



Research paper

Severity of anxiety– but not depression– is associated with oxidative stress in Major Depressive Disorder



Lisa R. Steenkamp^{a,b}, Christina M. Hough^a, Victor I. Reus^a, Felipe A. Jain^a, Elissa S. Epel^a, S. Jill James^c, Alexandra E. Morford^a, Synthia H. Mellon^d, Owen M. Wolkowitz^{a,*}, Daniel Lindqvist^{a,e}

^a Department of Psychiatry, University of California San Francisco (UCSF) School of Medicine, San Francisco, CA, United States

^b Institute of Psychology, Leiden University, Leiden, The Netherlands

^c Arkansas Children's Research Institute, Department of Pediatrics, University of Arkansas for Medical Sciences, Little Rock, AR, United States

^d Department of OB/GYN and Reproductive Sciences, University of California San Francisco (UCSF), School of Medicine, San Francisco, CA, United States

^e Lund University, Faculty of Medicine, Department of Clinical Sciences, Lund, Psychiatry, Sweden

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ABSTRACT

Background: Oxidative stress is implicated in both depression and anxiety, but it is currently unclear whether this relates to syndromal diagnoses or trans-diagnostic dimensional symptoms. We examined the relationship between oxidative stress and severity of depression and anxiety symptoms in individuals with Major Depressive Disorder (MDD).

Methods: Plasma oxidative stress markers F2-isoprostanes and oxidized glutathione (GSSG), and the antioxidant reduced glutathione (GSH), were assessed in 69 physically healthy, medication-free MDD subjects. Symptoms of anxiety and depression were assessed using the Hamilton Anxiety (HAM-A) and Hamilton Depression (HAM-D) Rating Scales. Total HAM-A and HAM-D scores, along with “core” anxiety and depression subscales, and individual HAM-D items “psychic anxiety” and “depressed mood,” were related to oxidative stress markers. Analyses controlled for age, sex, BMI, and smoking.

Results: Total HAM-A ratings were positively associated with F2-isoprostanes ($\beta = .26, p = .042$) and GSSG ($\beta = .25, p = .049$), but not GSH ($\beta = .05, p = .711$). Core anxiety severity was positively associated with F2-isoprostanes ($\beta = .34, p = .012$) and GSSG, although this did not reach significance ($\beta = .24, p = .074$). None of the biological markers were significantly associated with total HAM-D or core depression ratings (all $p > .13$). Subjects scoring high on “psychic anxiety” had elevated F2-isoprostanes ($p = .030$) and GSSG ($p = .020$). This was not seen with “depressed mood” scores (all $p > .12$).

Limitations: We assessed peripheral oxidative markers, but their relationship to the brain is unclear.

Conclusions: Oxidative stress is more closely related to anxiety than depression symptoms in MDD. This highlights the importance of relating oxidative stress to specific symptoms and could provide new insights into the biological correlates of affective disorders.

1. Introduction

Individuals with Major Depressive Disorder (MDD) have an increased risk of developing medical illnesses, including cardiovascular diseases, diabetes, obesity, Alzheimer's disease, and cancer (Penninx et al., 2013), conditions that have also been associated with oxidative stress (Ceriello and Motz, 2004; Emerit et al., 2004; Furukawa et al., 2004; Maritim et al., 2003; Reuter et al., 2010). Indeed, many studies have found evidence of increased oxidative stress in MDD, as well as increased gene expression regulated by oxidative stress, suggesting that

this is an important contributor to depression pathophysiology (Lindqvist et al., 2017; Maes et al., 2011; Mellon et al., 2016; Wolkowitz et al., 2011).

Oxidative stress occurs when an organism is not able to effectively detoxify or repair the harmful effects of free radicals, for example, when pro-oxidant species (e.g., F2-isoprostanes and oxidized glutathione) exceed the capacity of anti-oxidant defenses (e.g., reduced glutathione; Coyle and Puttfarcken, 1993). Oxidative stress has detrimental effects on cellular membranes, proteins, nucleic acids, and lipids. Although nearly all tissues are sensitive to oxidative damage, the brain is

* Correspondence to: Department of Psychiatry, University of California, San Francisco (UCSF), School of Medicine, 401 Parnassus Avenue, San Francisco, CA 94143, United States.
E-mail address: Owen.Wolkowitz@ucsf.edu (O.M. Wolkowitz).

Table 1
Characteristics of the study sample.

