CHAPTER 8

SUMMARY and GENERAL DISCUSSION
SUMMARY OF THE MAIN FINDINGS

Chapter 2 examines the determinants of the oxidative damage markers 8-OHdG and F2-isoprostanes in plasma in a subsample of adults without current psychopathology from the Netherlands Study of Depression and Anxiety (N=1117). 8-OHdG and F2-isoprostanes were weakly correlated (r=0.06, p=0.045). Both markers were positively associated with age (8-OHdG β=0.16, p<0.001; F2-isoprostanes β=0.07, p=0.043), and with cotinine levels, reflecting cigarette smoke exposure (8-OHdG β=0.09, p=0.034; F2-isoprostanes β=0.09, p=0.027). 8-OHdG was lower in females (β=-0.16, p<0.001). F2-isoprostanes were higher in females (β=0.15, p<0.001), heavy alcohol users (β=0.13, p<0.001), and in samples collected in spring (β=0.07, p=0.043), and lower in supplement users (β=-0.07, p=0.020), and those with more years of education (β=-0.10, p=0.001). Levels of both markers were lower in subjects who had adhered to fasting instructions (8-OHdG β=-0.06, p=0.034; F2-isoprostanes β=-0.07, p=0.014). F2-isoprostanes, but not 8-OHdG, were significantly positively associated with metabolic syndrome (β=0.08, p=0.013) and its individual components. Studies examining these oxidative damage markers in psychiatric and somatic disorders should incorporate these determinants in the study design.

Chapter 3 comprises a systematic review and meta-analysis of the literature on the association between oxidative DNA (8-OHdG) and lipid damage (F2-isoprostanes) and depressive disorders. For 8-OHdG 10 studies with 1,308 subjects were included, and for F2-isoprostanes 8 studies with 2,471 subjects. Both markers were higher in depression, with small to moderate effect sizes (8-OHdG Hedges’ g 0.31, p=0.01 and F2-isoprostanes Hedges’ g 0.48, p=0.001). There was high heterogeneity for both markers (I²=75% for 8-OHdG and I²=73% for F2-isoprostanes), reflecting a great level of inconsistency between the individual studies. Funnel plots and Egger’s tests (8-OHdG p=0.013 and F2-isoprostanes p=0.13) did not reveal evidence of publication bias.

Subgroup analyses within the F2-isoprostanate studies showed no differences between type of depression measurement (MDD diagnosis or depressive symptoms), biological specimens (urine, plasma), and laboratory methods or study quality. Subgroup analyses within the 8-OHdG studies showed stronger associations in plasma/serum vs urine samples (p<0.01) and in measurements performed with immuno-assays vs. chromatography-mass spectrometry (p<0.01), and weaker associations in high quality studies vs. low quality studies (p=0.02).

Overall this meta-analysis found both higher oxidative DNA and lipid damage in depressive disorders, however with a high level of inconsistency between the individual studies’ findings.

Chapter 4 compared plasma levels of F2-isoprostanes and 8-OHdG in 1619 subjects with current, 610 subjects with remitted MDD and/or anxiety disorders (of which 704 antidepressant users) with controls (N=612) in the Netherlands Study of Depression and Anxiety. Plasma F2-isoprostane levels did not differ between subjects with current or remitted MDD and/or anxiety disorders and controls, or by antidepressant use (p>0.50). Unexpectedly, plasma 8-OHdG levels were lower in subjects with current MDD and/or anxiety disorders (adjusted mean 8-OHdG 42.1 pmol/l, 95% CI 40.4-43.8) compared to controls (adjusted mean 8-OHdG 45.0 pmol/l 95% CI 42.9-47.2; p<0.001), however this association was no longer present after further adjustment for antidepressant use (p=0.562). When dividing the sample into controls, subjects with remitted disorders without antidepressants, subjects with current disorders without antidepressants, SSRI users, TCA users and other antidepressant users (with either remitted or current disorders), antidepressant users of all types had lower 8-OHdG levels than controls (and non-antidepressant users with disorders). Effect size Cohen’s d for the comparison between antidepressant users and controls was 0.21 p<0.001.

Within subjects with current MDD and/or anxiety disorder at baseline (N=992 with F2-isoprostane data and N=1130 with 8-OHdG data), neither 8-OHdG nor F2-isoprostanes predicted remission of MDD and/or anxiety disorders at two-year follow-up or chronicity of symptoms during two-year follow-up.

Contrary to the meta-analysis’ findings in Chapter 3 this study found no evidence of increased oxidative lipid damage in MDD and/or anxiety disorders. Antidepressant use was associated with lower oxidative DNA damage. Although this is an observational study, the findings suggest that the presence of psychopathology itself is not associated with oxidative damage levels, but that the use of antidepressant medication could lower oxidative DNA damage.

Chapter 5 compared levels of plasma antioxidant uric acid in 1648 subjects with current and 609 subjects with remitted MDD and/or anxiety disorders with those of controls in the Netherlands Study of Depression and Anxiety. Plasma uric acid was lower in current (adjusted mean uric acid 289 μmol/l), but not remitted MDD and anxiety disorders (adjusted mean uric acid 298 μmol/l) compared to controls (adjusted mean uric acid 299 μmol/l; Cohen’s d 0.10, p<0.001). There was a dose-response association with depressive (β =-0.05, p=0.012), anxiety (β =-0.04, p=0.009) and phobic symptom severity (β =-0.03, p=0.036), as well as with symptom duration (β =-0.04, p=0.009). This finding was independent of health and lifestyle factors, including metabolic syndrome and antidepressant use.

Plasma uric acid levels were lower in current MDD and/or anxiety disorders, following a dose-response gradient, suggesting the involvement of decreased antioxidant status in these disorders.
Analyses investigating the cumulative impact of the stress systems demonstrated that the number of systems with ≥1 marker in the high risk quartile showed a positive linear trend with both 8-OHdG (p=0.030) and F2-isoprostane levels (p =0.009).

Overall, markers of inflammation, the HPA-axis and ANS were found to be associated with oxidative DNA and/or lipid damage. Increased physiological stress was associated with increased oxidative damage in a dose-response fashion.

Chapter 6 comprises a study in the population sample of the Coronary Artery Risk Development in Young Adults Study (CARDIA) in which the association between plasma F2-isoprostanes, carotenoids and depressive symptoms (measured with the depressive symptom questionnaire CES-D) was studied. F2-isoprostanes were higher in subjects with depressive symptoms (CES-D ≥16, N=460) compared to subjects without (CES-D ≥16, N=2508; 55.7 vs. 52.0 pg/ml; Cohen's d 0.14, p=0.002) after adjustment for sociodemographics. The difference in F2-isoprostane levels after further adjustment for all health and lifestyle covariates was not significant (p=0.113). Further analyses revealed that the association was dependent on the lifestyle factors BMI, diet and smoking. Plasma carotenoids (zeaxanthin/lutein, β-cryptoxanthin, lycopene, α-carotene, β-carotene) were lower in subjects with depressive symptoms (CES-D ≥16, N=435) compared to controls (CES-D <16, N=2390), even after adjustment for all health and lifestyle factors (standardized sum of 5 carotenoids 238.7 vs. 244.0; Cohen’s d -0.16, p<0.001).

Depressive symptoms predicted higher F2-isoprostanes (β=0.04, p=0.048) and lower carotenoids (β=-0.05, p=0.002) at five year follow-up, in analyses adjusted for previous F2-isoprostane or carotenoid levels. The reverse was not the case; neither levels of F2-isoprostanes (p=0.095) nor carotenoids (p=0.560) were associated with depressive symptoms at five-year follow-up.

