CHAPTER

3

IS DEPRESSION ASSOCIATED with 
INCREASED OXIDATIVE STRESS? 
a SYSTEMATIC REVIEW and META-ANALYSIS

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ABSTRACT

Background
It has been suggested that depressed persons have increased oxidative stress and decreased anti-oxidant defences. 8-Hydroxy-2'-deoxyguanosine (8-OHdG) and F2-isoprostanes, measures of oxidative DNA and lipid damage respectively, are among the most reliable oxidative stress markers, but studies on their association with depression show conflicting results. This meta-analysis quantifies the association between depression and these markers and explores factors that may explain inconsistencies in the results.

Methods
A systematic literature search was conducted in PubMed, EMBASE and PsycINFO. Studies assessing the association of 8-OHdG or F2-isoprostanes with elevated depressive symptoms, major depressive disorder (MDD) or bipolar disorder (BD) were pooled in two random-effect models.

Results
The pooled effect size (Hedges’ g) for the association of depression with oxidative stress was 0.31 (p=0.01, I²=75%) for 8-OHdG (10 studies, 1308 subjects) and 0.48 (p=0.001, I²=73%) for F2-isoprostanes (8 studies, 2471 subjects), indicating that both markers are increased in depression. There was no indication of publication bias for either marker. The F2-isoprostane results did not differ by type of depression, biological specimen, laboratory method or quality, however subgroup analyses in the 8-OHdG studies showed significantly stronger associations in plasma/serum vs. urine samples (p<0.01), in measurements performed with immuno-assay vs. chromatography-mass spectrometry (p<0.01) and weaker associations in high quality studies vs. low (p=0.02).

Conclusion
This meta-analysis finds that oxidative stress, as measured by 8-OHdG and F2-isoprostanes, is increased in depression. Larger-scale studies are needed to extend the evidence on oxidative stress in depression, and examine the potential impact of treatment.

INTRODUCTION
Depression is a leading cause of morbidity worldwide (1). Depression is highly prevalent (2) and has a profound impact on functioning and quality of life (3) as well as on somatic health. Sufferers are at higher risk of diseases that are usually associated with increasing age such as cardiovascular disease (4), obesity (5), diabetes (6), cancer (7), cognitive impairment (8) and have a higher all-cause mortality rate (9). It is hypothesized that increased metabolic stress and accelerated cellular ageing may be underlying pathways that contribute to this poorer physical health in individuals with depression (10). A fast growing body of evidence suggests the involvement of a specific component of metabolic stress, oxidative stress, in the pathophysiology of depression (11).

Oxidative stress refers to the biologically damaging effects of free radicals (12). The production of free radicals, or reactive oxygen species (ROS), is a normal process in aerobic metabolism and ROS perform a number of physiological roles in cellular signalling and in the defence against pathogens. However, when present in excess, ROS cause damage to lipids, proteins and DNA, and can ultimately result in cell death. Oxidative stress is a well-recognized mechanism in ageing and disease. It has been shown to play a role in the pathophysiology of - amongst others - cardiovascular disease, diabetes mellitus, cancer and Alzheimer’s disease (12). Additionally, there is evidence suggesting that oxidative stress may be increased in a number of psychiatric disorders, including depression (13).

A recent meta-analysis pooling data from studies with different oxidative stress markers suggests oxidative stress is increased and antioxidant defences are decreased in depression (14). In line with these findings, increased nitric oxide (NO) and lipid peroxidation, as measured by thiobarbituric acid reactive substance (TBARS) assay, have also been found in patients with bipolar disorder, however these patients did not differ from controls in anti-oxidant enzymes levels (15). Overall, these studies suggest that oxidative stress is increased in major depressive disorder and bipolar disorder.

There is a wide range of oxidative stress biomarkers and laboratory techniques available, each of which has its own strengths and limitations (16). To date there is no consensus on the most appropriate biomarkers of oxidative stress in general and the validity of many of those in use is to still be established. ROS have a short half-life, making measurement difficult. Levels of antioxidants, vitamins or anti-oxidant enzymes are informative, but reflect only one side of redox homeostasis, leaving the question unanswered whether decreased levels are actually also indicative of increased oxidative damage. Studies show quite consistently that lipid peroxidation reflected by malondialdehyde (MDA) measured with the TBARS assay is increased in depression (14) (Palta et al. 2014) and in bipolar disorder (Andreazza et al. 2008). However this commonly used method...
also has well recognized limitations: MDA is not a specific product of lipid peroxidation, and the TBARS assay itself can generate MDA, causing overestimation of levels. MDA therefore cannot be considered an optimal representation of oxidative stress in vivo (16,17).

