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Peripheral antioxidant markers are associated with total hippocampal and CA3/dentate gyrus volume in MDD and healthy controls – Preliminary findings

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Abstract

Several psychiatric disorders, including major depressive disorder (MDD), are associated with increased blood markers of oxidative stress. The relevance of this to the oxidation-sensitive hippocampus (HC) is unknown. We investigated the relationship between peripheral oxidative stress markers and HC volume in unmedicated individuals with MDD (n=16) and healthy controls (n=19). To conserve power, our primary analysis was carried out in the combined group of subjects, and secondary analyses examined each group separately. Oxidative stress markers (oxidized glutathione) and antioxidants (reduced glutathione, glutathione peroxidase, Vitamin C) were assessed, and a "total net antioxidant score" was calculated. 4-T MRI estimated total HC volume and HC subfield (CA1, CA1-CA2 transition zone, subiculum and CA3/dentate gyrus [CA3&DG]) volumes. Across groups, the antioxidant score was significantly and positively correlated with total HC volume and CA3&DG subfield volume (normalized to total intracranial volume), adjusting for age and sex. Similar relationships were observed in each individual group but missed statistical significance, likely due to type II errors, with the exception of a significant correlation between the antioxidant score and CA3&DG volume in the MDD group. These

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preliminary data are consistent with oxidative stress being associated with smaller total HC and CA3&DG subfield volumes.

Keywords

oxidative stress; glutathione; Vitamin C; hippocampus; subfields; aging

1. Introduction

Oxidative stress occurs when the production of oxygen free radicals exceeds the body's antioxidant capacity (Figure 1). Among the oxidative systems implicated in psychiatric disorders, the glutathione system has a strong theoretical foundation (Berk et al., 2008). Glutathione is a ubiquitous cellular antioxidant found in the cytosol, mitochondria and cell nucleus (Berk et al., 2008). As shown in Figure 1, reactive oxygen species (ROS) are detoxified by reduced glutathione (GSH), which is oxidized to oxidized glutathione (GSSG) by the antioxidant enzyme glutathione peroxidase (Gpx). Similarly, the antioxidant Vitamin C (ascorbate) detoxifies ROS in its conversion to Dehydro-ascorbate. Oxidative stress is damaging to cells and tissues throughout the body, with the brain, and the hippocampus (HC) in particular, being especially sensitive due to their high oxygen consumption, rich content of easily oxidizable fatty acids and relatively low content of antioxidants (Evans, 1993). More specifically, experimental studies have shown that oxidative stress in the HC is associated with reduced neurogenesis, increased generation of glial cells, and increased neuronal death (Mattson, 2000; Huang et al., 2012). High levels of peripheral markers of oxidative stress have been reported in many, but not all, studies of Major Depressive Disorder (MDD) (Ng et al., 2008), and antidepressant treatment often ameliorates the oxidative stress (Behr et al., 2012). In this preliminary study, we sought to determine if peripheral markers of oxidative stress are related to magnetic resonance imaging (MRI)-based estimates of HC volume in unmedicated subjects with MDD and in healthy controls. Even though both peripheral and central signs of increased oxidative stress have been reported in MDD (Ng et al., 2008; Gawryluk et al., 2011), we are not aware of any previous studies investigating direct associations between peripheral markers of oxidative stress and hippocampal volume in MDD subjects and healthy controls.

Our use of high resolution MRI and in-house developed mapping protocols allowed us to additionally estimate the volume of individual HC subfields (Mueller et al., 2007). Certain HC subfields (CA1 and CA3 /dentate gyrus (CA3&DG)) are especially sensitive to oxidative damage (Chang et al., 2012; Uysal et al., 2012). Results from a post-mortem study on Alzheimer's disease suggest that an oxidative stress pathway starts in the CA3 subfield and then progresses to the CA1 subfield and subsequently to other parts of the brain (Cruz-Sanchez et al., 2010). In that study, increased oxidative stress was more evident in CA3 compared to CA1 and subiculum (Cruz-Sanchez et al., 2010). Furthermore, in an animal model of oxidative stress, glutathione deficiency caused a selective reduction of hippocampal parvalbumin-immunoreactive interneurons in CA3&DG (Steullet et al., 2010), suggesting that this hippocampal region is of particular importance in the interaction between oxidative stress and brain damage. Studies have also suggested some subfield

specificity of HC volume loss in MDD, implicating DG, CA1-CA3, and subiculum (Bearden et al., 2009; Huang et al., 2013), although these results are not consistent, and it is unknown if such subfield differences are related to oxidative stress