Depressive symptoms were cross-sectionally and longitudinally associated with higher F2-isoprostanes and lower carotenoids. Lifestyle factors are important determinants in this association, in particular for the F2-isoprostanes.

Chapter 7 examined the associations of the three major physiological stress systems, the inflammatory system, the hypothalamic-pituitary-adrenal axis (HPA-axis) and the autonomic nervous system (ANS) with oxidative damage markers 8-OHdG and F2-isoprostanes in the Netherlands Study of Depression and Anxiety (N=2858). All the associations were independent of antidepressant use and there were no interactions with the presence of psychopathology.

8-OHdG was positively associated with all inflammation markers (CRP β=0.047, p=0.008; IL-6 β=0.048, p=0.008, TNF-α β=0.050, p=0.005). 8-OHdG was associated with evening cortisol (β=0.073, p<0.001), but not significantly with the other measure of cortisol (AUCg β=0.045, p=0.053; AUCi β=0.033, p=0.051). 8-OHdG was not associated with the ANS measures of heart rate (p=0.503) and pre-ejection period (p=0.131), but was associated with respiratory sinus arrhythmia (RSA), however in the opposite direction than was hypothesized (β=0.076, p=0.001). F2-isoprostanes were associated with higher CRP, but not with IL-6 or TNF-α, or any of the measures of cortisol. There were significant associations between F2-isoprostanes and high ANS stress (heart rate β=0.064, p<0.001; RSA β=0.076, p=0.001).
Interestingly, 8-OHdG and F2-isoprostanes were only weakly positively correlated, indicating they reflect distinct aspects of oxidative damage and are not necessarily increased through the same pathways. This distinction might also be reflected in the most marked difference between the two markers; the clear association between F2-isoprostanes and metabolic syndrome, which was absent for 8-OHdG.

The weak correlation between the markers and some differences in the determinants suggest that covariates in oxidative stress studies need to be ascertained for each marker separately, as they may only partially overlap. The findings in this large sample taken as whole however confirm many of the factors known to impact on oxidative stress. This provides further validation of 8-OHdG and F2-isoprostanes as markers of oxidative stress and stresses the importance of including the identified determinants in research on oxidative stress in all fields of medicine.

Aim 2a. Is oxidative stress cross-sectionally associated with depression and anxiety disorders?

Oxidative damage markers

Based on the literature available at the outset of this thesis that was systematically reviewed and meta-analyzed in Chapter 3, both 8-OHdG and F2-isoprostanes are higher in depressive disorders, with small to moderate effect sizes. The meta-analysis was based on a relatively small number of studies with a limited number of subjects with depressive disorders or depressive symptoms (8-OHdG N=579 and F2-isoprostanes N=293), of which a minority had well-established diagnoses of MDD (8-OHdG N=141 and F2-isoprostanes N=149). Furthermore, analyses for both markers revealed high levels of heterogeneity, reflecting wide variation in the findings between studies.

Publication bias was ruled out as the main factor driving these differences. Subgroup analyses using the following factors, definition of depression (diagnosis of MDD vs. depressive symptom scale), source of sample (urine, plasma etc.) or laboratory methodology did not identify any of these as explanatory factors for the heterogeneity in the F2-isoprotane studies. It should be considered that the number of studies within which these subgroup analyses were conducted was small, and may have been underpowered to find these differences. For 8-OHdG, gold-standard laboratory techniques, urine (vs. plasma) and high study quality were identified as factors associated with smaller effects, and MDD diagnosis (vs. depressive symptoms) showed a larger effect.

Adjustment, or lack therefore, for health and lifestyle factors may have been the main reason for the inconsistent findings. Of the ten studies included for 8-OHdG only two included all relevant lifestyle factors, and only three out of eight studies on F2-isoprostanes did so. Importantly, the majority of 8-OHdG studies, and half of the F2-isoprostanes studies, did not report on or account for antidepressant use. This may have been a factor of particular importance as antidepressants at the time of the publication of this meta-analysis had already been demonstrated to affect levels oxidative markers.

The studies in Chapters 4 and 6 of this thesis were designed to overcome many of the limitations identified in the meta-analysis: both cohorts have large sample sizes and data on the majority of major health and lifestyle factors as well as on the use of antidepressant medication.

Chapter 4 on the NESDA study looks into 8-OHdG and F2-isoprostanes in 2229 subjects with MDD and anxiety disorders and reveals somewhat surprising findings. F2-isoprostanes were not associated with MDD or anxiety disorders as hypothesized and expected based on the results of the meta-analysis. This negative finding is not driven by confounding by health and lifestyle factors included in this study, or antidepressant use, as the association between F2-isoprostanes and psychopathology was neither
present in models with, nor in those without adjustment for these factors. Also contrary to the hypothesis, and contrary to the findings of the meta-analysis, the NESDA sample demonstrated lower 8-OHdG in current MDD and anxiety disorders, compared to remitted patients and to controls. However after further adjustment for antidepressant use there was no association between 8-OHdG and MDD and/or anxiety disorders. Further division of the sample by antidepressant use revealed antidepressant users of all types had lower levels of 8-OHdG compared to both controls, and non-antidepressant users. This unexpected finding raises a number of questions.

The association between lower 8-OHdG and antidepressant use is evident in this data and suggests antidepressants have antioxidant properties. From these cross-sectional findings it can however not be inferred that antidepressants are the causal factor. The possibility of confounding by indication should be kept in mind: antidepressant users may differ from other subjects in unknown and unmeasured ways that are related to lower 8-OHdG levels. The interpretation of lower plasma 8-OHdG levels, lower than those of healthy controls, is not clear-cut. Lower levels of 8-OHdG, reflecting lower DNA damage, might be considered an indicator of good health. However, lower levels of 8-OHdG in plasma could also reflect a lower rate of clearance from the cellular environment, meaning oxidized guanosine bases are not repaired, with possible cellular dysfunctions as a consequence (5).

There is evidence to suggest that antidepressants have antioxidant properties which might explain the lower levels of DNA damage in antidepressant users (6). Antidepressant treatment has been shown to lower oxidative stress makers and raise antioxidants (7). The fact that the finding is the same across all classes of antidepressants suggests a mechanism common to all types despite their pharmacological differences.

The evidence for antidepressant effects is limited to a small number of studies, and the effects of antidepressants on 8-OHdG specifically have not yet been studied in an intervention study. The effects of antidepressants on inflammatory markers have been more widely studied. SSRIs in particular have been shown to lower anti-inflammatory markers (8,9), which knowing how closely oxidative stress and inflammation are intertwined, would suggest they could do the same for measures of oxidative stress. The association found between antidepressant use and 8-OHdG is unexpected and requires replication both cross-sectionally as well as in intervention studies to provide more evidence for a causal relationship. This finding confirms the importance of accounting for antidepressant use when studying the association between oxidative stress and affective disorders, and implies that much of inconsistencies in previous literature may be due to inconsistent adjustment for antidepressant use.

Chapter 6 covers the association between depressive symptoms and F2-isoprostanes and carotenoids in the general population sample of the CARDIA study. This study found F2-isoprostanes were associated with depressive symptoms. This association however did not remain present after adjustment for health and lifestyle factors. Smoking, diet and BMI were found to be explanatory factors in the association between depressive symptoms and F2-isoprostanes.

This in itself is an important finding as it points to oxidative stress as a pathophysiological mechanism through which depressive symptoms and the poor lifestyle habits that can accompany them might lead to poor somatic health. It also highlights the importance of addressing these lifestyle factors in subjects with depressive symptoms.

