how these relate to antidepressant treatment response are not yet fully understood. Modulating brain monoamine activity, the cornerstone of most pharmacologic treatments of depression, is not sufficient for many patients, suggesting that other biological factors may be at play (Hindmarch, 2002). Inflammation and oxidative stress, the so called “evil twins of aging” (Joseph et al., 2005), may also be involved in the pathophysiology of MDD and may be related to treatment response (Berk et al., 2011; Dantzer et al., 2008; Maes, 2011; Miller, 2010; Ng et al., 2008; Raison et al., 2006; Schiepers et al., 2005; Uher et al., 2014).

Multiple studies have shown that depressed individuals have increased mean levels of pro-inflammatory cytokines in blood (Schiepers et al., 2005). Meta-analyses have shown that interleukin-6 (IL-6), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and C-reactive protein (CRP) are elevated in MDD (Dowlati et al., 2010; Howren et al., 2009). Although the exact mechanisms underlying the link between inflammation and depression are not fully understood, activation of the kynurenine pathway of tryptophan metabolism, triggered by inflammation and potentially leading to an accumulation of neurotoxic metabolites such as quinolinic acid, may be one pathway (Bryleva and Brundin, 2016; O'Connor et al., 2009). Also, increased gastrointestinal permeability leading to translocation of gram-negative bacteria may cause low-grade systemic inflammation and subsequently generate depressive symptoms (Maes et al., 2008), which is consistent with animal and human studies (DellaGioia et al., 2013; Yirmiya, 1996).

Oxidative stress is also elevated in MDD; a systematic review and meta-analysis found increased oxidative stress markers in MDD, with 8-OH 2-deoxyguanosine (8-OHdG) and F2-isoprostanes being the most prominent (Black et al., 2015). The mechanisms by which oxidative stress may be related to depressive symptoms are yet to be elucidated, although it has been noted that the brain is particularly vulnerable to oxidative damage due to high oxygen utilization and subsequent generation of free radical by-products, relatively weak antioxidant defenses, and the risk for oxidative cellular injury and necrosis (Ng et al., 2008).

Although several peripheral markers of oxidative stress and inflammation have been shown to be elevated in MDD, there is significant heterogeneity across studies (Black et al., 2015; Dowlati et al., 2010). This highlights the importance of careful phenotyping of the depressed and control subjects (Hiles et al., 2012), and controlling for, or screening out, subjects with variables that are known to influence markers of oxidative stress and inflammation, such as smoking, body mass index (BMI), age, sex, somatic co-morbidities, and somatic and psychiatric medication use (Behr et al., 2012; Dhalla et al., 2000; Hiles et al., 2012; Keaney et al., 2003; Le Lay et al., 2014; Schiepers et al., 2005; Yilmaz et al., 2004).

In addition to evaluating differences in inflammation and oxidative stress markers between MDD subjects and controls, several investigators have assessed whether these baseline levels in depressed individuals predict subsequent antidepressant outcome. According to a recent meta-analysis by Strawbridge et al., there was no significant relationship between baseline levels of TNF- $\alpha$ , CRP, or IL-6 and subsequent antidepressant treatment response (Strawbridge et al., 2015). There was, however, some evidence that persistently higher TNF- $\alpha$  levels over the course of treatment are associated with poorer response, whereas IL-6 levels may decrease following antidepressant treatment regardless of degree of antidepressant response (Strawbridge et al., 2015). To the best of our knowledge, only one study has investigated baseline oxidative

stress markers (e.g., Vitamin C and glutathione peroxidase (GPx) among others) as predictors of antidepressant treatment response, and that study had negative results (Sarandol et al., 2007). There is, however, evidence that successful antidepressant treatment may improve oxidative stress status (Jimenez-Fernandez et al., 2015; Mellon et al., 2016), although F2-isoprostanes and 8-OHdG, the two oxidative stress markers most often elevated in MDD, have never been investigated in this regard.

The primary aim of the present study was to investigate baseline levels of inflammatory and oxidative stress markers in a well-characterized, un-medicated, somatically healthy cohort of MDD subjects, controlling for relevant confounders. Based on previous meta-analyses pertaining to this, our *a priori* hypotheses were that the specific oxidative stress markers, 8-OHdG, F2-isoprostanes (Black et al., 2015), and the inflammatory markers IL-6, CRP and TNF- $\alpha$  (Dowlati et al., 2010; Howren et al., 2009), would be elevated in un-medicated MDD compared to controls. Since studies testing the relationship between MDD and the oxidative stress markers oxidized glutathione (GSSG), reduced glutathione (GSH), and antioxidants, GPx, and Vitamin C, have often produced negative results (Jimenez-Fernandez et al., 2015; Kotan et al., 2011; Sarandol et al., 2007), these biomarkers were not part of the *a priori* hypotheses, but were included in an exploratory analysis.

A secondary aim was to determine whether peripheral markers of oxidative stress and inflammation, in un-medicated MDD subjects, are associated with antidepressant treatment response. Although fewer studies have investigated markers of inflammation and oxidative stress in relation to antidepressant treatment response (Sarandol et al., 2007; Strawbridge et al., 2015), and results have been inconsistent, our exploratory hypothesis was that increased inflammation and oxidative stress would be associated with worse treatment response.