	MDD	n ^a
Age, years, mean (SD)	38.8 (13.9)	69
Sex, n men (%)	28 (40.6%)	69
Body mass index, mean (SD)	25.7 (4.5)	69
Smoking, n (%)		69
Not at all	52 (75.4%)	
Some days	9 (13.0%)	
Every day	8 (11.6%)	
HAM-D score, mean (SD)	20.0 (3.2)	69
Core Depression Factor Subscale score, mean (SD)	8.7 (1.8)	69
HAM-A score, mean (SD)	16.9 (4.9)	58
Hamilton Core Anxiety Scale score, mean (SD)	8.0 (2.8)	58
Psychic anxiety, n (%)		69
Low	47 (68.1%)	
High	22 (31.9%)	
Depressed mood, n (%)		69
Low	48 (69.6%)	
High	21 (30.4%)	
F2-isoprostanes, ng/ml, mean (SD)	.030 (.015)	65
GSSG, uM, mean (SD)	.198 (.090)	64
GSH, uM, mean (SD)	1.64 (.488)	64

^a Sample sizes are not all equivalent due to some subjects missing HAM-A or biochemical data. These missing data were handled using multiple imputation (discussed in Section 2.5).

especially sensitive, since it is a major consumer of oxygen and is rich in oxidizable lipids (Halliwell, 2006). Therefore, oxidative stress can result in altered neuronal functioning, neurotransmission, and overall brain activity (Bakunina et al., 2015; Cardozo-Pelaez et al., 1999; Wang and Michaelis, 2010).

Recent meta-analyses have concluded that peripheral oxidative stress markers are elevated in depression, while levels of antioxidants are decreased (Black et al., 2015; Palta et al., 2014). Although MDD has been the most widely studied psychiatric illness in this regard, other psychiatric illnesses, including anxiety disorders, are also characterized by increased oxidative stress (Atmaca et al., 2008; Bouayed et al., 2009; Kuloglu et al., 2002; Ozdemir et al., 2009), raising the possibility that oxidative stress may be a non-specific marker of psychiatric illness or that it may relate to certain trans-diagnostic dimensional symptoms, as opposed to specific syndromal diagnoses per se.

Depression and anxiety disorders are highly co-morbid, and there is a significant diagnostic overlap (Zbozinek et al., 2012). In fact, the majority of individuals with MDD suffer from a co-morbid anxiety disorder or subsyndromal anxiety symptoms (Zimmerman et al., 2000), often associated with poorer disease outcomes, including higher rates of suicide, more functional impairments, and worse response to antidepressant treatment (Belzer and Schneier, 2004). Although there are certain symptoms considered to be “core” features of anxiety and depressive disorders, there are also several shared symptoms, such as fatigue or loss of energy, difficulty concentrating, and sleep disturbances, leading to overlapping diagnostic boundaries (Zbozinek et al., 2012).

Most studies examining oxidative stress in MDD have focused on syndromally-defined MDD, and few, if any, have mapped oxidative stress onto specific symptom profiles, including symptoms of anxiety within MDD (Chung et al., 2012; Irie et al., 2005). Therefore, it is currently unclear whether the observed increase in oxidative stress in MDD is driven by symptoms of depression, anxiety, or both. It is important to better understand the psychiatric correlates of oxidative stress, as this could facilitate the development of novel anti-oxidant treatments in selected subjects with MDD.

F2-isoprostanes and oxidized and reduced glutathione (GSSG and GSH, respectively) are among the most studied oxidative stress markers in MDD. F2-isoprostanes are a highly accurate measure of lipid peroxidation and are considered a robust marker of oxidative stress

(Milne et al., 2005; Patrignani and Tacconelli, 2005). Lipid peroxidation leads to impaired cell functioning, loss of membrane elasticity and fluidity, and potentially cell death (Montuschi et al., 2004). GSH is a nonenzymatic antioxidant and is oxidized by reactive oxygen species to form GSSG (Schulz et al., 2000). GSH levels, therefore, are considered as an indication of antioxidant capacity, whereas GSSG is an indication of oxidative stress. The GSH system, one of the major antioxidant systems of the brain (Dringen, 2000), is essential for cell proliferation (Poot et al., 1995) and is involved in the regulation of apoptosis (Hall, 1999). In fact, accumulation of GSSG levels within cells has been found to directly trigger (neural) cell death (Filomeni et al., 2003; Park et al., 2009).

In the current study, we investigated the relationship between anxiety symptoms, depression symptoms, and the peripheral oxidative stress markers F2-isoprostanes and GSSG, as well as the antioxidant GSH, in unmedicated individuals with MDD. Based on previous findings of increased oxidative stress and reduced antioxidant capacity in MDD and anxiety disorders (Black et al., 2015; Ng et al., 2008; Sarandol et al., 2007; Smaga et al., 2015), we hypothesized that more severe anxiety and depression symptoms would be related to increased levels of F2-isoprostanes and GSSG, and decreased levels of GSH.