The meta-analysis’ findings, and the new findings from Chapter 4 and 6, are summarized in Table 1. This table also includes an updated result of the meta-analysis after including findings on 8-OHdG and F2-isoprostanes from the NESDA study (including subjects with current MDD, excluding those with only an anxiety disorder diagnosis, or remitted diagnoses) and on F2-isoprostanes from the CARDIA study. In line with methodology of the original meta-analysis, results adjusted for health and lifestyle factors, and antidepressant, were used and analyzed in a random effects model. 8-OHdG remains higher in subjects with depressive disorders compared to controls, although the effect size is slightly reduced (Hedges’ g 0.28 p<0.013). The same is true of F2-isoprostanes (Hedges’ g 0.34 p<0.001).

Including only studies with MDD diagnoses (excluding those on depressive symptoms) 8-OHdG levels did not differ significantly from controls (p=0.202). For F2-isoprostanes the effect sizes found in the subgroup analysis on studies with MDD diagnosis is considerably higher (F2-isoprostanes Hedges’ g 0.55 p<0.021) than that of the subgroup with depressive symptoms (F2-isoprostanes Hedges’ g 0.16 p=0.096).

The findings from the NESDA and CARDIA study demonstrate the importance of antidepressant use and lifestyle factors and it is therefore conceivable that some of the studies included in the meta-analysis may in fact be false positive (or negative) findings, confounded by health and lifestyle factors or antidepressant use. The strength of the evidence is therefore not only limited by the number of studies but also by heterogeneity that is likely in part due to this confounding. These inconsistencies in the findings warrant further research, with particular attention to antidepressant use.
Antioxidant markers

Plasma uric acid was lower in current MDD and/or anxiety disorders compared to controls in the NESDA sample (Chapter 4); findings in line with the hypothesis that depressive and anxiety disorders are associated with a lower antioxidant status. There was a dose-response relationship with both symptom severity and symptom duration, independent of antidepressant use, lifestyle factors and metabolic syndrome.

Uric acid is of particular interest in psychiatric disorders as it has neuroprotective effects owing to its antioxidant properties (10). Peripheral levels of uric acid have been demonstrated to be associated with the incidence and course of neurological disorders including Parkinson’s disease (11) and multiple sclerosis (12). Also, as the end product of purine metabolism, uric acid could be considered a marker of the purinergic system which has been implicated in the pathophysiology of mood disorders (13). In addition in bipolar disorder, uric acid has been found to be increased in manic phases, and treatment with uric acid lowering agent allopurinol, has been demonstrated to be an effective add-on treatment (14). A further advantage of uric acid, as opposed to markers of oxidative damage such as 8-OHdG and F2-isoprostanes is that it is determined in daily routine analysis, with low cost techniques available in all laboratories. One potentially important limitation of this study was lack of information on diet. A purine low diet, as might occur more often in depression, could influence (the strength of) this association.

In Chapter 6 a second important antioxidant marker, the carotenoids, were also demonstrated to be lower in depressive symptoms in the CARDIA study. The association between carotenoids and depressive symptoms remained present after adjustment for health- and lifestyle factors, including diet. However, diet is notoriously hard to measure reliably especially in large scale studies. It is possible that the dietary score used was not sensitive enough to pick up differences in carotenoid intake that could affect (the strength of) this association. Antioxidant levels might be lower as they become depleted in the defence against increased oxidative stress in depression. There is also evidence to suggest there are underlying genetic factors that make individuals susceptible to both low carotenoid levels and depression (15).

Limited evidence on anxiety disorders

Chapters 3 and 4 based on data from the NESDA study included subjects with anxiety disorders. Overall the findings for anxiety disorders were in the same direction as those for MDD; therefore the main results were reported for MDD and anxiety disorders combined in these chapters. The high comorbidity rates with MDD, and the fact our findings on F2-isoprostanes, 8-OHdG and uric acid were all in line with those for MDD, suggest that role of oxidative stress in anxiety is similar to that in depressive disorders. There are however very few studies covering oxidative stress in anxiety disorders specifically. The data from the NESDA study also does not cover obsessive compulsive disorder or post-traumatic stress disorder.

Overall, there was no association with F2-isoprostanes and anxiety disorders, 8-OHdG was lower in anxiety disorders compared to controls but attributable to antidepressant use. Uric acid was lower in current anxiety disorders, independent of health and lifestyle factors.

Effect sizes for oxidative stress in depression and anxiety disorders

The effect sizes for each of the sociodemographic, lifestyle and other health determinants studied in chapter two are significant but small (ranging from $\beta=0.06$ to $\beta=0.16$), as were those on the association with the physiological stress systems (ranging from $\beta=0.044$ to $\beta=0.076$). All the effect sizes for the association between oxidative stress markers and depression/anxiety in the studies in this thesis were, although statistically significant, small in size (meta-analysis Hedges’ $g$ 0.31-0.48; NESDA, 8-OHdG Cohen’s d 0.21, uric acid Cohen’s d 0.10; CARDIA, carotenoids Cohen’s d -0.016). This raises the questions on the (clinical) relevance of these findings. It illustrates the fact that oxidative stress levels are determined by multiple factors, and depressive and/or anxiety disorders are not the main ones.

These effect sizes are in line with effect sizes in previous studies on oxidative stress (16) in depression. They are also in the same range as effect sizes for other biological measures in depressive disorders, including inflammatory markers (17), HPA-axis markers (18) and measures of cellular ageing such as telomere length (19).

How these effect sizes are related to the clinical relevance, or lack thereof, of these findings is discussed in the paragraph “Clinical implications” in this chapter.

Markers of oxidative stress beyond the scope of this thesis

There are a great number of markers of oxidative stress and antioxidants not covered or discussed in detail in this thesis. Findings on many of these markers in depression have been summarized in recent meta-analyses (7,16,20). Their conclusions are similar to those for this thesis; there are a limited numbers of studies available for most oxidative stress markers in depression, most with relatively small sample sizes, findings are suggestive of higher oxidative stress and lower antioxidants, with small to moderate effect sizes and there is considerable heterogeneity.
The studies in chapters 4, 5 and 6 in the NESDA and CARDIA samples are however to our knowledge, the largest on any marker of oxidative stress and depressive disorders conducted to date. The sample sizes of the NESDA and CARDIA study are greater than all previous studies on the markers they cover combined, and include data on most major potential confounding health and lifestyle factors.

The number of intervention studies addressing the effects of antidepressants on measurements of oxidative stress is still small (11 studies) but suggests that antidepressants affect levels of oxidative stress markers (7). Antidepressants have been shown to raise non-enzymatic antioxidants, but not enzymatic antioxidants, and lower the oxidative damage marker MDA.

### Conclusion 2a

**The cross-sectional association between oxidative stress and depression and anxiety disorders**

Based on all available literature and meta-analysis, markers of oxidative damage 8-OHdG and F2-isoprostanes are higher in depressive disorders. Heterogeneity between studies is however very high, likely in part due to differences in adjustment for important health and lifestyle factors and antidepressant use. The largest studies including most potential health, lifestyle confounders, in particular antidepressants, demonstrated that markers of oxidative damage are either not associated with depression and anxiety (F2-isoprostanes in NESDA, Chapter 4), higher in depressive symptoms as expected but attributable to lifestyle factors (F2-isoprostanes in CARDIA, Chapter 6), or lower but attributable to antidepressant use (8-OHdG in NESDA, Chapter 4).

The antioxidant uric acid is lower in MDD and/or anxiety disorders, and carotenoids are lower in depressive symptoms, independent of antidepressant use and lifestyle factors.