## 2. Methods

### 2.1. Study participants

Fifty un-medicated MDD subjects and 55 healthy controls were enrolled in the study. The study enrolment procedures are described in a CONSORT flow diagram (supplementary information). Subjects were recruited by flyers, bulletin board notices, Craigslist postings, newspaper ads, and clinical referrals. All procedures were approved by the Committee on Human Research of the University of California, San Francisco (UCSF) (protocol #10-00825). Study participants gave written informed consent to participate in this study and were compensated for participating. Antidepressant treatment was provided at no cost to MDD subjects who participated in this portion of the study (described below).

Depressed subjects were diagnosed with current MDD, without psychotic features, and scored >17 on the 17-item Hamilton Depression Rating Scale (HDRS) (Hamilton, 1960). All diagnoses were made according to the Structured Clinical Interview for DSM IV-TR Axis I Disorders (SCID) (First, 1997), which was the DSM version in use while this study was performed, and were confirmed by clinical interview with a board-certified psychiatrist. Exclusion criteria for MDD subjects were: bipolar disorder, psychotic symptoms during their current major depressive episode, history of psychosis outside of a mood disorder episode, any eating disorder or post-traumatic stress disorder within one month of entering the study, and substance abuse or dependence (including alcohol) within six months of entering the study. Co-morbid anxiety disorders (except PTSD) were not exclusionary if MDD was considered the primary diagnosis. Control subjects had no history of any DSM-IV-TR Axis I disorder, also confirmed by SCID interview. Further, none of the study participants had acute illnesses or infec-

tions, chronic inflammatory disorders, neurological disorders, or any other major medical conditions considered to be potentially confounding (e.g. cancer, HIV, diabetes, history of cardiovascular disease or stroke, etc.), as assessed by history, physical examinations and routine blood screening (complete chemistry panel, including electrolytes, kidney and liver function, protein and albumin, high sensitivity C-reactive protein, complete blood count with differential count, lipid panel, folate, fasting glucose, HbA1c, and thyroid function tests). All subjects were free of any psychotropic medications (including antidepressants), hormone supplements, steroid-containing birth control or other potentially interfering medications, and had not had any vaccinations, for at least six weeks prior to enrollment in the study. None were taking vitamin supplements above the U.S. recommended daily allowances (e.g., 90 mg/day for vitamin C). Short-acting sedative-hypnotics were allowed as needed in the MDD subjects, up to a maximum of three times per week, but none within one week prior to participation. On the day of each study visit, all subjects had to pass a urine toxicology screen (marijuana, cocaine, amphetamines, phencyclidine, opiates, methamphetamine, tricyclic antidepressants, and barbiturates) and a urine test for pregnancy in women of child-bearing potential.

## 2.2. Antidepressant treatment

Twenty-two of the 50 MDD subjects underwent eight weeks of open-label outpatient treatment with an SSRI antidepressant (NCT00285935). Funding was not adequate to offer treatment to the remaining 28 MDD subjects. Since the decision to offer treatment was based solely on the amount of funding remaining, there was no systematic bias in offering treatment or not to subjects. To limit the range of potential mechanism of action of antidepressant treatment, the choice of medication was limited to an SSRI. The decision regarding the specific SSRI prescribed was made based on clinical grounds such as medical history, family history, and potential side effects. Outpatient compliance with the medication regimen, as well as clinical evaluations and assessments of drug tolerability, were assessed by a telephone check-in at the end of week 1 and an in-person check-in at the end of week 4 and week 8, at which times pill counts were performed. To assess treatment outcome, the HDRS ratings were repeated at the end of treatment, at week 8. Fifteen subjects were treated with sertraline, two with fluoxetine, two with citalopram, and three with escitalopram. Medication dosages were increased over the course of treatment as tolerated and as warranted by clinical response. Sertraline dosing began with 25 mg per day and increased to a maximum of 200 mg per day; fluoxetine and citalopram dosing began with 10 mg per day and increased to a maximum of 40 mg per day; escitalopram dosing began with 10 mg per day and increased to a maximum of 20 mg per day. MDD subjects were rated with the HDRS at baseline and after eight weeks of SSRI treatment. “Responders” were defined as subjects with greater than or equal to 50% improvement on HDRS ratings at week 8 compared to baseline; “Non-responders” had a lesser degree of improvement. There was no significant difference in final SSRI dose (sertraline equivalents) between Responders and Non-responders ( $p = 0.65$ ).

## 2.3. Blood draw preparations

Subjects were admitted as outpatients to the UCSF Clinical and Translational Science Institute between 8:00 a.m. and 11:00 a.m., having fasted (except water) since 10:00 p.m. the night before. Subjects were instructed to sit quietly and relax for 25–45 min before blood samples were obtained for assessment of inflammatory and oxidative stress markers, and routine clinical labs to determine

overall health. The severity of depressive symptoms was then rated in depressed subjects using the 17-item HDRS (Hamilton, 1960).