In this study, we therefore additionally sought to relate peripheral oxidative stress markers to these particular HC subfield volumes. We hypothesized that the net amount of antioxidants (GSH, Gpx and Vitamin C) relative to markers of oxidation (GSSG) would be correlated with larger total HC volume in un-medicated MDD subjects and controls. Based on previous experimental studies (Cruz-Sanchez et al., 2010; Steullet et al., 2010; Chang et al., 2012; Uysal et al., 2012), we also explored the additional hypotheses that volumes of the CA1 and CA3&DG subfields would be significantly correlated with oxidative stress. In the absence of data to the contrary, our null hypothesis was that unmedicated MDD subjects and controls would show similar relationships between oxidative stress markers and HC volume.

2. Methods

2.1. Ethics statement

The Committee on Human Research of the University of California, San Francisco (UCSF) approved the study protocol. All study participants gave written informed consent to participate in this study and were compensated for participating.

2.2. Study participants

The study sample described in the present report has also been used to test a different set of hypotheses reported elsewhere (Dhabhar et al., 2009; Wolkowitz et al., 2011; Wolkowitz et al., 2012; Rawdin et al., 2013). Sixteen MDD subjects (6 men and 10 women, mean age 33.6 + 7.2) and 19 healthy controls (7 men and 12 women, mean age 36.5 + 12.1) were recruited for the study. Depressed subjects were all outpatients; they and the controls were recruited by flyers, bulletin board notices, Craigslist postings, newspaper ads and, in the case of depressed subjects, clinical referrals. DSM-IV diagnoses of MDD were established by the Structured Clinical Interview for DSM IV-TR (SCID) and were verified in a separate diagnostic evaluation by a Board-certified psychiatrist. MDD subjects were required to have a minimum 17-item Hamilton Depression Rating Scale (HDRS) rating of >17. Potential subjects in either group were excluded if they met SCID criteria for alcohol or substance abuse within 6 months prior to entering the study. Exclusion criteria for the MDD group also included any history of psychosis or bipolar disorder or recent post-traumatic stress disorder, but other anxiety disorders were allowed if the MDD diagnosis was considered the primary diagnosis. Five of the MDD subjects fulfilled additional criteria for at least one secondary co-morbid diagnosis; one had generalized anxiety disorder, one had obsessive-compulsive disorder, one had binge eating disorder, and two were diagnosed with anxiety disorder not otherwise specified. Healthy control subjects were also screened by means of the SCID, and exclusion criteria were any past or present DSM-IV Axis I diagnosis.

Physical examinations, review of systems and routine blood screening showed no signs of somatic illnesses that were likely to influence levels of oxidative stress markers. Study participants had no acute illnesses or infections, and had not had any vaccinations within 6 weeks prior to entering the study. All subjects were free of psychotropic medications,

hormone supplements, steroid-containing birth control or other potentially interfering medications or vitamin supplements above the U.S. recommended daily allowances (e.g., >90 mg/day for Vitamin C) for at least 6 weeks prior to enrollment in the study. Short-acting sedative-hypnotics were allowed as needed up to a maximum of 3 times per week, but none within 1 week prior to participation in the study.

2.3. Blood sampling and assays

Blood draws were performed on subjects at 8:00 am at the UCSF Clinical and Translational Science Institute after a night of fasting except water. Urine toxicology screening and urine pregnancy test (when applicable) were performed on all subjects and confirmed negative on the morning of testing. Plasma was collected with a lavender EDTA Vacutainer tube for GPx analysis, and serum was collected into serum separator Vacutainer tube for vitamin C analysis. Whole blood was collected and processed for GSH and GSSG measurements using the following procedure: 1) blood was drawn into a lavender EDTA tube which was set on ice and processed within 30 minutes, 2) 400μL of ice-cold 5% MPA solution and 100μL whole blood were added into a 1.5mL microcentrifuge tube, 3) microcentrifuge tube was capped and vortexed for 5 seconds immediately, 4) the microcentrifuge tube was incubated on ice for 5 minutes, 5) the microcentrifuge tube was centrifuged at 4°C for 2 minutes at 13000 RPM in a microcentrifuge, and 6) the supernatant was aliquoted to a vial and stored at -80°C until assay at the Kronos Science Laboratory.