### Aim 2b. Is oxidative stress longitudinally associated with depression and anxiety disorders?

The longitudinal associations between oxidative stress and depression and anxiety disorders are of interest, as they may provide some insight into the direction of the association. If oxidative stress precedes symptoms of depression and anxiety that would provide a stronger basis to implicate oxidative stress as causative mechanism in the development of these disorders. Alternatively if oxidative stress follows the emergence of symptoms, it might more likely be a consequence of the disorders, for example through the lifestyle behaviors that were identified in chapters 2, 4, and 6, as discussed above, which are known to be associated with depression.

To gain insight into the direction of the association between oxidative stress and depressive and anxiety disorders longitudinal analyses were conducted in both the NESDA (Chapter 3) and CARDIA (Chapter 6) samples.

Within subjects with a current disorder at baseline in the NESDA study neither 8-OHdG nor F2-isoprostanes predicted symptom chronicity during a 2 year follow-up period, or remission rates at 2 year follow-up (Chapter 4). In the CARDIA study (Chapter 6) neither marker (F2-isoprostanes or carotenoids) predicted depressive symptoms at follow-up, before or after correction for baseline depressive symptoms. The CARDIA study design also allowed examination of the question whether depressive symptoms are associated with future oxidative stress levels. Depressive symptoms were associated with higher F2-isoprostanes and lower carotenoids at 5 year follow-up, even with the conservative estimation method in which analyses were corrected for previous F2-isoprostane and carotenoids levels. From these findings we might tentatively conclude that depressive symptoms precede oxidative stress. Interpreting these findings with those from the cross-sectional analyses in which lifestyle factors were shown to be important confounders, we might infer that depressive symptoms, lead to poor lifestyle behaviors that in turn promote oxidative stress. However a previous study addressing the association between carotenoids and depressive symptoms longitudinally in a geriatric cohort found higher carotenoid levels predicted a reduced risk of depressive symptoms at six year follow-up (21).

As there are so few studies addressing the association between oxidative stress, antioxidants and depression longitudinally more research is needed to draw a substantiated conclusion on the direction of the association. Based on what is known on the pathophysiology it is likely that the association will be bi-directional.
Conclusion 2b

The longitudinal association between oxidative stress and depression and anxiety disorders

Very few studies address the longitudinal association between oxidative stress and depression and anxiety disorders. F2-isoprostanes, 8-OHdG and carotenoids did not predict subsequent depression or anxiety (Chapters 4&6). However, some evidence was found that depressive symptoms predict higher F2-isoprostanes and lower carotenoid levels at 5 year follow-up (Chapter 6).

Aim 3. Is oxidative stress cross-sectionally associated with physiological stress?

The study in Chapter 7 on 8-OHdG and F2-isoprostanes and its association with the three major physiological stress systems, the inflammatory system, the HPA-axis and the autonomic nervous system was to our knowledge the first of its kind. Oxidative stress is increasingly being studied in somatic and psychiatric disorders to unravel their pathophysiology, identify predictors of course and new treatments. To achieve these goals it is necessary to understand how these systems are related.

The study showed associations between 8-OHdG and the inflammatory, HPA-axis and autonomic nervous system. The F2-isoprostanes were associated with markers of inflammation and the ANS, but not cortisol. Contrary to the hypothesis that increased physiological stress would be associated with oxidative damage, 8-OHdG was significantly associated with lower ANS stress as measured by respiratory sinus arrhythmia. This unexpected finding, if replicated, deserves further investigation.

These findings lend credence to the hypothesis that increased physiological stress, or allostatic overload, causes cellular damage, as measured by markers of oxidative damage. It should be noted however that a causal relationship cannot be inferred from these cross-sectional analyses. There was however evidence of a cumulative impact both within, and across stress systems of increasing stress on oxidative damage for both markers. These findings also provide further validation for the oxidative damage markers throught the associations with more well-established markers of physiological stress. The interrelations between the stress systems highlight how understanding physiological stress in disease requires attention to all systems, and their possible interactions with one another.

A potential limitation of the study might be the overrepresentation of subjects with psychiatric disorders. There were however no significant interactions with the presence of psychiatric disorders, and analyses were adjusted for use of antidepressant medication. The nature of sample may also have been an advantage as depression and anxiety have been associated with increased physiological stress, and this sample therefore comprises a broad range of stress levels, that allow these associations to be identified.

Conclusion 3

The association between physiological stress and oxidative damage

Oxidative DNA/lipid damage are associated with plasma markers of inflammation, salivary markers of the HPA-axis and markers of autonomic nervous system functioning. Increased physiological stress, both within and across systems, is associated with increasing oxidative damage in a dose-response fashion.
Table 1. Summary of cross-sectional and longitudinal associations of major depressive disorders, depressive symptoms with oxidative DNA and lipid damage (8-OHdG, F2-isoprostanes) and antioxidants (uric acid, carotenoids)

<table>
<thead>
<tr>
<th>Oxidative damage</th>
<th>Cross-sectional</th>
<th>Longitudinal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F2-isoprostanes</td>
<td>8-OHdG</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>Hedges' g</td>
</tr>
<tr>
<td>Chapter 3</td>
<td>Meta-analysis</td>
<td>MDD depressive symptoms</td>
</tr>
<tr>
<td>Chapter 4</td>
<td>NESDA</td>
<td>MDD*</td>
</tr>
<tr>
<td>Chapter 6</td>
<td>CARDIA</td>
<td>MDD</td>
</tr>
<tr>
<td>New</td>
<td>Meta-analysis</td>
<td>MDD depressive symptoms</td>
</tr>
<tr>
<td></td>
<td>Meta-analysis</td>
<td>MDD depressive symptoms</td>
</tr>
<tr>
<td></td>
<td>Meta-analysis</td>
<td>MDD depressive symptoms</td>
</tr>
<tr>
<td></td>
<td>Subgroups</td>
<td>MDD (DSM or ICD)</td>
</tr>
<tr>
<td>Antioxidants</td>
<td>Uric acid</td>
<td>Carotenoids</td>
</tr>
<tr>
<td>Chapter 5</td>
<td>NESDA*</td>
<td>MDD</td>
</tr>
<tr>
<td>Chapter 6</td>
<td>CARDIA*</td>
<td>MDD</td>
</tr>
</tbody>
</table>

* Result for current MDD (with or without comorbid anxiety), adjusted for socio-demographics, health, lifestyle and antidepressant use. *BD studies on 8-OHdG only, no available studies on F2-isoprostanes in BD. MDD=major depressive disorder; BD=bipolar disorder; CARDIA=Coronary Artery Risk Development in Young Adults Study; DSM=diagnostic statistical manual; ICD= international classification of diseases. NESDA=Netherlands Study of Depression and Anxiety.

METHODOLOGICAL CONSIDERATIONS

Measuring oxidative stress and antioxidants

Increased oxidative stress has been demonstrated in dozens of disorders, ranging from cardiovascular to auto-immune diseases (22) and other psychiatric disorders, including psychosis (23) and bipolar disorders (24). Halliwell, a leading expert in the field of oxidative stress in biology and medicine, points out: “The fact that there is more of something in a disease does not make it important.” Why? Because every form of tissue injury is associated with ROS formation; it is much more often a consequence than a cause. Halliwell has described four requirements that should be met to conclude oxidative stress is a relevant mechanism in a disease (25):

1. ROS or oxidative damage should be demonstrable at the site of injury.
2. The time course of ROS or oxidative damage formation should be consistent with the time course of tissue injury or accompanying disease.
3. Direct application of ROS to the tissue at concentrations found in vivo should reproduce the tissue injury and oxidative damage observed.
4. Removing or inhibiting ROS should diminish the tissue injury to an extent related to the degree of inhibition of the oxidative damage. (25)

In the case of depression and anxiety disorders we are currently still establishing the first point. Many of the peripherally measured markers may not reflect damage at the relevant site of injury, the central nervous system. This emphasizes how much work there still is to be done to truly establish the role of oxidative stress in these disorders.
Measuring a dynamic process with single measurements of single markers

Oxidative stress is an ongoing dynamic biological process and its complexities cannot be captured by single peripheral measurements of free radicals, antioxidants or products of oxidative damage. This limitation applies to all studies in all fields of medicine using markers of oxidative stress, including those in this thesis.