The current study focusses on two important measures of oxidative damage that have already been widely studied in somatic disease and are the subject of an increasing number of recent publications on depression: 8-hydroxy-2′-deoxyguanosine (8-OHdG) and F2-isoprostanes. The majority of the currently available literature on these markers in depression was not included, or not yet available for inclusion, in the previous meta-analyses on this subject. 8-OHdG and F2-isoprostanes reflect oxidative damage to DNA and lipids respectively. 8-OHdG is an oxidized derivative of deoxyguanosine and it is both the most abundant and most investigated DNA lesion. It has recognized mutagenic properties and has been linked to - among others - the development of cancer (18). F2-isoprostanes, oxidized derivatives of arachidonic acid, have come to be considered the preferred approach to assess oxidative stress in vivo and lipid peroxidation in particular (19).

Several studies have found elevated levels of F2-isoprostanes (20–22) and 8-OhdG (23,24) in patients with depression, but these findings have not been consistent (25,26). Earlier studies did not systematically explore to what extent the (conflicting) findings are due to e.g. the laboratory methods, biological specimens used for oxidative stress, or the extent to which studies took potential confounders such as health and lifestyle factors into account. The present study extends the current evidence-base by systematically meta-analysing the literature on two robust markers of oxidative stress, 8-OHdG and F2-isoprostanes, and their association with depression (major depressive disorder, bipolar disorder and elevated depressive symptoms). In addition, by conducting subgroup analyses based on type of depression, biological specimen, laboratory method used to measure oxidative stress, correction for confounders and the quality of studies, this study aims to identify factors that contribute to the inconsistent findings of individual studies.

**METHODS**

**Literature search and study selection**

Systematic searches of the literature were conducted in the databases PubMed, EMBASE and PsycINFO up to January 8th 2014 with search terms covering major depressive disorder, bipolar disorder and depressive symptoms combined with 8-OHdG and F2-isoprostanes respectively, taking into account a wide range of synonyms used for these markers. A full list of search terms is reported in Appendix A. No limitations in the search strategy were set. The search results were reviewed by two independent reviewers (CNB and MB) by screening title and abstract, followed by a full text review. Disagreements were settled by discussion. Studies were eligible for inclusion if they:

1. Contained a measurement of 8-OHdG or F2-isoprostanes in any body fluid or tissue in live human adult subjects;
2. Defined major depressive disorder (MDD) or bipolar disorder (BD) according to DSM-IV or ICD-10 criteria or assessed depressive symptoms using a validated instrument;
3. Reported (or were able to provide) sufficient information to calculate an effect size for the difference between levels of the oxidative stress markers in control subjects and subjects with depression.

An assessment of the references of the included studies as well as a search of their citations in the PubMed database was performed to identify any additional studies.

**Data extraction and risk of bias assessment**

Two authors (CNB and MB) independently extracted the study characteristics (including among others biological specimen, age and sex distribution) and results from each study using a predesigned collection form (see Appendix B). The results extracted included means, standard deviations of oxidative stress markers in patient and control groups (or alternative results sufficient to calculate an effect size) and an assessment of correction for potential confounders (age, sex, ethnicity, socio-economic status, smoking, alcohol use, body mass index (BMI), physical activity, somatic disease and antidepressant or mood stabilizer use). Authors of studies that did not contain sufficient information (means, standard deviations or standard errors and number of subjects) to calculate an effect size were contacted with a request for additional data. Authors of studies who only reported results on depressive symptoms as continuous variables were requested to perform additional analyses using a dichotomized classification of a depressed and control group based on the appropriate cut-off for the instrument used to assess depressive symptoms. Authors of studies whose original articles did not contain adjusted results were contacted with a request to perform additional analyses adjusting for as many of the following confounders if available: age, sex, socio-economic status or education, ethnicity, smoking, alcohol use, BMI, physical activity, presence of somatic disease and antidepressant or mood stabilizer use. If multiple adjusted analyses had been conducted, the results corrected for the largest number of potential confounders were included in the meta-analysis.

The quality of the studies was determined by assessing the risk of bias by two independent reviewers (CNB and MB) in three domains: selection bias, information bias and confounding. Disagreements were settled by discussion. Risk of bias assessment in observational studies requires consideration of the risks specific to the subject of study. For this purpose we adapted a tool by Hayden et al. (27) (see Appendix B). Low risk of bias was defined as a score of 4.5 or higher (on a 5 point scale).
**Meta-Analysis**

Analyses were performed with Comprehensive Meta-Analysis (CMA) software version 2.2.064. Effect sizes (Hedges’ g) were calculated and pooled using a random effects model, as considerable heterogeneity was expected. A p-value <0.05 was considered significant.