2. Methods

2.1. Subjects

The study was approved by the University of California, San Francisco (UCSF) Committee on Human Research. All subjects gave written informed consent to participate. Subjects were recruited by clinical referrals, newspaper advertisement, flyers, bulletin board notices, and Craigslist postings.

We enrolled 69 subjects with unipolar MDD who were medication-free for at least six weeks. Oxidative stress data from subsets of this sample have been previously reported in relation to hypotheses not explored in the present study (Lindvist et al., 2017; Rawdin et al., 2013). MDD diagnoses were determined using the Structured Clinical Interview for the DSM-IV (First et al., 1997). Subjects were required to have a minimum score of 17 on the 17-item Hamilton Depression Rating Scale (HAM-D; Hamilton, 1960).

Subjects with current psychosis or a history of bipolar disorder or psychotic symptoms outside of a major depressive episode were excluded. In addition, subjects were excluded if they had an eating disorder or post-traumatic stress disorder (PTSD) within one month of entering the study, or alcohol or substance dependence or abuse within six months of entering the study. Depressed subjects with co-morbid anxiety disorders were allowed (except PTSD) if the primary diagnosis was MDD.

The presence of medical conditions was assessed by medical history, physical examinations, and routine blood screening. Subjects were excluded if they had acute illnesses or infections, neurological disorders, chronic inflammatory disorders, or other major medical conditions that were considered potentially confounding.

All subjects were free of any psychotropic medication, steroid-containing contraceptives, hormone supplements, and any other potentially confounding medications for at least six weeks prior to participation, and none were taking vitamin supplements above the United States Recommended Daily Allowances. Subjects were allowed to use short-acting sedative hypnotics for sleep up to three times a week, but no sedative hypnotics were allowed within one week prior to entering the study.

2.2. Procedures

After having fasted (except water) since 2200 h the night before, subjects were admitted as outpatients to the UCSF Clinical and Translational Science Institute between 0800 and 1100 h. First, subjects

were required to test negative on a urine toxicology screening and, if applicable, on a urine pregnancy test. Blood was drawn after 25–45 min of relaxation. Plasma for oxidative stress markers was collected in lavender-top EDTA tubes and was placed on ice immediately after being drawn. Plasma was separated by centrifugation at 14,000 rpm at 4 °C for 15 minutes in a refrigerated centrifuge within 15–20 min after the blood draw.

2.3. Assay of oxidative stress markers

Due to differing recruitment periods (2006–2008 and 2011–2014), samples were assayed in separate batches. As noted below (Section 2.5), this factor was statistically corrected for in all analyses.

F2-isoprostanes were quantified at Vanderbilt University, Eicosanoid Laboratory. The F2-isoprostanes were extracted and purified using solid-phase extraction and thin layer liquid chromatography. Subsequently, they were converted into trimethyl-ether derivatives and analyzed using chromatography–mass spectrometry (GC–MS), which is described elsewhere (Milne et al., 2007; Morrow and Roberts, 2002).

For GSSG and GSH assessment, the use of different assay batches necessitated a change in assay laboratories and methods over the course of the study.

The first batch consisted of samples from 16 MDD subjects. Samples were processed by adding 400 µl ice cold 5% meta-phosphoric acid and 100 µL of whole blood into a 1.5 mL microcentrifuge tube. Immediately, the microcentrifuge tube was capped and vortexed for 5 s and then centrifuged at 4 °C for 2 min at 13,000 RPM in a microcentrifuge. The supernatant was aliquoted to a vial and was stored at –80 °C until assay at the Kronos Science Laboratory. The measurements were carried out using LC-MS/MS on a system that consisted of two Shimadzu LC-10AD pumps, a Shimadzu degasser (Shimadzu Scientific Instruments, Columbia, MD), and a Perkin Elmer autosampler (Perkin Elmer LLC, Norwalk, CT) directly interfaced with a triple-stage quadrupole mass spectrometer (API2000, Applied Biosystems, Foster City, CA) equipped with a TurbolonSpray ionization source. Nitrogen was used as the collision gas. Glutathione under MRM mode was measured using Positive ionization methods. GSSG and GSH were monitored with ion pairs (m/z) of 613/355 and 308/179, respectively. A seven-point linear calibration curve was established for each analyte using internal and external standards. Three 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 coefficients of variance ranged from 3% to 10%.