As described in the introduction measuring free radicals themselves is extremely challenging. Antioxidants, both the enzymatic and the non-enzymatic, are more readily available. Markers of oxidative damage, such as those included in our studies, reflect the outcome of the balance between oxidative stress and antioxidants. These too, however, are only a fraction of the products of oxidative damage that occur. For example in this thesis we studied plasma F2-isoprostanes, determining both the free and the esterified F2-isoprostanes, but specifically the isomer 8-isoprostaglandin F2α (iPFGα-III), that has been mostly widely studied. There are a few studies that cover multiple isomers of F2-isoprostanes, and although they are highly correlated, they do not have identical associations with health, lifestyle factors or disease (26). This example illustrates the complexity of measuring oxidative stress and the necessity to include multiple types of markers, covering as wide a range of markers as possible.

Pros and cons of oxidative stress and antioxidant markers

To date there is no consensus on which markers are the most reliable or valid, and which are most suitable may differ based on the research question. Expert opinion in the field leans towards F2-isoprostanes and 8-OHdG as current markers of choice (27).

It is clear that some widely used markers are not valid representations of oxidative damage. Malondialdehyde (MDA) or thiobarbituric reactive substances (TBARS) are examples of this. These are often used as markers of oxidative lipid damage. They are however not specific products of oxidative stress and auto-oxidation can take place during the measurement of these markers (28). Other very non-specific measures are sometimes available. Markers of oxidative damage, such as those included in our studies, reflect the outcome of the balance between oxidative stress and antioxidants. These too, however, are only a fraction of the products of oxidative damage that occur. For example in this thesis we studied plasma F2-isoprostanes, determining both the free and the esterified F2-isoprostanes, but specifically the isomer 8-isoprostaglandin F2α (iPFGα-III), that has been mostly widely studied. There are a few studies that cover multiple isomers of F2-isoprostanes, and although they are highly correlated, they do not have identical associations with health, lifestyle factors or disease (26). This example illustrates the complexity of measuring oxidative stress and the necessity to include multiple types of markers, covering as wide a range of markers as possible.

Validation of oxidative stress markers is an ongoing process. A recent animal study suggests that 8-OHdG may not come from genomic DNA, but instead be a product of oxidatively damaged guanosine bases from the nucleotide pool (29). This would imply that although 8-OHdG is a product of oxidative damage, it is not a product of intracellular damage and may therefore not be as relevant to cellular functioning and health as previously assumed. Nevertheless, the many studies that have shown it is associated with disease, cancer in particular, point to its potential clinical relevance (30).

These examples illustrate that many aspects and details of oxidative stress markers are still unknown. Many details, such as diurnal and other sources of intra-individual variation, have yet to be established. Increasing insight into these factors will allow more methodologically sound use of these markers and a better interpretation of the findings.

Source of the sample and measurement techniques

Oxidative stress and antioxidant markers are usually determined in urine or blood samples, as is the case in the studies in this thesis. The advantages of these samples are their relative accessibility and acceptability to study participants. Samples from these sources are thought to represent a “full body” measurement of oxidative damage markers. They may however fail to pick up more subtle changes in specific organs or cell types. Particularly relevant for studies on psychiatric disorders is whether these peripheral measures reflect levels in the central nervous systems. For most markers this is unknown and there is reason to believe this may not necessarily be the case.

As described in the introduction, measurement techniques used for the assays vary in their precision and validity. Some studies have shown reasonable correlations between immunoassays and liquid or gas chromatography mass-spectrometry methods, but commercial kits may vary in their quality. Chromatography mass-spectrometry methods are therefore still considered the gold-standard, although they are labor intensive and therefore costly. Expert opinion warns against the use of some commercially available kits (27).

Confounding

For many markers the sampling, sociodemographic, health, lifestyle and environmental determinants have not been established. In this thesis we report on many of these factors in Chapter 2, but should also point out how many conflicting findings there are in the literature for important basic factors such as sex and age, even within the literature on 8-OHdG and F2-isoprostanes.

Smoking is a factor that is well-known to increase oxidative stress. Its long-term oxidative damage effects may however not be captured by the same markers that are acutely influenced after smoking a cigarette. Most of these details and nuances in oxidative stress research have yet to be studied and established. Another example is the effect of diet on many markers. Diet is a difficult and time-consuming to measure reliably, especially in large samples. Therefore for many markers the effects of diet are not known. The same applies to physical activity; self-report measurements of physical activity have limited reliability and more reliable measures using actigraphy for example are more costly and
labor intensive. It is however likely that levels of physical activity influence the basal levels of enzymatic antioxidant defenses. Physical activity causes an acute burst of oxidative stress; in response to which cells upregulate their antioxidant defenses, which has ongoing benefits for cellular protection beyond the moments spent exercising (31).

There are a great number of potential environmental sources of oxidative stress (32), some of which may not have been identified yet. Therefore it is possible that some findings in oxidative stress research are affected by residual unmeasured confounding. This implies that it is especially important to consider which factors might be relevant in every study on oxidative stress markers and include these factors as confounders where available. Presenting multiple models adjusted for different categories of confounders also provides insight into which factors affect the association and to what degree. Identifying these factors is an important finding itself and can also help inform future research.

**Oxidative stress is not all bad, antioxidants are not all good**

It is important to be aware of the fact that reactive oxygen species fulfill a number of essential roles in human biology; free radicals are used to target micro-organisms, and in controlled cell death, and there is increasing evidence to suggest that they are involved in intracellular signaling (22). Antioxidants are not necessarily beneficial to health. A well-known example is the use of carotenoids in a high risk population of smokers to prevent the onset of lung cancer. Researchers found that those using the carotenoids were at increased risk (33), most likely due to the fact that the supplements prevented the destruction of malignant cells by oxidative mechanisms.

The enzymatic and non-enzymatic antioxidants together form an intertwined antioxidant defence system, in which the level and activity of each compound affects the other. This means that interpreting levels of single antioxidant marker provides limited information on the functioning of the antioxidant defence system as a whole.

Interpretation of studies on oxidative stress, in this thesis, and in all fields of medicine requires consideration of the factors and limitation described here. This highlights the complexity of studying and understanding oxidative stress and the role it plays in health and disease.

**MEASURING DEPRESSION AND ANXIETY**

Currently all psychiatric diagnoses are classifications of signs and symptoms, rather than clearly delineated diseases with distinct identifiable biological substrates, such as is the case for (many, but certainly not all) somatic disorders recognized in general medicine. The most widely used classification system, the Diagnostic and Statistical Manual of Mental Disorders (DSM), is a system based on clinical observations, field research to ascertain the reproducibility and validity of these observations by different practitioners across different settings. Ultimately the classifications are a consensus of expert opinion, arrived at by discussions between members of the DSM-task force, overseen by the American Psychiatric Association (APA).