## 2.4. Inflammatory markers assay

A high sensitivity multiplexed sandwich immunoassay was used to quantify IL-6 and TNF- $\alpha$  concentrations (Mesoscale Discovery, Gaithersburg, MD). The intra-assay coefficients of variation (CV) were: IL-6 (4.4%) & TNF- $\alpha$  (4.2%). The inter-assay CV were: IL-6 (5.5%) & TNF- $\alpha$  (3.0%). The sensitivity for each cytokine was IL-6 (0.07 pg/ml) & TNF- $\alpha$  (0.10 pg/ml). Cytokine assays were performed in the lab of Dr. Firdaus Dhabhar at Stanford University. CRP was assayed with a latex-enhanced immunoturbidimetric method (Sonora Quest Laboratories). The sensitivity of this assay was 0.23 mg/L, intra-assay CV was <10%, and inter-assay CV was <12%.

## 2.5. Oxidative stress assays

Plasma was collected with a lavender EDTA vacutainer tube for F2-isoprostanes, GPx, 8-OHdG, GSH, and GSSG analyses, and serum was collected into serum separation vacutainer tube for vitamin C analysis. Samples were stored at  $-80^{\circ}\text{C}$  until assay.

Levels of F2-isoprostanes were quantified by the Eicosanoid Laboratory at Vanderbilt University. F2-isoprostanes 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 et al., 2002). The CV was 12% for this assay and Lower Limit Of Quantification (LLOQ) was 0.003 ng/ml.

Glutathione peroxidase activity (BioVision, Inc., Milpitas, California, USA) was measured in duplicate from plasma, using a colorimetric assay according to the instructions from the manufacturer. CV was <10% and LLOQ was 0.5 nmol NADPH/ml/min.

Vitamin C was measured using an Agilent 1100 Series high performance liquid chromatograph (HPLC) system with a diode array detector as described previously (Margolis and Schapira, 1997). Briefly, serum sample was preserved by adding an equal volume of metaphosphoric acid and treated with dithiothreitol. The resulting supernatant was injected into the HPLC systems equipped with a  $250 \times 4.6$  mm Capcell Pak NH<sub>2</sub> column (Shiseido, Tokyo, Japan). The column was equilibrated at  $40^{\circ}\text{C}$  at a flow-rate of 1 ml/min with a mobile phase composed of monobasic potassium phosphate/H<sub>2</sub>O/acetonitrile. Vitamin C was analyzed using external standards with UV spectrophotometric detection at 243 nm wavelength. Two quality-control samples were injected at the beginning, the end, and after every 10 samples to monitor intra- and inter-day assay accuracy and precision. Recoveries were consistently in excess of 90% and CV was 4.8%. LLOQ was 0.2 nmol/ml.

For free GSH and GSSG determination, 200  $\mu\text{l}$  ice cold 10% metaphosphoric acid was added to 200  $\mu\text{l}$  of plasma and the sample incubated for 30 min on ice to precipitate proteins. After centrifugation at 14,000 rpm for 15 min at  $+4^{\circ}\text{C}$ , the supernatant was filtered through a 0.2 mm filter (PGC Scientific, Frederic, MD) and a 20  $\mu\text{l}$  aliquot was injected into the HPLC-electrochemical detection system and ESA autosampler (model 542). The analyses were accomplished using HPLC with an ESA solvent delivery system (model 580) and a reverse phase Capcell pak C<sub>18</sub> column (3 mm;  $4.6 \times 150$  mm, Phenomenex, Inc., Torrance, CA, USA). All plasma metabolites were quantified using a 16 channel CouloArray electrochemical detector (ESA, Inc., Chelmsford, MA) equipped with a high sensitive four sensor cell (model 6210). The concentrations of plasma metabolites were calculated from peak areas and standard calibration curves using HPLC software. The inter-assay CVs

were 5% for GSH and 8% for GSSG. The LLOQ for both analytes were 0.02  $\mu\text{M}$ .

To determine the concentration of 8-OHdG in plasma, 250  $\mu\text{l}$  of ice cold 10% meta-phosphoric acid was added to 200  $\mu\text{l}$  plasma and the sample incubated for 20 min on ice for protein precipitation. After centrifugation at 18,000g for 15 min at +4 °C, the supernatant was filtered through a 0.2  $\mu\text{m}$  filter (PGC Scientific, Frederic, MD) and a 10  $\mu\text{l}$  aliquot was injected into the HPLC–MS system. The mobile phase consisted of 7 mM ammonium acetate, 1.5% methanol and 0.1% formic acid. The chromatography was performed using a Dionex HPLC–UV system coupled to an electrospray ionization (ESI) tandem mass spectrometer (Thermo-Finnigan LCQ) with a Phenomenex Gemini column (C18, 150  $\times$  2.0 mm, 3  $\mu\text{m}$  particle size) at a flow rate 0.3 ml/min and HPLC–UV wavelength of 254 nm. To determine a concentration of 8-OHdG in plasma, a standard curve with known concentrations of 8-OHdG was created and software automatic calculation was performed. CV was 6.3% and LLOQ is 0.2 pmol/column.