Possible publication bias was tested by inspecting the funnel plot, by the statistical significance of the Egger’s test of the intercept (28) and Duval and Tweedie’s trim and fill procedure (29).

To examine heterogeneity, the I²-statistic was calculated. Values of 25%, 50% and 75% indicate low, moderate and high heterogeneity, respectively (30). The 95% confidence intervals around I² (31) were calculated using the non-central chi-squared-based approach within the heterogi module for STATA version 11.0 for Mac.

When a minimum of three samples per subgroup was available, analyses were performed categorized by: type of depression (MDD, BD, depressive symptoms above the questionnaire cut-off level), laboratory method for measuring oxidative stress (chromatography [coupled to either mass-spectrometry or electrochemical detection] vs. immunoassay), biological specimen used (urine, blood [product] or other), low or high risk of bias score, and adjusted versus unadjusted results. Studies that accounted for at least age, sex and one life-style variable (either by matching, restriction, adjustment or testing for baseline group differences) were defined as corrected for confounding.

**RESULTS**

**Search results, study characteristics and risk of bias scoring**

**8-OHdG**

After removal of duplicates, 79 records were assessed based on title and abstract. The full-text of 39 of these records was retrieved for further assessment. Of these studies 6 did not report sufficient information in the original article to calculate an effect size and none was provided by authors on request (32–37). In 2 studies both 8-OHdG and depression were measured but the association between the two was not the focus of study and therefore not analysed (38,39), nor was this data provided on request. In total 10 studies met all criteria for inclusion; 4 studies on depressive symptoms (25,40–42), 2 studies on MDD (23,24), 3 studies on BD (43–45) and 1 study with both MDD and BD patients (46) (Figure 1). Two of the included studies (25,46) provided additional data on request.

The 10 studies (23–25,40–46) included a total of 579 subjects with depression (332 with depressive symptoms [scoring above the cut-off of the instrument used], 141 with MDD, 106 with BD) and 729 controls. All included studies were published between 2005 and 2014. The studies include samples from the general population (N=2), psychiatric in- and outpatient clinics (N=4), hospital oncology (N=2) and heart failure departments (N=1) and 1 unreported source (Table 1). Risk of bias was scored as low in 3 studies and high in 7 studies (Appendix C).

**F2-isoprostanes**

After removal of duplicates, 221 records were assessed based on title and abstract. The full-text of 30 of these records was retrieved for further assessment. Of these studies 1 did not report sufficient information in the original article to calculate an effect size and none was provided by authors on request (47). In one study both F2-isoprostanes and depression were measured but the association between the two was not the focus of study and therefore not analyzed nor provided on request (48). The samples of Rawdin 2013 (26) and Wolkowitz 2011 (49) overlap, leaving 8 original samples eligible for inclusion, 3 on depressive symptoms (Chung et al. 2009; Segal et al. 2012; Milaneschi et al. 2013) and 5 on MDD (Figure 1). Six of the included studies provided additional data upon request (21,22,49–52).

The 8 studies (20–22,49–53) include a total of 293 subjects with depression (144 subjects with depressive symptoms, 149 with MDD) and 2178 controls. All included studies were published between 2008 and 2013. The studies include samples of adults from the general population (N=2), elderly adults from the general population (N=3), psychiatric outpatients (N=1), systemic lupus erythematosus (SLE) patients (N=1) and fibromyalgia patients (N=1) (Table 2). Risk of bias was scored as low in 3 studies and high in 5 studies (Appendix C).
8-OHdG in depression

PubMed 41 records  
EMBASE 73 records  
PsycInfo 15 records

129 records in total  
79 records after duplicates removed

79 records screened on title and abstract

39 full-text articles assessed for eligibility

10 studies included in the meta-analysis

40 records included:  
21 no humans  
12 no depression  
3 no 8-OHdG  
3 review  
1 no adults

29 records excluded:  
2 no 8-OHdG  
8 no depression  
2 post-mortem  
3 duplicate data  
5 review  
8 no data on association depression and 8-OHdG

F2-isoprostanes in depression

PubMed 140  
EMBASE 145  
PsycInfo 6

291 records in total  
221 records after duplicates removed

221 records screened on title and abstract

30 full-text articles assessed for eligibility

8 studies included in the meta-analysis

191 records included:  
115 no humans  
65 no depression  
6 no F2-isops  
4 review  
1 post-mortem