The classifications used in the thesis are those of the DSM-IV-R, which for MDD and anxiety disorders have remained largely unchanged in the latest edition, the DSM-5. The presentation of the latest edition led to renewed criticism both of the classifications themselves as well as the procedures used to arrive at them.

**Critique of the procedure to establish DSM diagnoses**

The lack of transparency concerning the procedures to establish the DSM has been the focus critique. Members of the taskforce were initially required to sign non-disclosure agreements. Neither the names of members of the working groups, nor their possible conflicts of interest, were made public. Critics pointed out that the lack of disclosures made it impossible for clinicians and patients to discern whether the new classifications were free of bias, and in particular not biased towards the interests of the pharmaceutical industry. The APA made some efforts to address these criticisms by providing more information on the involved individuals and their professional and financial ties. But many feel that the current level of transparency is not enough and should also cover indirect financial ties, such as grants to the institutes where individuals are employed and financial ties of family members to relevant companies or organizations. The disclosures that were made revealed that industry ties are the rule not the exception among DSM working group members, with 70% reporting direct links to industry (an increase of 14% compared to the DSM-IV working groups), leading critics to argue that disclosure alone is insufficient to ensure that the process of creating the classification is free from financial and industrial bias (34).
Limitations and concerns about the DSM diagnostic categories

Reliability and validity

The core of the criticism of the DSM is the questionable validity and reliability of the diagnostic categories. Over 50 organizations including The British Psychological Society and the American Counseling Association endorsed a petition critiquing the DSM-5 (35). Concerns focused on the lowering of diagnostic thresholds that would unnecessarily label more people mentally ill. The inclusion of new disorders in the absence of a scientific basis, possibly leading to inappropriate treatment was also a point of critique. In addition they stated that the lack of recognition of social causes of mental health complaints in the DSM wrongly emphasizes the biological causes.

The DSM-5’s own task force’s field trials revealed that many of the diagnoses had poor interrater reliability. MDD and anxiety disorders scored particularly weakly, with kappa statistics for MDD and generalized anxiety disorder (0.28 and 0.20 respectively), reflecting “questionable agreement” between clinicians (36). This poor reliability is likely in part due to the heterogeneity of patients who fall with the diagnostic category of MDD, and the high rates of comorbidity within it. Major depressive disorder encompasses everything from relatively mild symptoms, in people who may not feel the need to seek treatment for them, to those crippled by severe melancholic symptoms. The diagnostic criteria allow for some specifications on severity but still include patients with wildly different symptoms, presentations, functioning and severity within one diagnosis. In an effort to address the high levels of comorbidity between depression and anxiety the DSM-5 included an “anxious-distress” specifier. The previous addition of the “mixed anxiety and depression” category in the DSM-IV was not successful in solving the problem of reliability; its kappa score was 0 (37). First results on the DSM-5 specifier are more promising (38,39).

Following the DSM’s diagnostic criteria two individuals can have the same MDD diagnosis without having a single symptom in common. Symptoms include both insomnia and its opposite hypersomnia, and increased as well as decreased appetite. Within the diagnosis major depressive disorder a number of subtypes based on symptom profiles have been identified, following the lead of clinical experience as well as through data driven approaches. These phenotypically distinct subtypes may also have distinct etiology and possibly also distinct biological substrates. The melancholic and atypical subtype differ phenotypically most markedly on the sleep, appetite and weight change symptoms, with the melancholic subtype showing decrease, and atypical showing an increase. They also display differences in biological profiles; the atypical is accompanied by high rates of metabolic syndrome and inflammation, whereas the melancholic subtype has more pronounced HPA-axis dysregulation (40).

The dichotomous nature of the categories, wherein a patient either does or does not meet the criteria for diagnosis, presuming them to be either diseased or healthy does not capture the more subtle reality of symptom severity, which is likely more adequately reflected by a continuous measure. The diagnostic categories rely on symptom counts, but more symptoms do not necessarily correlate with the level of distress (41,42), nor do they take into account the circumstances of the individuals in which these symptoms manifest.

The design of the NESDA study on which most of this thesis was based, overcomes some of these limitations of the DSM classification (43). The recruitment procedure ensured inclusion of people with all levels of severity, precisely to be able to study the differences between them. Incorporating both depression and anxiety DSM diagnoses in the study allows assessments in both disorder groups separately as well as in subjects with comorbidity. The study also includes numerous measures of symptom severity for depressive, anxious, phobic, (hypo)manic and somatic symptoms. In addition the study includes a range of measures on functioning in work and social life as recent life events, and childhood traumatic experiences. All of these measures together allow for more multifaceted approach.

In this thesis various measures of symptom severity (for depressive, anxious and phobic symptoms) as well as measures of duration were used to study the relationship with oxidative stress, beyond the dichotomous diagnostic categories.

Diagnostic tools: structured interviews and self-report questionnaires

Structured interviews are the gold-standard diagnostic tools for ascertaining psychiatric diagnoses. The SCID (Structured Clinical Interview for DSM disorders) and CIDI, used by the studies in this theses are among the most widely used. These interviews have some important limitations (44). Interviewees may not recognize their own experience in the clinically worded, direct questions as they are posed in these interviews. It could also be the case that subjects are not aware that a certain experience does apply to them until it is articulated in a more unstructured open form of conversation. Naturally, there are many other reasons interviewees may choose to intentionally or unintentionally give answers that do match their actual experience; shame, fear, embarrassment, politeness, attention seeking, doubt, distrust, animosity, dependence, disinterest, eagerness to please, misunderstanding, mischievousness, pride, indifference etc. The circumstances and purpose of the interview may also be crucial. An interview to ascertain whether someone is fit for a job they desire or the social welfare they need, may yield different answers than an interview for research purposes only.
Structured interviews require specially trained staff and are costly and time-consuming to conduct on a large scale. One of the most important strengths of the NESDA study is that these interviews have been conducted in such a large sample. Many studies however must rely on cheaper and easier to implement self-report symptoms questionnaires, such as the CES-D used in CARDIA study in this thesis. Self-report questionnaires may have a low predictive value for CIDI classifications when used in a general population sample (45), but likely perform better in settings with higher base rates of MDD. Nevertheless studies on markers of biological stress such as inflammatory and metabolic markers have also shown associations with depressive symptoms, with slightly smaller effect sizes, as would be expected, compared to studies using diagnoses established with clinical interviews (46).

Cultural bias in the DSM classifications, effects on biology?
The majority of people suffering from mental health disorders worldwide live in developing, overwhelmingly non-western societies. The DSM, developed under authority of the American Psychiatric Association has been criticized as having Western-centric outlook. In religious, non-Western, illiterate or poorly educated people the reliability of the CIDI has not been established. A study in Turkish and Moroccan immigrants in the Netherlands demonstrated there was construct bias, with mental health problems being seen as taboo or personal failing; method bias, reflected in the interview situation as being experiences as awkward; and item bias, relating amongst others to questions about suicide or reference to distinct episodes of mental health problems (47). Psychiatrists approaching research from a global health perspective argue that even in the case of reproducibility or acceptability of diagnostic categories in a certain setting, this does not equal legitimacy of the constructs.

This is unlikely to affect the data used in this thesis that was collected in the western world, the CARDIA study in the United States and the NESDA study in the Netherlands (in which over 95% of the sample reported being of North-European heritage). It does however have potential implications for studying psychiatry as a whole, and also for underlying pathophysiological mechanisms including oxidative stress. Kleinman (48) suggested that cultural differences in how people understand the self and the body could create differences in how psychopathology manifests or is presented across cultures.