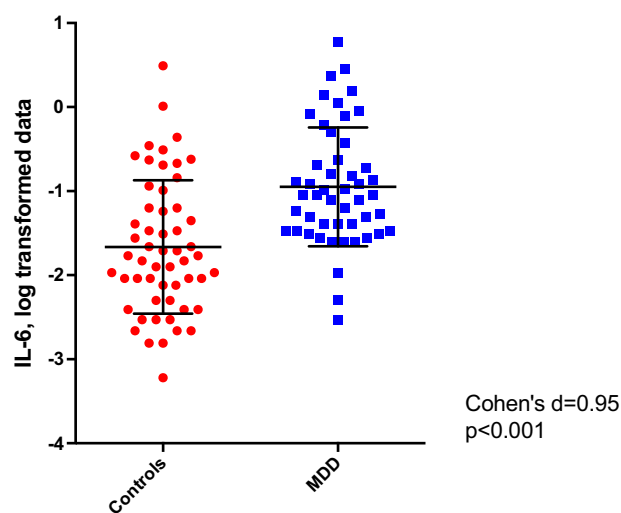
## 2.6. Statistics

All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS, version 23 for Mac). All tests were 2-tailed with  $\alpha=0.05$ . For the primary hypothesis (between-group differences at baseline), given the current sample size with an  $\alpha$  of 0.05 (two-tailed) we were able to detect a large effect size (0.8) with a power of 0.98 and a medium effect size (0.5) with a power of 0.72.

All biological marker distributions except GSH were skewed, therefore we used log-transformation to achieve normality. In cases when log-transformation was insufficient (vitamin C and GSSG), we used Blom transformation (Blom, 1958), a statistical procedure replacing each raw score with its rank value and adjusting the scale distances between the ranks to achieve a normal distribution. Some subjects were missing certain biomarker values due to assay issues, and missing data were not replaced. Therefore, sample sizes vary somewhat between analyses. Effect size was calculated using Cohen's  $d$  on normalized data. For unadjusted group-wise comparisons, we used Student's  $T$ -test; when controlling for potential confounds, analysis of covariance (ANCOVA) was used. Due to the small number of subjects receiving antidepressant treatment and to conserve power, we opted to use Student's  $T$ -test (as opposed to ANCOVA) when comparing biological markers between SSRI Responders and Non-responders. For this purpose, we used residuals of biological markers (regressed on age, sex, BMI and smoking). Paired samples  $T$ -tests using normalized data were used to investigate changes in oxidative stress and inflammatory markers over the course of antidepressant treatment in Responders and Non-responders separately.

Our *a priori* hypothesis was that MDD subjects have higher levels of oxidative stress markers 8-OHdG and F2-Isoprostanes and inflammatory markers CRP, IL-6, TNF- $\alpha$ . Exploratory analyses included (i) Group-wise comparisons (MDD vs controls) in Vitamin C, GPx, GSH, and GSSG levels and (ii) Group-wise comparisons (Responders vs Non-Responders) in all oxidative stress and oxidative stress markers. For our *a priori* hypothesis, we adjusted the significant  $p$ -value according to the Bonferroni method ( $p < 0.025$  for the 2 oxidative stress markers and  $p < 0.017$  for the 3 inflammatory markers). We did not correct for multiple comparisons in the exploratory analyses (Bender and Lange, 2001).

Our *a priori* measure of treatment outcome was the standard definition of Responder vs Non-responder. This definition has been used in most other studies examining the relationship of treatment outcome to these biomarkers (Sarandol et al., 2007; Strawbridge et al., 2015), thus using this definition of outcome enhances the comparability of our findings with the majority of other studies



**Fig. 1.** Interleukin-6 (IL-6) (pg/ml) in subjects with Major Depressive Disorder (MDD) and controls; log-transformed data. Error bars indicate  $\pm 1$  SD. Analysis of covariance adjusted for body mass index, age, sex and smoking.

examining the relationship between inflammation and oxidative stress vs treatment outcome. As exploratory analyses, we also tested the relationship between absolute change in HDRS scores and levels of inflammation and oxidative stress using Pearson's  $r$ .

## 3. Results

### 3.1. Demographics

Demographic characteristics are summarized in Table 1. MDD subjects and controls were well-matched in regards to age and sex distribution, but MDD subjects tended towards higher BMI ( $p=0.07$ ) and were more likely to be smokers ( $p < 0.01$ ). Responders and Non-responders were well-matched on all demographic variables, although BMI tended to be higher in Non-responders ( $p=0.12$ ). Based on their known association with oxidative stress and inflammation, age, sex, BMI, and tobacco use were included as covariates in the group comparisons below.

### 3.2. Baseline group comparisons, MDD subjects vs controls

Oxidative stress, antioxidant, and inflammatory markers in MDD and controls are summarized in Table 2 and Figs. 1–4. As hypothesized, MDD subjects had significantly higher levels of IL-6 ( $p < 0.001$ , Fig. 1), TNF- $\alpha$  ( $p < 0.001$ , Fig. 2), 8-OHdG ( $p = 0.018$ , Fig. 3), and F2 isoprostanes ( $p = 0.012$ , Fig. 4) compared to controls.

Vitamin C, GSSG, GSH, GPx, and CRP were not significantly different between groups (Table 2).