The second and third batches of GSSG and GSH consisted of samples from 48 MDD subjects. Samples were processed by adding 200 µl ice cold 10% meta-phosphoric acid to 200 µl of plasma, after which the sample was incubated for 30 min in order to precipitate proteins. The sample was centrifuged at 14,000 rpm for 15 min at 4 °C, after which the supernatant was filtered through a .2 mm filter (PGC Scientific, Frederic, MD). A 20 µl aliquot was injected in the HPLC-electrochemical detection system and the ESA autosampler (model 542). HPLC with an ESA solvent delivery system (model 580) and a reverse phase Capcell pak C18 column (3 mm; 4.6 × 150 mm, Phenomenex, Inc., Torrance, CA, USA) were used for the analyses. A 16 channel CouloArray electrochemical detector (ESA, Inc., Chelmsford, MA) with a high sensitive four-sensor cell (model 6210) was used to determine the plasma metabolites. Using HPLC software, the concentrations of the plasma metabolites were calculated from peak areas and standard calibration curves included with each run. The coefficients of variation were 5% and 8% for GSH and GSSG, respectively.

2.4. Anxiety and depression measures

The 17-item HAM-D was used to determine the severity of depression (Hamilton, 1960). To assess severity of anxiety, we used the

Hamilton Anxiety Rating Scale (HAM-A; Hamilton, 1959). The HAM-A is a widely used clinician-rated scale consisting of 14 items that are each scored on a scale of 0 (not present) to 4 (severe). The HAM-D and HAM-A have good reliability (Bagby et al., 2004) and validity (Cusin et al., 2009; Maier et al., 1988).

It is commonly acknowledged that the HAM-A and HAM-D assess certain overlapping symptoms of anxiety and depression, making it difficult to parse out the differences between anxiety and depression (Zbozinek, et al., 2012). To address this, we used subscales of the HAM-A and HAM-D to investigate the relationship between oxidative stress and “core” symptoms of anxiety and depression. To measure core anxiety, we used the Hamilton Core Anxiety Scale (HAM-A₆; Bech, 2006; Bech et al., 2005; Kent et al., 2016). The HAM-A₆ includes the items anxious mood, tension, fears, intellectual, somatic (muscular), and anxious behavior at interview. To measure core depression, we used the Core Depression Factor Subscale of the HAM-D (Bech, 2006; Detke et al., 2002; Perahia et al., 2006). We specifically chose this subscale because it does not include the item “psychic anxiety” that is frequently present in other subscales of core depression. The core depression factor subscale includes the HAM-D items depressed mood, feelings of guilt, suicide, work and activities, and psychomotor retardation (Bech, 2006).

Another approach to capture specific symptomatology is to select a single item that best reflects the primary state of the pathology. We selected the HAM-D item “psychic anxiety” to capture the emotion of “pure” anxiety (Russell et al., 2007; Thase et al., 2014). We classified subjects into one of two subgroups, according to their scores on the single item psychic anxiety: absent to mild anxiety (score 0 or 1) or moderate to severe anxiety (score 2, 3, or 4; Russell et al., 2007). To capture the emotion of “pure” depression, we selected the HAM-D item “depressed mood” (Hieronymus et al., 2016). Subjects were classified into one of two subgroups according to their score on the single item depressed mood: low to mild depressed mood (score 1 or 2) or moderate to severe depressed mood (score 3 or 4).

2.5. Data analysis

Statistical analyses were performed with IBM SPSS Statistics, version 23. All tests were 2-tailed with an alpha = .05. Since oxidative stress markers were analyzed in separate assay batches (two for F2-isoprostanes and three for GSH and GSSG), we transformed biomarker levels into z-scores before combining data across batches. We examined the distributions for normality, and non-normal distributions were either natural log transformed (HAM-A and HAM-D) or Blom transformed in cases where log transformation was not feasible (F2-isoprostanes and GSH; Blom, 1958).

There were missing values for both HAM-A (16% missing data) and oxidative stress markers (6–7% missing data), due to clinical constraints and difficulties while obtaining blood samples, respectively. These randomly missing data were handled in two ways: First, multiple imputation was used, employing the fully conditional approach (FCS) and predictive mean matching (PMM), with 100 maximum iterations and 20 imputed data sets (as described by Rubin (1986, 1987)). Second, we performed a sensitivity analysis using the original data, in which subjects with missing data were excluded.

To investigate the relationship between symptom scores (independent variable) and oxidative stress markers (dependent variable), we performed a series of linear regression analyses, one model for each pair of symptom scores and oxidative stress markers. We used residual values of the oxidative stress markers that were adjusted for age, sex, BMI, and tobacco use (Black et al., 2016a; Block et al., 2002).

We performed independent *t*-tests to investigate whether the subgroups low vs. high anxiety and low vs. high depression (single-item measures) differed in levels of oxidative stress markers (using residual values corrected for age, sex, BMI, and tobacco use).

3. Results

3.1. Demographics

Table 1 displays the characteristics of the study sample. HAM-D scores ranged from 17 (moderate depression) to 34 (very severe depression), with an average score of 20, indicating that overall, our sample was moderately to severely depressed. HAM-A scores ranged from 8 (mild anxiety) to 33 (severe anxiety), with an average score of 17, indicating a mild to moderate overall anxiety severity in our sample.