These differences may however reflect more than a cultural difference in experience or choice of words. They may actually also reflect a difference in pathophysiology, shaped by the cultural, social and environmental setting of a population. Research into perimenopausal symptoms, such as hot flushes, revealed that they may not only differ across cultures due to cultural expectations or meaning given to menopause, but could also be influenced by lifestyle, diet or other environmental factors that shape human biology (49). It is conceivable that a similar mechanism might also affect the association between oxidative stress and depression across cultures. In a population exposed to major environmental sources of oxidative stress, for example from air pollution, the relatively small contribution of depression to oxidative stress levels may be masked. In such a population the presentation of depression may focus on somatic symptoms exacerbated by the effects of pollution. In a population with a particularly antioxidant rich diet the contribution of oxidative stress to depression may also be unmeasurable, buffered by the antioxidant intake. The beneficial effects of the antioxidant intake may perhaps also prevent some of the somatic symptoms associated with depression.

These suggestions are purely hypothetical and speculative, but serve to illustrate the possibility of multiple interactions between environment, biology, brain, culture and social context that could shape the experience and presentation of mental disorders, as well as their underlying pathophysiology.

CLINICAL IMPLICATIONS
The ultimate goal of studies on the associations of oxidative stress and depression and anxiety disorders is not only to interpret the pathophysiology of these disorders, but to change them. This paragraph focuses on the implications of this thesis’ findings for diagnostics, prevention and treatment of affective disorders.

Oxidative stress and antioxidants in depression and anxiety: cause or consequence?
As has already been mentioned in this chapter, increased oxidative stress is a very non-specific finding as tissue damage of any kind is followed by release of ROS. Therefore increased oxidative stress does not necessarily imply that oxidative stress is the mechanism behind this damage.

Oxidative stress as consequence
The results from the CARDIA study (Chapter 7) in particular demonstrated the important impact of health- and lifestyle factors on F2-isoprostanes levels in depressive symptoms. The longitudinal associations also pointed to depressive symptoms predicting higher F2-isoprostanes at follow-up, not vice versa. This could suggest that increased oxidative stress is a consequence of depressive symptoms. Depressive symptoms may cause or exacerbate poor health behaviors such as smoking, or an unbalanced diet, which in turn promote oxidative damage. The importance of healthy lifestyle for overall health is of course not novel. These findings only serve to confirm what is already known; poor lifestyle
causes the body harm and those with depression are at particular risk. Lifestyle changes, however, are difficult to achieve and sustain. People with depression have poorer self-care and higher non-compliance to treatment plans and medications. Extra attention on how to help people with depression to maintain a healthy lifestyle, and research into what support is best suited to help them achieve this is warranted.

**Oxidative stress as cause**

There are however studies suggesting that oxidative stress is a primary causative mechanism in affective disorders. There is increasing evidence to suggest mitochondrial dysfunction might be present in psychiatric disorders, in particular in bipolar disorder (50). The increased exposure to ROS, may further damage the already poorly functioning mitochondria, worsening the problem. There is also emerging evidence of mitochondrial dysfunction in affective disorders (60). This increase in physiological stress is likely accompanied by an increase in oxidative stress. The findings in Chapter 7 demonstrated a cumulative impact of increased levels of physiological stress on oxidative markers and support this notion. Increased physiological stress and oxidative damage are most likely intertwined events, and not a clear cut case of one element of physiological stress or oxidative damage preceding the other. Rather, affective disorders may be better understood, in part, as psychological manifestations of increased physiological stress in multiple systems.

The brain is particularly vulnerable to oxidative damage due to its high oxygen consumption and relatively weak antioxidant defenses (61). Oxidative damage sustained during a depressive episode may make individuals more susceptible to developing a next. This has been hypothesized to underlie the relapsing course MDD can take, and the observation that episodes often become more frequent, severe and chronic as time goes on (62).

**Oxidative stress as treatment target in depression and anxiety disorders**

No marker of oxidative damage or antioxidant, or any other biological markers, can currently discriminate between controls and people with MDD or an anxiety disorders. Findings are based on differences at group level and effect sizes are generally small. An effect size of 0.1 or 0.2 (as found in most of the studies in this thesis) means that 54-58% of the control group would have oxidative stress levels below the depression and/or anxiety group (63). That means markers with effect sizes in these ranges perform only slightly better than chance in discriminating between groups. Biomarkers that differentiate controls and patients however are not the aim of these studies, as disorders as heterogeneous as depression should be expected to have an equally heterogeneous pathophysiology. More likely is that multiple biological measurements together with a range of clinical factors will provide tools that are predictive of clinical course or treatment response in subgroups within these disorders.

The most pressing clinical question however is, does oxidative stress provide an avenue to treat depression and anxiety disorders effectively? To answer that question it is worthwhile to first review the available evidence on the effectiveness antioxidant treatment in general medicine.

**Antioxidant treatment in somatic disorders**

Disappointingly, antioxidant treatments in general medicine have so far proved largely unsuccessful (64). The fields of medicine in which they have been most widely studied are cancer treatment and prevention of neurodegenerative disease. There are a number of reasons why this has been the case thus far (65). The most important reason for the lack of success of antioxidant treatment is that oxidative stress in most disorders, although it may be associated with the presence of the disease, is not the a major contributing factor or the driving pathophysiological mechanism of the disorder. Therefore, intervening with antioxidant treatment does not affect the main underlying mechanism or cause.

Secondly, dietary antioxidant intake by and large does not affect oxidative damage levels as their levels are regulated to a certain maximum regardless of the amount of intake (27). On the whole this is beneficial as it allows ROS to be present at sufficient
levels to perform their necessary and beneficial functions as defence against pathogens, in programmed cell death and cellular signaling. It does however render extra antioxidant intake, even at high levels from supplements, largely ineffective in altering oxidative stress levels. Thirdly, antioxidant treatment is usually administered systemically, even though its intended target is a specific tissue, cell type or even cell compartment. To have the levels sufficiently high at the site of intended action would mean having to create levels so high systemically that toxicity becomes a problem (65). The fourth problem is the disruption to the balance and interplay within the antioxidant defense system itself, as well as in relation to ROS, that introducing high doses of a single compound can cause. Its damaging effects could then mask and outweigh any of the possible benefits.

In addition to these obstacles, lack of adequate selection of patients for trials (those who have evidence of increased levels of oxidative damage, not only the diagnosed disorder) as well as lack of specific antioxidant compounds for specific profiles of oxidative damage, have been identified as reasons for the lack of success.

This has lead some experts to suggest that treatment with pro-oxidants might prove more effective in some cases (66). Increased exposure to low grade oxidative stress might upregulate the body’s antioxidant enzymes and so protect against oxidative damage. This is the mechanism through which regular physical activity exerts some of its beneficial health effects, as during physical activity the body is exposed to a burst of oxidative stress (67). This mechanism could conceivable be exploited by administering it in a supplement form to achieve the same benefits.

**Antioxidant treatment for depression**

There is some emerging evidence for the use of antioxidant supplements as (add-on) treatment for depression. A trial on N-acetylcysteine (NAC), a redox-active glutathione precursor, in 252 patients with MDD showed some benefits over placebo on secondary outcomes, but did not provide clear evidence of effectiveness (68). A few trials into ascorbic acid (vitamin C) supplementation have conflicting results (69,70). Coenzyme q10 treatment improved depressive symptoms in multiple sclerosis patients (71). A meta-analysis of curcumin supplementation including 6 trials showed beneficial effects on depression (72). Zinc exhibits antioxidant properties by activating enzymatic antioxidant systems (73) and supplementation has shown to be effective as stand-alone and add-on treatment for depression, however current evidence is limited to 6 trials (74).