### 3.3. Comparison between responders and non-responders

#### 3.3.1. Prediction of future response based pre-treatment biomarker levels

Twenty-two of the 50 subjects received antidepressant treatment, and none dropped out before the end of the study. Non-responders had significantly higher baseline levels of F2-isoprostanes compared to Responders ( $t = 3.10$ ,  $p = 0.006$ ). At week 8, Non-responders continued to have significantly higher levels of F2-Isoprostanes ( $t = 2.37$ ,  $p = 0.031$ ). None of the other oxidative stress markers or inflammatory markers were significantly different between Responders and Non-responders at baseline or at week 8 (all  $p > 0.150$ ). Absolute change in HDRS scores over 8 weeks of

**Table 1**  
Demographic characteristics of all subjects and divided by Responder/Non-responder status.

	MDD (n = 50)	Controls (n = 55)	P-value	Responders (n = 12)	Non-responders (n = 10)	P-value
Age (years, mean $\pm$ SD)	39.6 $\pm$ 14.7	37.6 $\pm$ 13.9	0.47	39.4 $\pm$ 12.0	38.5 $\pm$ 15.5	0.88
Sex (% female)	54%	60%	0.54	67%	70%	0.87
BMI, (mean $\pm$ SD)	26.1 $\pm$ 4.5	24.4 $\pm$ 4.9	0.07	26.8 $\pm$ 5.5	29.8 $\pm$ 3.0	0.12
Smoking status (% smokers)	26%	6%	<0.01	33%	30%	0.87
Education (years, mean $\pm$ SD)	16.0 $\pm$ 2.3	17.0 $\pm$ 1.7	0.02	16.5 $\pm$ 1.7	15.3 $\pm$ 2.7	0.24
<b>Ethnicity (%)</b>			0.72			0.51
Caucasian	64.0%	61.8%		83.3%	70.0%	
Asian	14.0%	21.8%		0%	0%	
Black/African American	8.0%	5.5%		0%	10%	
Other	14.0%	10.9%		16.7%	20.0%	
–						
HDRS score (mean, SD)	20.2 $\pm$ 3.3	N/A	N/A	19.8 $\pm$ 2.9	19.7 $\pm$ 3.6	0.92

Abbreviations: MDD = Major Depressive Disorder; BMI = Body Mass Index; HDRS = Hamilton Depression Rating Scale.

**Table 2**  
Oxidative stress, antioxidant, and inflammatory markers in MDD and controls.

	MDD (n = 50)	Controls (n = 55)	F-value	P-value	Effect size
IL-6 (pg/ml)	0.35, 0.23–0.59	0.17, 0.10–0.30	15.37	<0.001	0.95
MDD, n = 49					
Controls, n = 55					
TNF-alpha (pg/ml)	2.75, 2.21–3.01	2.10, 1.82–2.33	15.30	<0.001	0.89
MDD, n = 49					
Controls, n = 55					
CRP (mg/L)	0.48, 0.25–1.30	0.60, 0.20–1.70	0.47	0.49	0.03
MDD, n = 49					
Controls, n = 54					
8-OHdG (pmol/ $\mu$ g DNA)	11.63, 8.37–16.68	9.06, 6.75–12.88	5.79	0.02	0.57
MDD, n = 49					
Controls, n = 55					
F2-isoprostanes (ng/ml)	0.023, 0.018–0.033	0.019, 0.015–0.028	6.53	0.01	0.38
MDD, n = 47					
Controls, n = 55					
GSSG ( $\mu$ M)	–0.14, –0.91–0.78	–0.07, –0.72–0.77	0.01	0.91	0.01
MDD, n = 48					
Controls, n = 51					
GSH ( $\mu$ M)	–0.36, –0.99–0.53	–0.06, –0.70–0.55	0.15	0.70	0.14
MDD, n = 48					
Controls, n = 51					
Vitamin C (nmol/ml)	41.07, 36.58–44.44	37.34, 34.35–42.64	1.90	0.17	0.23
MDD, n = 49					
Controls, n = 55					
GPx (nmole NADPH/ml/min)	0.10, –0.49–0.56	0.03, –0.87–0.77	0.01	0.93	0.05
MDD, n = 49					
Controls, n = 48					

Data were normalized prior to analyses, however, raw data (median, IQR) are presented. Glutathione peroxidase (GPx), oxidized glutathione (GSSG), and reduced glutathione (GSH) were run in two separate assay batches and was therefore transformed into z-scores before combined across batches, thus, positive or negative values may be seen. ANCOVAs were used adjusting for BMI, age, sex, and smoking. Effect size was calculated using Cohen's d on normalized data.

Abbreviations: MDD = Major Depressive Disorder; IL-6 = Interleukin-6; TNF-alpha = Tumor Necrosis Factor-alpha; 8-OHdG = 8-OH 2-deoxyguanosine; GSSG = oxidized glutathione; GSH = reduced glutathione; CRP = C-Reactive Protein.

treatment did not correlate significantly with baseline levels of any of the biomarkers (all  $p > 0.192$ ).