22 records excluded:  
8 no F2-isoprostanes  
3 no depression  
1 duplicate data  
8 review/letter  
8 no data on association depression and F2-isoprostanes  
or insufficient data for effect size

Meta-analyses

8-OHdG

The overall effect size (Hedges’ g) including all 10 studies on 8-OHdG in the meta-analysis was 0.31 (95% CI 0.06, 0.56), I²=75% (95% CI 58- 86%) indicating 8-OHdG is significantly increased in depression (Figure 2a and Table 3). Egger’s test for publication bias was not significant (p=0.69). The effect size adjusted for publication bias by Duval and Tweedie’s trim and fill procedure (two trimmed studies) increased marginally to 0.37 (95% CI 0.13, 0.61) indicating no or little effects of publication bias (see Appendix D for funnel plot).

Subgroup analyses in the 8-OHdG studies showed significantly larger effect sizes for studies that were conducted in plasma or serum vs. those in urine (p<0.001) and studies that were performed with immuno-assays vs. those with chromatography (p=0.006). Studies with a low risk of bias showed a significantly lower effect size compared to studies with a higher risk of bias (p=0.02). Subgroup analyses by type of depression (depressive symptoms or BD vs. MDD) and results corrected and uncorrected for confounders revealed no significant differences (Table 3).

F2-isoprostanes

The overall effect size (Hedges’ g) including all 8 studies on F2-isoprostanes in the meta-analysis was 0.48 (95% CI 0.19, 0.77), I²=73% (95% CI 47-86%), indicating that F2-isoprostanes are significantly increased in depression (Figure 2b and table 3). Egger’s test was not significant (p=0.13) and the effect size adjusted for publication bias by Duval and Tweedie’s trim and fill procedure (no trimmed studies) was unchanged, indicating no significant effects of publication bias (see Appendix D for funnel plot).

A sensitivity analysis was performed excluding one study (53) as this study measured oxidative stress centrally (in cerebrospinal fluid) as opposed to peripheral measurements (blood and urine) used in all others. Exclusion of this study did not affect the overall results (Hedges’ g 0.44, p=0.004; Table 3). Subgroup analyses in F2-isoprostanes showed a trend level difference between studies assessing depressive symptoms vs. MDD with the MDD studies showing a larger effect size (p=0.08). Subgroup analyses by biological specimen used, laboratory method for oxidative stress measurement and risk of bias score revealed no significant differences (Table 3). No subgroup analyses were performed based on correction for confounders as only one study was defined as uncorrected.
<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Sample Description</th>
<th>Depressive Disorder</th>
<th>Controls N</th>
<th>Method</th>
<th>8-OHdG Analysis</th>
<th>Confounders</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forlenza 2006</td>
<td>CC</td>
<td>general population</td>
<td>MDD</td>
<td>62</td>
<td>DSM-IV (DISH)</td>
<td>Serum</td>
<td>ELISA</td>
<td>+ + + + +</td>
</tr>
<tr>
<td>Irie 2005</td>
<td>CC</td>
<td>psychiatric outpatients</td>
<td>MDD</td>
<td>30</td>
<td>DSM-IV</td>
<td>Label-</td>
<td>ELISA</td>
<td>- - - - -</td>
</tr>
<tr>
<td>Jorgensen 2013</td>
<td>CC</td>
<td>psychiatric department</td>
<td>MDD^1</td>
<td>27</td>
<td>DSM-IV (MINI)</td>
<td>Urine</td>
<td>ELISA/MSC</td>
<td>+/- +/- +</td>
</tr>
<tr>
<td>Ceylan 2012</td>
<td>CC</td>
<td>not reported</td>
<td>BD</td>
<td>14</td>
<td>DSM-IV</td>
<td>Serum</td>
<td>ELISA</td>
<td>- - - - -</td>
</tr>
<tr>
<td>Soeiro-de-Souza 2013</td>
<td>C</td>
<td>psychiatric outpatients</td>
<td>MDD</td>
<td>50</td>
<td>DSM-IV</td>
<td>Plasma</td>
<td>ELISA</td>
<td>+/- +/- +</td>
</tr>
<tr>
<td>Huzayyin 2014</td>
<td>CC</td>
<td>heart failure outpatients</td>
<td>BD</td>
<td>38</td>
<td>DSM-IV</td>
<td>Serum</td>
<td>ELISA</td>
<td>- - - - -</td>
</tr>
<tr>
<td>Kupper 2009</td>
<td>C</td>
<td>oncology department</td>
<td>BD</td>
<td>52</td>
<td>HAM-D ≥ 20</td>
<td>Serum</td>
<td>ELISA</td>
<td>+/- +/- +</td>
</tr>
<tr>
<td>Wei 2009a</td>
<td>C</td>
<td>oncology department</td>
<td>BD</td>
<td>63</td>
<td>HAM-D ≥ 20</td>
<td>Serum</td>
<td>ELISA</td>
<td>+/- +/- +</td>
</tr>
<tr>
<td>Yi 2012</td>
<td>C</td>
<td>municipal workers</td>
<td>DS</td>
<td>105</td>
<td>CES-D ≥ 16</td>
<td>Urine</td>
<td>HPLC/ED</td>
<td>+/- +/- +</td>
</tr>
<tr>
<td>Females</td>
<td>C</td>
<td>municipal workers</td>
<td>DS</td>
<td>74</td>
<td>CES-D ≥ 16</td>
<td>Urine</td>
<td>HPLC/ED</td>
<td>+/- +/- +</td>
</tr>
</tbody>
</table>