3.2. Hamilton anxiety and depression rating scales

Fig. 1 shows the relationship between HAM-A ratings (panel A and C), HAM-D ratings (panel B and D), and the oxidative stress markers F2-isoprostanes and GSSG. As hypothesized, HAM-A ratings positively predicted levels of F2-isoprostanes ($\beta = .26$, 95% CI [.010, .506], $p = .042$) and levels of GSSG ($\beta = .25$, 95% CI [.003, .487], $p = .049$). HAM-A ratings did not, however, significantly predict GSH levels ($\beta = .05$, 95% CI [−.205, .300], $p = .711$).

HAM-D ratings did not significantly predict levels of F2-isoprostanes ($\beta = .13$, 95% CI [−.123, .374], $p = .323$), GSSG ($\beta = .13$, 95% CI [−.118, .369], $p = .314$), or GSH ($\beta = -.12$, 95% CI [−.357, .126], $p = .347$).

3.3. Core anxiety and core depression

We next sought to determine if higher “core” anxiety severity was related to increased oxidative stress. Core anxiety severity positively predicted levels of F2-isoprostanes ($\beta = .34$, 95% CI [.081, .606], $p = .012$). Core anxiety severity also positively predicted levels of GSSG, but this did not reach significance ($\beta = .24$, 95% CI [−.024, .506], $p = .074$). Further, we found no significant correlation between core anxiety severity and levels of GSH ($\beta = .04$, 95% CI [−.257, .327], $p = .803$).

Core depression severity did not significantly predict any of the oxidative stress markers; F2-isoprostanes ($\beta = .19$, 95% CI [−.054, .425], $p = .130$), GSSG ($\beta = .18$, 95% CI [−.053, .422], $p = .128$), and GSH ($\beta = .08$, 95% CI [−.165, .315], $p = .540$).

3.4. Low vs. high anxiety and low vs. high depression (single-item analysis)

Oxidative stress markers in individuals with high vs. low “psychic anxiety” and high vs. low “depressed mood” items are summarized in Table 2. We found significantly higher levels of F2-isoprostanes and GSSG in the high anxiety subgroup compared to the low anxiety subgroup, with medium effect sizes (Cohen's $d = .54$ and $.62$, respectively; Cohen, 1988). There were no significant differences in GSH levels between subjects with high anxiety compared to subjects with low anxiety.

With regard to depression severity, we found no significant differences in F2-isoprostanes, GSSG, and GSH between the low and high depression subgroups.

The sensitivity analyses (in which cases with missing data were excluded) resulted in nearly identical findings, with only a small difference in significance values for the relationship between GSSG and HAM-A ratings ($p = .049$ for multiply imputed data, and $p = .059$ for original, non-imputed data), and the relationship between GSSG and core anxiety ($p = .074$ for multiply imputed data, and $p = .030$ for original, non-imputed data).

4. Discussion

In the present study, we examined the relationship between oxidative stress, anxiety, and depression symptoms in somatically

healthy, medication-free individuals with MDD. Our main finding was that MDD subjects with more severe anxiety, but not subjects with more severe depression, showed increased oxidative stress, after controlling for age, sex, BMI, and smoking.

Specifically, we found that higher plasma levels of F2-isoprostanes and GSSG were associated with higher total HAM-A scores, higher core anxiety scores (HAM-A_c), and higher scores on the single HAM-D item “psychic anxiety.” The group differences in oxidative stress markers between those with low vs. high anxiety were of medium effect size (Cohen's $d = .54$ and $.62$). Similarly, the continuous relationships between severity of anxiety symptoms and oxidative stress marker levels showed r values ranging from $.24$ to $.34$, indicating small to medium effect sizes (Cohen, 1988). Surprisingly, F2-isoprostanes and GSSG levels showed no significant associations with depression severity, as quantified by total HAM-D scores, core depression scores, and scores on the single HAM-D item “depressed mood”. As opposed to the oxidative stress markers, the antioxidant GSH was not significantly associated with either anxiety or depression severity.

To the best of our knowledge, this is the first study to show a relationship between increased oxidative stress and more severe symptoms of anxiety, but not depression, in medication-free individuals with MDD. This conclusion is supported by the concordance of our findings across three different characterizations of anxiety and depression symptoms. Our findings raise the possibility that the increased oxidative stress reported in many studies of MDD may be more attributable to anxiety rather than to depression *per se*, although this needs to be confirmed in future studies. Our findings may also point to the possibility that increased oxidative stress contributes to, or is a consequence of, the worse illness course seen in MDD patients with comorbid anxiety compared to those with more “pure” depressive symptoms (Belzer and Schneier, 2004).