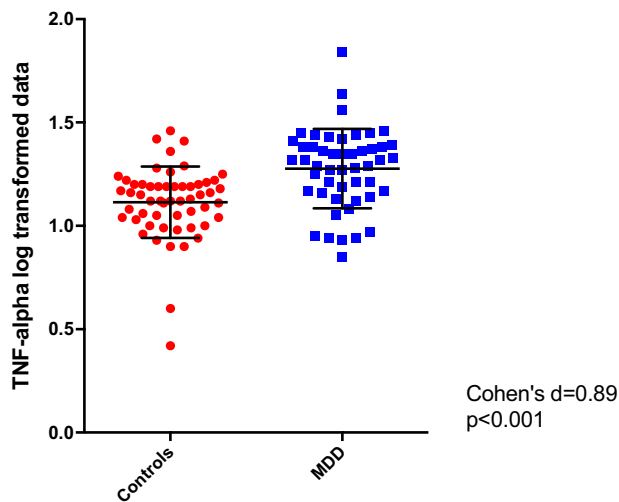
### 3.3.2. Pre- to post- change in biomarkers based on treatment response

In all depressed subjects combined, there was a significant increase in levels of 8-OHdG between baseline and week 8 ( $t = 2.99$ ,  $p = 0.008$ ). This effect was largely driven by an increase in 8-OHdG levels among the Non-responders ( $t = 2.98$ ,  $p = 0.021$ ), whereas no significant change was seen among the Responders ( $t = -1.51$ ,  $p = 0.162$ ). Responders showed a significant decrease in IL-6 levels over the course of treatment ( $t = 2.81$ ,  $p = 0.019$ ), whereas Non-responders showed a nominal, but not statistically significant, increase in IL-6 ( $t = 1.76$ ,  $p = 0.130$ ). None of the other biological markers showed a significant change over the course of treatment in all subjects or when Responders and Non-responders were analyzed separately (all  $p > 0.10$ ). Absolute change in HDRS score did

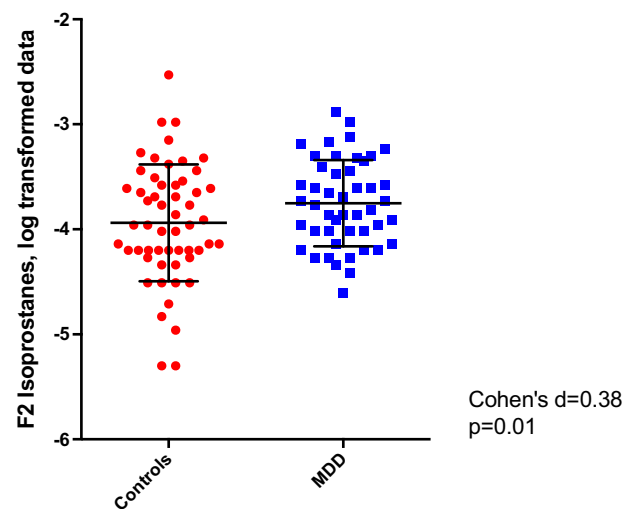
not correlate significantly with change in any of the biomarkers ( $p > 0.166$ ).

## 4. Discussion

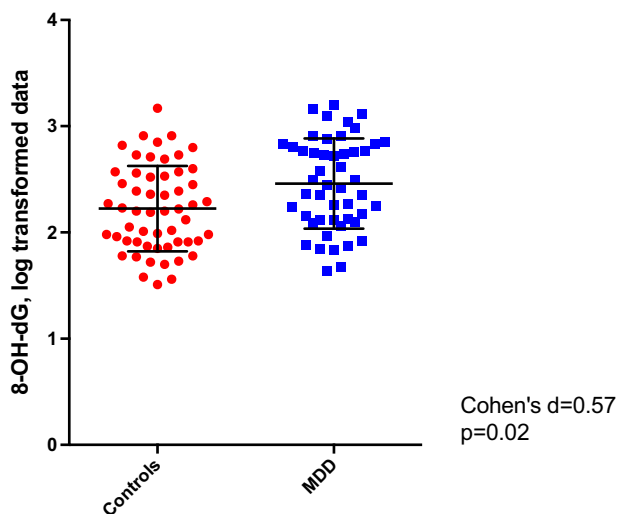
The present study found significantly increased blood levels of inflammatory and oxidative stress markers in a well-characterized, somatically healthy and unmedicated sample of MDD subjects compared to healthy controls. Specifically, we found that blood levels of IL-6, TNF- $\alpha$ , 8-OHdG and F2-isoprostanes were significantly increased in the MDD group, and these group effects remained significant after controlling for the effects of BMI, age, sex, and smoking status. The effect sizes for the group differences ranged from small (F2-isoprostanes), moderate (8-OHdG), to large (IL-6 and TNF- $\alpha$ ). Moreover, we found that high baseline levels of F2-isoprostanes were significantly related to poorer response to SSRI treatment. Interestingly, levels of F2-isoprostanes remained significantly higher in Non-responders over eight weeks of SSRI



**Fig. 2.** Tumor Necrosis Factor (TNF)-alpha (pg/ml) in subjects with Major Depressive Disorder (MDD) and controls; log-transformed data. Error bars indicate  $\pm 1$  SD. Analysis of covariance adjusted for body mass index, age, sex, and smoking.