BD= bipolar disorder; BDI= Beck Depression Inventory; C= cohort; CC= case-control; CES-D= Center for Epidemiological Studies Depression Scale; DISH= Depression Interview and structured Hamilton Interview; DSM= Diagnostic and Statistical Manual of Mental Disorders; ELISA= enzyme-linked immunosorbent assay; GC/MS= gas chromatography/mass spectrometry; HAM-D= Hamilton Depression Rating Scale; HPLC/ED= high-performance liquid chromatography/electrochemical detector; ICD-10= International Statistical Classification of Diseases and Related Health Problems; MDD= major depressive disorder; MINI= Mini International Neuropsychiatric Interview; NOS= not otherwise specified; SADS-L= Schedule for Affective Disorders and Schizophrenia, lifetime version; SCID= structured clinical interview for DSM-IV; UPLC/MS= Ultra performance liquid chromatography/mass spectrometry.

* subsample of patients in Jorgensen et al. 2013 described in the article as M-DEP (moderately depressed) patients; ^ subsample of patients in Jorgensen et al. 2013 described in the article as SD-EF (severely depressed, unipolar only); ^ subsample of patients in Yi et al. 2013 described in the article as S-DEP (severely depressed, bipolar only); ^ Socio-demographics include age, sex, socio-economic status (income, education or other), ethnicity, lifestyle: smoking, alcohol, BMI, physical activity. Somatic disease: presence of (chronic) disease (cardiovascular, infectious, auto-immune or malignancy) that may influence oxidative stress levels. Antidepressant / mood stabilizers: current use.

+ confounders is accounted for; – confounder is not accounted for; +/- some, but not all of the confounders in the category have been accounted for. Studies are defined as corrected (+) if the have accounted for age, sex and at least one lifestyle variable.
Table 2. Study characteristics of the included studies on the association between F2-isoprostanes and major depressive disorder and depressive symptoms

<table>
<thead>
<tr>
<th>F2-isops</th>
<th>Study Design</th>
<th>Sample Description</th>
<th>Depressive disorder</th>
<th>Patients N</th>
<th>Controls N</th>
<th>Diagnostic method</th>
<th>Biological specimen</th>
<th>F2 analysis</th>
<th>Confounders</th>
<th>Effect size</th>
</tr>
</thead>
<tbody>
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<td>Chung 2013</td>
<td>CC</td>
<td>general population</td>
<td>MDD</td>
<td>18</td>
<td>36</td>
<td>DSM-IV (SCID)</td>
<td>Urine</td>
<td>GC/MS</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td>Dimopoulos 2008</td>
<td>CC</td>
<td>general population &gt;60 years</td>
<td>MDD</td>
<td>33</td>
<td>33</td>
<td>DSM-IV, psychiatrist</td>
<td>Plasma</td>
<td>ELISA</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td>Pomara 2012</td>
<td>CC</td>
<td>general population &gt;60 years</td>
<td>MDD</td>
<td>28</td>
<td>19</td>
<td>DSM-IV (DSH)</td>
<td>CFS</td>
<td>ELISA</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td>Yager 2010</td>
<td>CC</td>
<td>general population</td>
<td>MDD</td>
<td>57</td>
<td>74</td>
<td>DSM-IV (DSH)</td>
<td>Serum</td>
<td>ELISA</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Wolkowitches 2011a</td>
<td>CC</td>
<td>psychiatric outpatients</td>
<td>MDD</td>
<td>13</td>
<td>14</td>
<td>DSM-IV (DSH)</td>
<td>Plasma</td>
<td>GC/MS</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Chung 2009</td>
<td>CC</td>
<td>fibromyalgia patients</td>
<td>DS</td>
<td>28</td>
<td>20</td>
<td>CES-D ≥36</td>
<td>Urine</td>
<td>GC/MS</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Milaneschi 2013</td>
<td>CC</td>
<td>general population 30-79 years</td>
<td>MDD</td>
<td>31</td>
<td>996</td>
<td>GDS 2.5 and/or AD use</td>
<td>Urine</td>
<td>RIA</td>
<td>+</td>
<td>+</td>
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<tr>
<td></td>
<td>C</td>
<td>general population</td>
<td>DS</td>
<td>52</td>
<td>896</td>
<td>Urine</td>
<td>RIA</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<td>Segal 2012</td>
<td>CC</td>
<td>SLE patients</td>
<td>DS</td>
<td>33</td>
<td>90</td>
<td>CES-D ≥36</td>
<td>Plasma</td>
<td>GC/MS</td>
<td>+/-</td>
<td>+/-</td>
</tr>
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AD= antidepressant; C= cohort; CC= case-control; CES-D= Center for Epidemiological Studies Depression Scale; CFS= cerebrospinal fluid; DISH= Depression Interview and structured Hamilton Interview; DS= depressive symptoms; DSM-IV= Diagnostic and Statistical Manual of Mental Disorders IV; ELISA= enzyme-linked immunosorbent assay GC/MS= gas chromatography/mass spectrometry; GDS= geriatric depression scale; ICD-10= International Statistical Classification of Diseases and Related Health Problems; MDD= major depressive disorder; RIA= radio immuno assay; SCID= structured clinical interview for DSM-IV; SLE= systemic lupus erythematosus