Peripheral markers of increased oxidative stress, as well as decreased antioxidants, have been reported in individuals with MDD (Black et al., 2015; Lindqvist et al., 2017; Palta et al., 2014) and in individuals with anxiety disorders, including panic disorder, social phobia, and obsessive compulsive disorder (Atmaca et al., 2008; Kuloglu et al., 2002; Ozdemir et al., 2009). To our knowledge, only two prior studies examined the relationship between oxidative stress and specific symptom dimensions of MDD. Chung et al. (2013) examined the relationship between oxidative stress and the Profiles of Mood State (POMS) questionnaire in MDD subjects, but found no significant associations with any of the subscales. Irie et al. (2005) found similar results as in the present study, namely, a positive relationship between oxidative stress and core anxiety severity (“tension-anxiety” subscale of the POMS), but not core depression severity (“depression-rejection” subscale of POMS) in female MDD subjects. The effect sizes of the relationship between anxiety and oxidative stress reported by Irie et al. (2005) were medium to large in size, which are larger in magnitude than the effect sizes found in this study. However, the results of the present study were based on a substantially larger sample of depressed individuals, all of whom were medication-free, and analyses controlled for potential confounders, in contrast to the two prior studies.

A dimensional approach in psychiatric research has been recently advocated for, exemplified by the introduction of the RDoC criteria by the NIMH (Cuthbert and Insel, 2013). In this paradigm, anxiety symptoms are part of the “Threat” construct in the Negative Valence Systems domain, and depression symptoms are part of the “Loss” construct and “Reward” constructs in the Negative and Positive Valence Systems domains, respectively (Woody and Gibb, 2015). Although the present study did not employ an RDoC design, it did attempt to parse out anxiety vs. depression components in relation to oxidative stress.

Both preclinical (Bouayed et al., 2007; Rammal et al., 2008; Vollert et al., 2011) and clinical studies (Atmaca et al., 2008; Kuloglu et al., 2002) have found a positive relationship between oxidative stress and severity of anxiety symptoms. Interestingly, several animal studies have