**Fig. 4.** F2-isoprostanes (pmol/ml) in subjects with Major Depressive Disorder (MDD) and controls; log-transformed data. Error bars indicate  $\pm 1$  SD. Analysis of covariance adjusted for body mass index, age, sex, and smoking.



**Fig. 3.** 8-OH 2-deoxyguanosine (8-OH-dG) (pmol/ $\mu$ g DNA) in subjects with Major Depressive Disorder (MDD) and controls; log-transformed data. Error bars indicate  $\pm 1$  SD. Analysis of covariance adjusted for body mass index, age, sex, and smoking.

treatment. Finally, we found that Non-responders to SSRI treatment showed a significant increase in 8-OHdG during the course of treatment, which was not seen in treatment Responders. Additionally, IL-6 decreased significantly in Responders during the course of SSRI treatment, but not in Non-responders.

Elevations of pro-inflammatory cytokines IL-6 and TNF- $\alpha$ , as we report here, have frequently been reported in MDD (Dowlati et al., 2010). Our study, however, adds to the existing literature by including a well-characterized clinical sample, strict exclusion criteria (precluding medical illness, vaccinations, interfering medications and psychotropic medication including antidepressants within the preceding six weeks), and the assessment of important covariates such as age, sex, BMI and smoking status. In contrast to our a priori hypothesis, CRP was not elevated in the MDD sample. In previous studies from our group in PTSD (Lindqvist et al., 2014b) and other groups in MDD (Simon et al., 2008), as well as in several studies in medically ill patients (Emami Ardestani and Zaerin, 2015; Giannoudis et al., 1998) it is fairly common to observe elevations in one cytokine or CRP but not in others. The reasons for this are not

known, but significant elevations in any inflammatory cytokine or CRP are generally taken as evidence of a pro-inflammatory state, since these compounds have somewhat redundant, and at times synergistic, inflammatory effects (Kelso, 1994; Nicola, 1994). Even though a meta-analysis found an association between high CRP and depression in clinical and community samples (Howren et al., 2009), other studies have suggested that this elevation may be more pronounced in atypical compared to non-atypical depression (Hickman et al., 2014). We were not able to determine if this was the case also in the present study, since it was not designed to distinguish between atypical and non-atypical MDD.

In addition to elevations in pro-inflammatory cytokines, we also found that MDD subjects had significantly higher blood levels of oxidative stress markers F2-isoprostanes and 8-OHdG compared to controls. We did not, however, see significant group differences in GSH, GSSG, GPx or vitamin C, which is consistent with a prior pilot study from our research group conducted in a separate group of MDD subjects (Lindqvist et al., 2014a). F2-isoprostanes and 8-OHdG are two reliable markers of oxidative damage to lipids and DNA respectively (Kadiiska et al., 2005; Wu et al., 2004). In line with the findings of the present study, a recent meta-analysis showed that these are the two oxidative stress markers most consistently elevated in depression, usually with small to moderate effect sizes (Black et al., 2015). F2-isoprostanes and 8-OHdG are elevated not only in MDD, but also in various somatic disorders such as cardiovascular disease, cancer and diabetes (Dalle-Donne et al., 2006). Moreover, several behavioral factors such as smoking and BMI may be associated with increased markers of oxidative stress (Keaney et al., 2003; Wu et al., 2004). In the present study sample, we actively excluded subjects with somatic disorders that could potentially influence oxidative stress markers. Furthermore, smoking status and BMI, along with age and sex, were controlled for in the analyses. Differences in oxidative stress markers between MDD subjects and healthy controls remained highly statistically significant after controlling for these variables, suggesting that some factor intrinsic to depression or, alternatively, the psychological stress frequently associated with depression, may account for this significant group difference in oxidative stress markers. In support of the notion that increased oxidative stress is something intrinsic to MDD, we have previously reported that genes regulated by Nuclear factor erythroid-derived 2-like 2 (NRF-2), a transcription factor regulated by oxidative stress, are elevated in MDD and

decrease after successful antidepressant treatment (Mellon et al., 2016).