*a* Socio-demographics include age, sex, socio-economic status (income, education or other), ethnicity, lifestyle: smoking, alcohol, BMI, physical activity. Somatic disease: presence of (chronic) disease (cardiovascular, infectious, auto-immune or malignancy) that may influence oxidative stress levels. Antidepressant /mood stabilizers: current use.

+ confounder is accounted for; +/- confounder is not accounted for; +/− some, but not all of the confounders in the category have been accounted for. Studies are defined as corrected (+) if the have accounted for age, sex and at least one lifestyle variable.
Figure 2a. Meta-analysis of 8-OHdG in depression (major depressive disorder [MDD], bipolar disorder [BD], and depressive symptoms [DS]) with effect sizes Hedges’ g and 95% confidence intervals for the comparison of 8-OHdG levels with controls.

F2-isops lower in depression 8-OHdG lower in depression

Figure 2b. Meta-analysis of F2-isoprostanes in depression (major depressive disorder [MDD] and depressive symptoms [DS]) with effect sizes Hedges’ g and 95% confidence intervals for the comparison of F2-isoprostane levels with controls.

Table 3. Results of meta-analyses on the association of 8-OHdG and F2-isoprostanes with depressive symptoms, major depressive disorder and bipolar disorder

<table>
<thead>
<tr>
<th>8-OHdG</th>
<th>N studies</th>
<th>Hedges’ g</th>
<th>95% CI</th>
<th>p</th>
<th>I²</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>All studies (depressive symptoms, MDD, BD)</td>
<td>10</td>
<td>0.31</td>
<td>0.06, 0.56</td>
<td>0.01</td>
<td>75</td>
<td>58-86%</td>
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</table>

Egger’s test for publication bias

Subgroup analyses

<table>
<thead>
<tr>
<th>Hedges’ g</th>
<th>95% CI</th>
<th>p</th>
<th>I²</th>
<th>95% CI</th>
</tr>
</thead>
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<td>All studies (depressive symptoms, MDD, BD)</td>
<td>10</td>
<td>0.69</td>
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</table>

Subgroup analyses

<table>
<thead>
<tr>
<th>N samples</th>
<th>Hedges’ g</th>
<th>95% CI</th>
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<table>
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<tr>
<th>F2-isoprostanes</th>
<th>N studies</th>
<th>Hedges’ g</th>
<th>95% CI</th>
<th>p</th>
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<td>All studies (depressive symptoms, MDD)</td>
<td>8</td>
<td>0.48</td>
<td>0.19, 0.77</td>
<td>0.001</td>
<td>73</td>
<td>47-86%</td>
</tr>
</tbody>
</table>

Egger’s test for publication bias

Subgroup analyses

<table>
<thead>
<tr>
<th>Hedges’ g</th>
<th>95% CI</th>
<th>p</th>
<th>I²</th>
<th>95% CI</th>
</tr>
</thead>
</table>

* subsample of patients in Jorgensen et al. 2013 described as M-DEP (moderately depressed);