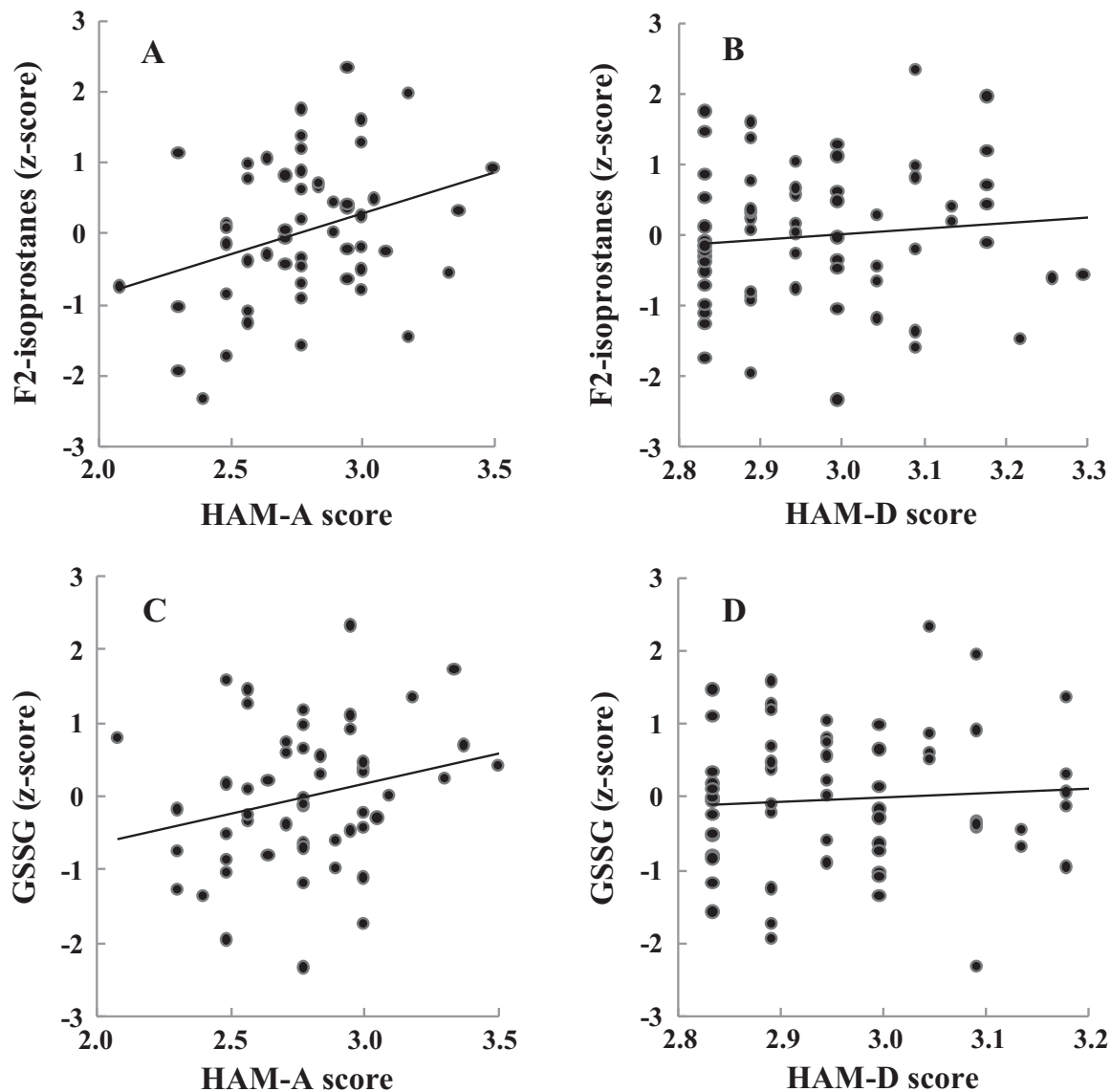


Fig. 1. Scatter plots of the relationship between HAM-A scores (x-axis) and levels of (a) F2-isoprostanes and (c) GSSG (y-axis), and the relationship between HAM-D scores (x-axis) and levels of (b) F2-isoprostanes and (d) GSSG (y-axis). HAM-A and HAM-D scores are log transformed and F2-isoprostane and GSSG levels are (Blom) normalized and regressed on confounders age, sex, BMI, and smoking. Scatter plots display the data without multiply-imputed data, whereas statistical analyses include multiply-imputed data for missing values, as described in the text.

shown that *induction* of oxidative stress results in anxiety-like behavior (Masood et al., 2008 and Salim et al., 2010). Moreover, a genomics study showed that overexpression of genes involved in oxidative stress metabolism (glutathione reductase 1 and glyoxalase 1) in the mouse brain increased anxiety-like behavior, whereas inhibition of glyoxalase 1 expression had the opposite effect (Hovatta et al., 2005). In contrast, we are not aware of animal studies that have examined causality of oxidative stress in depression-like behaviors (Salim, 2014).

Whether oxidative stress is the cause or consequence of anxiety, or if

the two are jointly related to a third factor (e.g., genetics or lifestyle factors; Hovatta et al., 2010), is still unknown. Nonetheless, the hypothesis that oxidative stress is involved in the genesis of anxiety has been widely proposed (Bouayed et al., 2009; Hassan et al., 2014). The neurobiological mechanisms may involve brain regions such as the amygdala and the hippocampus, which are especially sensitive to oxidative damage (Masood et al., 2008; Salim, 2016; Salim et al., 2010) and which are implicated in anxiety-related disorders (Shin and Liberzon, 2010). Oxidative stress may lead to neuroendocrine altera-

Table 2

Levels of oxidative stress markers for low vs. high anxiety and low vs. high depression (single item) subgroups.

	Low anxiety (n = 47)	High anxiety (n = 22)	t-value	p-value	Low depression (n = 48)	High depression (n = 21)	t-value	p-value
F2-isoprostanes, ng/ml (mean, SD)	-.16 ± .12	.35 ± .23	-2.17	.030	-.03 ± .12	.07 ± .13	-.41	.685
GSSG, uM (mean, SD)	-.18 ± .14	.39 ± .18	-2.32	.020	-.12 ± .13	.28 ± .23	-1.58	.115
GSH, uM (mean, SD)	-.05 ± .14	.11 ± .24	-.58	.560	-.06 ± .15	.15 ± .22	-.78	.436

Abbreviations: SD = standard deviation. Levels of oxidative stress markers are normalized residuals corrected for age, sex, BMI, and smoking. Accordingly, values can be positive or negative.

tions in the amygdala, including dendritic shrinkage and amygdala hyperactivity (Salim, 2016). Moreover, oxidative damage to the hippocampus might disrupt the neural circuitry involved in anxiety and fear conditioning (Hassan et al., 2014). Additional mechanisms proposed to mediate the oxidative stress/ anxiety relationship include impaired secretion of brain-derived neurotrophic factor (BDNF; Salim et al., 2011) and pathologic alterations in NMDA receptor activity, mitochondrial functioning, and neurogenesis (Haxaire et al., 2012; Hovatta et al., 2010; Rai et al., 2013). However, any inferences about central nervous system effects, based on our data, are speculative since we only studied peripheral oxidative stress markers. Further, none of these explanations would predict a relationship exclusively with anxiety without also predicting a relationship with other psychiatric symptoms such as depression.