Relatively higher levels of F2-isoprostanes were also related to worse antidepressant treatment response. Except for one negative study by Sarandol et al. (Sarandol et al., 2007), this is the first time, to our knowledge, that baseline oxidative stress markers have been investigated in relation to antidepressant treatment response. Comparability between that study and the present one is, however, limited since Sarandol et al. did not measure F2-isoprostanes or 8-OHdG, but a number of other oxidative stress markers and antioxidants (including vitamin C and GPx and others). Moreover, Sarandol et al. used a variety of different antidepressants (often SNRIs or a combination of SNRIs and SSRIs), while we used SSRIs only. In further support of a link between increased oxidative stress and worse antidepressant treatment response, we found that Non-responders had a significant increase in 8-OHdG over the course of treatment. Although we are not aware of any previous clinical studies investigating the relationship between changes in 8-OHdG over the course of antidepressant treatment, there is one animal study pertaining to this. Abdel-Wahab et al., showed that venlafaxine reduced serum and hippocampal levels of 8-OHdG in stressed mice (Abdel-Wahab and Salama, 2011). One potential biological mechanism by which oxidative stress may promote depressive symptoms and antidepressant treatment resistance is *via* increased neurodegeneration (Luca et al., 2013). Neuronal cells are particularly vulnerable for oxidative stress, and free radicals were early on implicated in the pathophysiology of a large number of neurodegenerative disorders such as Alzheimer's and Parkinson's disease (Jesberger and Richardson, 1991). Under pathological conditions, oxidative stress may help induce neurodegeneration *via* various mechanisms causing apoptosis, excitotoxicity, and axonal damage (Chiurciu et al., 2016). Of note, a recent animal study showed that oxidative stress at physiological levels may cause hippocampal dysfunction, specifically astrocyte defects, even before apoptosis was detected (Ishii et al., 2016). These findings might be relevant for the link between oxidative stress and treatment response, since smaller hippocampal volume has been associated with slower antidepressant response in late-life depression (Sheline et al., 2012). We have previously shown that increased peripheral oxidative stress was associated with smaller hippocampal volume in MDD and healthy controls (Lindqvist et al., 2014a). The present results suggest an oxidative stress mechanism *via* which treatment resistance may emerge and suggest a possible (but as yet unconfirmed) link to hippocampal volume. Importantly, though, our oxidative stress markers were entirely based on peripheral blood samples, and their relationship to brain levels is not known.

Although the observed association between high F2-isoprostanes levels and worse treatment outcome are noteworthy, they should be considered preliminary. Due to the small number of subjects in the treatment arm of our study, larger-scale replication studies are warranted. Baseline levels of CRP, IL-6 and TNF- $\alpha$  were not associated with subsequent treatment response. This is in line with a recent meta-analysis by Strawbridge et al., where no significant differences in baseline levels of CRP, IL-6 or TNF- $\alpha$  were found between Responders and Non-responders (Strawbridge et al., 2015). Individual studies such as the one by Uher et al. did, however, find an association between higher baseline CRP levels and worse response to an SSRI (Uher et al., 2014). In search for an explanation for the divergent results between this study and ours, we note that Uher et al. used only one type of SSRI (escitalopram), treated the subjects for 12 weeks, and had a much larger sample size than we did. Despite no associations between baseline levels of inflammatory markers and subsequent treatment response, we found that among Responders, but not Non-responders, IL-6 levels significantly decreased over the course of treatment. Strawbridge et al. also noted a reduction in IL-6 with antidepressant treatment,

although this was not correlated with improvement in depressive symptoms (Strawbridge et al., 2015).

Despite associations between markers of inflammation and oxidative stress and the well-established categorical definition of treatment response (Responder vs Non-responder), we did not find any significant associations between absolute change in HDRS and biomarker levels. The reasons for this are not known. This may be due to the small sample size or conceptual differences between the different definitions of treatment outcomes. In particular, the dichotomous distinction of "Responder" vs "Non-Responder" accords with the widely accepted definitions of clinically meaningful, but not necessarily complete, improvement (Bobo et al., 2016). The dichotomous distinction of "Remitter" vs "Non-remitter" accords with more complete treatment outcome and a lower chance of relapse, yet the number of remitters was too small in this study to perform meaningful analyses. The disadvantage of dichotomous approaches is that the definitions are somewhat arbitrary. Analyzing absolute change in HDRS ratings has the advantage of using continuous, rather than dichotomous measures, with enhancement of statistical power, but also has disadvantages, such as essentially equating a change in HDRS ratings from, for example, 30–20, with a change of, for example, 17–7, which arguably yield quite different clinical outcomes and degrees of patient satisfaction.

Among the strengths of our study are our strict exclusion criteria (e.g. excluding subjects taking antidepressants within the preceding six weeks), thorough diagnostic assessments, covarying for potential confounders and the addition of a treatment arm in a subset of subjects. Further, even though our strict exclusion of subjects with co-morbidities is a strength, this approach may have resulted in a sample not likely representative of community MDD in general. Moreover, a relatively small number of subjects received antidepressant treatment. Thus, our study was likely underpowered to detect anything other than a large effect size when testing the relationship between biomarkers and antidepressant treatment response, and these results should be considered preliminary and in need of replication in larger cohorts. Also, the open label study design precludes any firm conclusions regarding the association between changes in biomarkers and medication-associated treatment effect, since a potential placebo effect was not controlled for. Another important caveat in interpreting our data is that inflammation and oxidative stress markers were measured in peripheral blood samples. Therefore, while these biological markers were significantly higher in MDD compared to controls, it is unclear how they are related to inflammation and oxidative stress pathology in the brain.

The results from the present study confirm the findings from several previous reports showing increased levels of some, but not all, markers of inflammation and oxidative stress in MDD. These results were likely not confounded by co-morbid somatic conditions, medications, obesity or smoking, all factors known to be associated with inflammation and oxidative stress. Furthermore, signs of increased peripheral lipid oxidative stress may be a predictor of worse SSRI antidepressant treatment response, although this finding is in need of replication in larger studies.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.psyneuen.2016.11.031>.

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