The current limited evidence for antioxidants as effective treatment for depression should be separated from the question whether individuals suffering from depression could benefit from antioxidant supplements. Clinicians should be aware, that although severe antioxidant vitamin deficiencies are rare in the general population of western societies, people with psychiatric disorders constitute an at risk group (75). Poor diet, due to lack of self-care and/or poverty, especially in the case of comorbid substance abuse, or in the elderly can cause deficiencies in people with depression. Clinicians should be mindful of the need to ensure patients have an adequate diet, if necessary with low dose supplementation, to treat or prevent such deficiencies.

Although there is little evidence that antioxidant supplementation reduces the risk of depression in healthy populations (76), prevention of depression in high risk individuals through dietary strategies or supplementation may well prove beneficial (77), although these effects are not solely attributable to antioxidant activity.

**Antidepressants as antioxidants**

Antidepressant medications may exert some of their action through antioxidant effects (6). In Chapter 4 the association between antidepressant use and lower oxidative damage was demonstrated in a cross-sectional study, but there is also evidence from intervention studies that antidepressants affect oxidative damage levels and increase antioxidants levels (6,7,20). These antioxidant effects may be related to a reduction of inflammatory markers which induce oxidative stress. Serotonin itself acts as a free radical scavenger and increased levels may therefore reduce oxidative stress (78). The finding that antidepressant use reduces oxidative stress may however not necessarily be related to the effect of the medication. Increased physical activity, less smoking or better diet due to improvement of the depressive symptoms may reduce oxidative stress.

The knowledge that antidepressants may exert some of their effects through antioxidant mechanisms could be used to predict who will respond to antidepressant treatment. Currently clinicians and patients have to rely on trial and error and patients can go through many months of starting medications only to find they are ineffective, or to which they respond only partially. Future research on markers of oxidative stress pre-treatment might become part of a clinical assessment that helps predict whether antidepressant use is likely to be effective.

**Antioxidants are big business**

Vitamin supplements are big business. Dutch consumers spend 300 million euro per year on over the counter supplements in the belief they are improving their health (79). Many manufacturers advertise their products with claims of health benefits for which there is no or little evidence. “Vitamin C strengthens your immune system”, is one such claim, which led the Dutch consumers’ organization (Consumentenbond) to file a complaint for misleading advertising (80).
False claims of health benefits are problematic, but more worrying perhaps are potential hazards of the use of over the counter supplements. A Cochrane systematic review and meta-analysis including almost 300,000 subjects from 56 studies concluded there was no evidence to support antioxidant supplements for primary or secondary prevention of mortality, and beta-carotene, vitamin E and high doses of vitamin A may actually increase mortality (81).

The right of well-informed, affluent consumers to spend money on the growing range of products and treatments that the “detoxing”, wellness and dietary supplement industries have on offer is not in dispute. But clinicians and researchers however do have a responsibility to inform their patients and the public, and provide reliable evidence on the safety and efficacy of these products and therapies. In a global political climate that is increasingly hostile to the values of science active engagement of professionals in this area is required. Offers of scientifically unproven treatments, with potential risks, for which people are required to cover the often high costs from their own pockets, are targeted specifically at people for whom conventional medical treatment has failed (82). People with psychiatric disorders, in their need to alleviate their suffering, are particularly vulnerable in this respect and clinicians and researchers should advocate for regulation and quality control measures where necessary to protect them, and the public at large.

**RECOMMENDATIONS FOR FUTURE RESEARCH**

As has become clear in this chapter, much work is still to be done to establish the role of oxidative stress in the pathophysiology of depression and anxiety, and its potential as an avenue for treatment.

**Oxidative measurements in general**

As described in paragraph 3 of this chapter relatively little research has been conducted on the sources of intra- and inter-individual variability of most oxidative stress markers. Information on the diurnal variation and the stability over time could prove vital in interpreting research on these markers. For psychiatric disorders in particular, information of the association between peripheral measures and those in the central nervous system are important to know whether the easily accessible peripheral measures provide relevant information on oxidative stress levels centrally.

Smaller scale studies in healthy individuals with detailed measurements on factors such as diet, physical activity and smoking from who measurements of oxidative stress are obtained in plasma, urine and cerebrospinal fluid over multiple time points would provide information on which markers are both feasible and reliable to use in larger scale studies.

**Oxidative stress and antioxidants in depression**

The studies in this thesis are the largest of their kind, but replication of these findings is still necessary. Ideally, intervention studies including multiple markers of oxidative stress and antioxidants as well as detailed data on the health and lifestyle factors identified in chapter 2 should be conducted. Measurements done both pre- and post-treatment could examine the effects of different interventions on oxidative stress, those of antidepressants as well as psychotherapy, physical activity and nutrition interventions, and also demonstrate whether changes in psychiatric symptoms are correlated with changes in oxidative stress.

Large-scale intervention studies into the effectiveness of antioxidants as (add-on) therapy for depression and anxiety are necessary. However the obstacles that have been identified in trials on antioxidants in somatic disease should be taken into account. Selection of high risk patients, those with evidence of a marked increase in oxidative damage or depleted antioxidant status could be selected and treated with dietary and physical activity programs and/or antioxidant supplements.

In all these studies, attention should be paid to potential confounding health and lifestyle factors as well as the effects of antidepressant use.

**Research beyond DSM classifications**

The director of the NIMH (National Institute of Mental Health) in the United States Thomas Insel, announced in 2013 that projects that relied exclusively on DSM criteria would no longer be eligible for funding, because the field trials had highlighted their lack of validity (83). This will have a major impact on the design of future studies in mental health research as applicants will have to re-invent their diagnostic methods and broaden the focus of their research to be eligible for funding from this important source.

As mentioned, increased oxidative stress is not specific to depression and anxiety disorders, as it has also been demonstrated in other psychiatric disorders, including psychotic disorders and bipolar disorder (84). An approach that selects individuals with psychiatric symptoms and evidence of increased oxidative damage might uncover similarities in pathophysiology despite the variations in clinical presentation.
TO CONCLUDE

It is clear that depression and anxiety disorders are highly heterogeneous. Oxidative stress is a complex and dynamic process, that is not the core feature, but one of many mechanisms involved in the pathophysiology of these disorders. No single therapeutic approach is going to be effective for all the individuals whose symptoms fall within the confines of the diagnostic categories of major depressive and anxiety disorders. Treatments that focus on increasing antioxidant capacity and reducing oxidative damage, either through lifestyle interventions, antioxidant supplements or new compounds that exert their effects through redox-regulation are promising avenues worthy of further investigation.

The evolution of antioxidant defenses hundreds of millions years ago allowed the development of the large and complex human brain. This is humanity's blessing, and its curse. It has made us capable of great advances, and given us the capacity to suffer immensely in ways other creatures cannot. In the same way our biology strives to maintain redox homeostasis, our minds strive to maintain emotional equilibrium. Both hold many secrets we have yet to uncover.

This thesis adds a few pieces to a large puzzle. My hope is others will find it useful and build on it, as we collectively search for answers that help us relieve suffering.

REFERENCES


35. Nelkin DN. Open letter to the DSM 5 task force [Internet]. Coalition for DSM-5 Reform, Division 32 committee on DSM 5. Available from: http://ds5-reform.com/the-open-letter-to-dsm-5-task-force/


82. Revitalis, Heb jij een gezondheidsprobleem waarvoor ze geen oplossing hebben? [Internet]. Available from: http://revitalis.nl/