Our findings are in contrast to some previous studies showing a positive relationship between oxidative stress and overall depression severity (Bilici et al., 2001; Dimopoulos et al., 2008; Forlenza and Miller, 2006; Irie et al., 2005; Jorgensen et al., 2013; Sarandol et al., 2007; Yanik et al., 2004). The discrepant findings may be explained by the large heterogeneity of oxidative stress measures across studies. In fact, two out of three previous MDD studies that examined the relationship between F2-isoprostanes and depression severity were, as the present study, negative (Chung et al., 2012; Yager et al., 2010). Further, any differences between our study and others could be explained by our stringent inclusion and exclusion criteria, resulting in a sample of somatically healthy and medication-free individuals. This, and our decision to adjust for the potentially confounding influences of age, sex, smoking, and BMI, are in contrast to most prior studies examining the relationship between oxidative stress and depression symptom severity (Bilici et al., 2001; Dimopoulos et al., 2008; Chung et al., 2012; Irie et al., 2005; Jorgensen et al., 2013; Sarandol et al., 2007; Yanik et al., 2004).

The finding that GSH was not related to either anxiety or depression severity is in contrast to our hypothesis that decreased antioxidant activity would be related to more severe symptoms. However, our finding is in line with a previous report from our group that GSH levels were not decreased in MDD subjects compared to healthy controls, despite the fact that F2-isoprostanes were elevated (Lindqvist et al., 2017). Our findings may indicate that chronic oxidative stress results in a compensatory but inadequate increase in GSH synthesis in attempt to reduce further oxidative damage (Andreazza et al., 2009), but this is purely hypothetical.

Our study has several limitations. Firstly, we assessed oxidative stress markers at only one time point on a single day. F2-isoprostanes are chemically stable and day-to-day variability, as well as diurnal variability, are relatively limited (Montuschi et al., 2004). GSSG levels also have a minimal diurnal variation, but, on the other hand, GSH levels show fluctuations over the day, especially related to food intake (Blanco et al., 2007). This might be another possible reason for not finding correlations between symptom severity and GSH levels. However, we obtained blood samples between 8 and 11 AM in all subjects to minimize diurnal variability, and subjects had fasted since the night before to diminish the effect of food intake on plasma oxidative stress levels. Secondly, we only assessed the oxidative markers in peripheral blood samples, therefore extrapolations to brain oxidative stress would be purely conjectural. However, peripheral markers of oxidative stress have recently been correlated with decreased hippocampal CA3/dentate gyrus volume in unmedicated individuals with MDD (Lindqvist et al., 2014). Thirdly, although we controlled for the potential confounding effects of age, sex, BMI, and tobacco use, we cannot rule out that other variables, not accounted for in our analyses, may influence oxidative stress in MDD. Dietary patterns have recently been identified as potential confounders in the association between oxidative stress and depression (Black et al., 2016c). Future studies should include dietary measures to further examine the role of this factor in the relationship between MDD and oxidative stress. Fourthly,

the relationship between oxidative stress and non-pathological levels of anxiety and depression, such as in healthy controls, was not addressed in this study. Lastly, due to our strict inclusion and exclusion criteria, the findings may not be generalizable to real-world MDD patients seen in clinical practice.

Strengths of the study are that we used somatically healthy depressed patients who had all been free of antidepressants and other possibly confounding drugs for a minimum of six weeks. The use of antidepressants may influence oxidative stress markers and may thus be a serious confounder (Black et al., 2016b; Mellon et al., 2016). Another strength is that we used several strategies to parse out anxiety and depression symptomatology in relation to oxidative stress. The congruence of findings among these approaches strengthens the overall conclusions and provides face validity to our results.

To conclude, the present study showed a relationship between increased peripheral oxidative stress and more severe anxiety, but not depression symptoms, in physically healthy, medication-free individuals with MDD. Our findings highlight the importance of mapping oxidative stress, and perhaps other biomarkers, onto specific dimensional symptoms in addition to syndromal diagnoses. Future studies should expand the range of symptom dimensions surveyed (i.e., using the RDoC) and the number and type of oxidative stress measures studied. Moreover, future studies should test whether successful treatment of underlying symptoms diminishes oxidative stress. Although not addressed in the current study, it might be possible that antioxidant treatments could play a role in ameliorating anxiety symptoms in individuals with MDD (Balmus et al., 2016; Deepmala et al., 2015). Also, to the extent anxiety symptoms are directly correlated with oxidative stress, it will be important to determine whether a causal relationship exists, and, if so, its directionality. Distinguishing these possibilities could lead to a better recognition of the biological underpinnings of affective disorders.

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