Bipolar disorder (BD) and depressive symptoms (DS)

Depression

Laboratory method

Correction for confounders

Risk of bias score

“low” risk of bias

“high” risk of bias

BD= bipolar disorder; DS= depressive symptoms; MD= major depressive disorder; NA = not applicable; no or insufficient studies

Risk of bias score

Correction for confounders

Laboratory method

Biological specimen

Table 3. Results of meta-analyses on the association of 8-OHdG and F2-isoprostanes with depressive symptoms, major depressive disorder and bipolar disorder

8-OHdG

Egger’s test for publication bias

Subgroup analyses

<table>
<thead>
<tr>
<th>N samples</th>
<th>Hedges’ g</th>
<th>95% CI</th>
<th>p</th>
<th>I²</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>All studies (depressive symptoms, MDD, BD)</td>
<td>10</td>
<td>0.31</td>
<td>0.06, 0.56</td>
<td>0.01</td>
<td>75</td>
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</tbody>
</table>

Egger’s test for publication bias

Subgroup analyses

<table>
<thead>
<tr>
<th>N samples</th>
<th>Hedges’ g</th>
<th>95% CI</th>
<th>p</th>
<th>I²</th>
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</thead>
</table>

<table>
<thead>
<tr>
<th>F2-isoprostanes</th>
<th>N studies</th>
<th>Hedges’ g</th>
<th>95% CI</th>
<th>p</th>
<th>I²</th>
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Egger’s test for publication bias

Subgroup analyses

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<th>95% CI</th>
</tr>
</thead>
</table>

* see Appendix D for funnel plots.

** Jorgensen et al. 2013 is counted in two categories because it describes stratified analyses of both MDD and BD patients.

*** Results of sensitivity analyses, excluding Pomara et al. 2012 with specimens from CSF (cerebrospinal fluid): All studies: Hedges’ g 0.44, p 0.004; Egger’s test: p 0.22. MDD: Hedges’ g 0.66, p between subgroups 0.17. Immunoassay: Hedges’ g 0.44, p between subgroups 0.75. BD= bipolar disorder; DS= depressive symptoms; MD= major depressive disorder; NA = not applicable; no or insufficient studies to perform the subgroup analysis.
**DISCUSSION**

This meta-analysis found that both oxidative stress markers, 8-OHdG and F2-isoprostanes, are increased in subjects with depression (major depressive disorder, bipolar disorder and depressive symptoms) compared to controls, with effect sizes in the small to moderate range (54). In addition, subgroup analyses of the 8-OHdG studies revealed that some of the variation in the results may be explained by the type of the biological specimen and/or the laboratory method for oxidative stress measurement, and that studies with lower risk of bias reported significantly smaller effect sizes. Findings for F2-isoprostanes however, did not differ when analysed by type of depression, biological specimen, laboratory method or quality.

The results indicate that depression is associated with increased oxidative damage to DNA and lipids (reflected by 8-OHdG and F2-isoprostanes respectively). These findings are in line with previous meta-analytic studies that reported decreased anti-oxidants and anti-oxidant enzymes in unipolar depression, and increased oxidative stress in both uni- and bipolar depression (14,15). Previously DNA damage in bipolar disorder has been reported measured by increased levels of DNA strand breakage with the comet assay (55). Oxidative damage to proteins determined by the protein carbonyl assay has not yet been as widely studied, but increased levels have been reported in MDD and BD (56,57), however not consistently (58,59). The oxidative stress markers used in this meta-analysis are among the most robust makers of oxidative stress currently available and have recognized roles in the pathophysiology various somatic diseases such as cardiovascular disease, cancer and diabetes (16,18). 8-OHdG is a biologically important mutagenic DNA lesion, while F2-isoprostanes are known to be increased in atherosclerotic lesions and may also be biologically active in the pathogenesis of atherosclerosis (60). It should be noted that although both markers reflect oxidative damage they are not necessarily associated with each other. One study included in our meta-analysis (21) found no correlation between the two, suggesting that 8-OHdG and F2-isoprostanes reflect specific aspects of oxidative imbalance.

Although the main findings of this study are unlikely to be greatly influenced by publication bias, considerable heterogeneity between studies was found for both 8-OHdG and F2-isoprostanes. This heterogeneity may be explained by several factors. In the studies that examined 8-OHdG, patients in three bipolar disorder studies (43–45) were not all currently depressed, but some were in a manic or euthymic state. This may account for the fact that two of these studies’ results have the highest and lowest effect sizes included in the meta-analysis. In addition, the subgroup analyses in 8-OHdG studies demonstrated significant differences between studies based on risk of bias, with the studies with lower risk of bias finding smaller effects. There was also a significant difference in effect size in the subgroup analyses by biological specimen (higher in plasma/serum than in urine) and by laboratory method used to measure oxidative stress (higher in immuno-assays than chromatography). With only one exception however, all the measurements using chromatography were done in urine samples, whereas all the immuno-assays for the measurement of 8-OHdG in were done in plasma or serum. Therefore, it cannot be determined whether this difference is based on the biological specimen or laboratory technique. 8-OHdG levels determined by immuno-assays are higher than those measured by chromatography, with the latter considered the gold standard. The correlation between the two methods is generally high (61) and so may not necessarily affect the strength of the association with depression. It has been reported that urinary 8-OHdG levels are more stable than those in plasma or serum, and therefore possibly more reliable (62). Although the subgroup analyses may help to explain the heterogeneity observed, the findings should be interpreted with considerable caution as the number of studies in the subgroups is small.

This meta-analysis confirms oxidative stress markers are increased in subjects with depression in cross-sectional studies, but the underlying mechanisms explaining this link need to be examined further. Many behavioural factors that are related to increased exposure to ROS (smoking, alcohol use, overweight, physical activity)(12,16,63) are also associated with depression (5,64–66). It cannot be ruled out that the association is partially driven by these or other lifestyle confounders. The majority of the included studies took some, but few took all of these potential confounders into account. The results of studies that did correct for confounders did not differ significantly from studies that did not, strengthening the observation that an association between depression and oxidative stress is present independent of these life-style factors. The observed increased levels of oxidative stress in depression might be understood within the concepts of allostasis and allostatic load. The former refers to the physiological adaption to physical, psychological, social and environmental stressors. The latter refers to the physical “wear and tear” induced by prolonged exposure to the stress response (67). Depression has been found to be associated with increased “wear and tear” or accelerated cellular ageing reflected by decreased telomere length in depressed patients (68). Dysregulations in the major stress systems (hypothalamic-pituitary-adrenal axis activity, autonomic nervous system function and inflammatory functions) have been demonstrated in depression (69) and these could be contributing to increased oxidative stress. Oxidative stress is closely related to the inflammatory pathway in particular. Pro-inflammatory cytokines are produced in reaction to oxidative stress and oxidative stress in turn amplifies the inflammatory response. High cortisol levels have been associated with increased levels of oxidative damage (70) (Joergensen et al. 2011). The damage caused by this allostatic load experienced during mood episodes is hypothesized to render an individual more vulnerable to developing a following episode and at higher risk to develop somatic disease (11,71). The brain is particularly vulnerable to oxidative damage due to its high oxygen consumption and low anti-oxidants defences. There is evidence from post-mortem studies suggesting that in depression oxidative stress is increased (72–74) and anti-oxidants are decreased (75) in the brain.
This meta-analysis' strength lies in the focus on robust oxidative stress markers and in the comprehensive search that was conducted to identify all studies on the association of 8-OHdG and F2-isoprostanes with depression. Through additional data requests the number of studies eligible for inclusion was increased, and where possible adjusted data was obtained mitigating the effects of possible publication bias and increasing the reliability of the overall results.

There are also a number of important limitations. As is apparent from the number of included studies and subjects the evidence on 8-OHdG and F2-isoprostanes in depression is limited. The studies that were excluded because they only reported on depressive symptoms as a continuous measure (32,34,36) all found a positive association. Therefore it is most likely that they would not have altered the overall result. Furthermore, the covariates used in the adjusted analyses varied widely. Therefore it was not possible to assess the effects of adjustment on the results satisfactorily. Information on diet and anti-oxidant supplement use was not available for most studies and therefore it cannot be ruled out this factor may have had an effect. In addition, the use of antidepressants or mood stabilizers could also be sources of confounding that were often not investigated. There is some evidence to suggest that antidepressants, lithium and other mood stabilizers protect against oxidative stress (76–78). Studies addressing their effects on F2-isoprostanes however found an increase (22) or no effect on oxidative stress levels (26).

In conclusion, the finding that both 8-OHdG and F2-isoprostanes are increased in depression strongly suggests that depression is accompanied by increased oxidative damage. This finding supports the hypothesis that increased metabolic stress is present in depression, which could potentially contribute to the higher risk of somatic morbidity and mortality in sufferers. There is a need for future larger scale studies on oxidative stress in depression, in which the role of treatment effects should be addressed.

Appendices A, B, C, D. Supplementary data
Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.psyneuen.2014.09.025.

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Conflict of interest
All authors confirm they have no financial or other conflicts of interest to disclose.

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