GPx activity was quantified by a colorimetric method similar to the Cayman GPx assay kit (Cayman Chemical Company, Ann Arbor, MI) developed at the Kronos Science Laboratory. Each sample was analyzed in duplicate. The intra and inter precision of the procedure were monitored by two levels of controls. The intra- and inter-assay coefficients of variance were 4% and 10%, respectively.

Vitamin C was measured using an Agilent 1100 Series high performance liquid chromatograph (HPLC) system with a diode array detector as described previously (Margolis and Schapira, 1997). Briefly, serum samples were preserved by adding an equal volume of metaphosphoric acid and treated with dithiothreitol. The resulting supernatant was injected into the HPLC systems equipped with a 250×4.6 mm Capcell Pak NH_2 column (Shiseido, Tokyo, Japan). The column was equilibrated at 40°C at a flow-rate of 1 ml/min with a mobile phase composed of monobasic potassium phosphate/H_2O/acetonitrile. The vitamin C was analyzed using external standards with UV spectrophotometric detection at 243 nm wavelength. Two quality-control samples were injected at the beginning, the end, and after every 10 samples to monitor intra- and inter-day assay accuracy and precision. Recoveries were consistently in excess of 90% and coefficients of variance ranged from 4% to 13%.

GSH and GSSG measurements were carried out using LC-MS/MS on a system consisting of two Shimadzu LC-10AD pumps, a Shimadzu degasser (Shimadzu Scientific Instruments, Columbia, MD), and a Perkin Elmer autosampler (Perkin Elmer LLC, Norwalk, CT) directly interfaced with a triple-stage quadrupole mass spectrometer (API2000, Applied Biosystems, Foster City, CA) equipped with a TurboIonSpray ionization source. Nitrogen was used as the collision gas. Positive ionization methods were used to measure glutathione under MRM

mode. GSH and GSSG were monitored with ion pairs (m/z) of 308/179 and 613/355 respectively. A seven-point linear calibration curve was established for each analyte using both internal and external standards. Three quality-control samples were injected at the beginning, the end, and after every 10 samples to monitor intra- and inter-day assay accuracy and precision. Recoveries were consistently in excess of 90% and coefficients of variance ranged from 3% to 10%.

2.4. MRI Acquisition

All imaging was performed on a Bruker MedSpec 4 Tesla system. The following sequences were acquired. (1) For the measurement of hippocampal subfields, a high-resolution T2-weighted fast spin echo sequence (TR/TE: 3,990/ 21 ms, echo train length 15, 18.6-ms echo spacing, 160° flip angle, 100% oversampling in ky direction, 0.4×0.4 mm in plane resolution, 2-mm slice thickness, 24 interleaved slices without gap, acquisition time 5:30 min, and angulated perpendicular to the long axis of the hippocampal formation). (2) For the measurement of total hippocampal volume, a volumetric T1-weighted gradient echo MRI (MPRAGE) (TR/TE/TI = 2,300/3/950 ms, 7° flip angle, $1.0 \times 1.0 \times 1.0$ mm^3 resolution, and acquisition time, 5:17 min) and 3. For the determination of the intracranial volume (ICV), a T2-weighted turbospin echo sequence (TR/ TE 8,390/70 ms, 150° flip angle, $0.9 \times 0.9 \times 3$-mm nominal resolution, 54 slices, and acquisition time 3:06 min).

2.5. Hippocampal Subfield and Hippocampus Volumetry

The method used for subfield marking, including assessment of measurement reliability and its limitations, has been described in detail previously (Mueller et al., 2007; Mueller et al., 2010). The high resolution sequence does not allow distinguishing details on the resolution of a histological preparation. Therefore a set of arbitrarily defined hippocampal landmarks was used to assign different regions to different subfields. We do not claim that this subfield assignment actually corresponds to the histological subfields but merely that it provides a good and reproducible approximation. To briefly summarize, the marking scheme depends on anatomical landmarks, particularly on a hypointense line representing myelinated fibers in the stratum moleculare/lacunosum (Eriksson et al., 2008), which can be reliably visualized on these high-resolution images. The distance between this hypointense line and the outer boundary of the hippocampus provides an estimate of the cortical thickness of the hippocampus at this point. Additional external and internal hippocampal landmarks are used to further subdivide the hippocampus into subiculum, CA1, CA1-2 transition zone (CA1-2 transition), CA3, and DG. The latter two are lumped together (CA3&DG), because there are no macroscopic landmarks to separate them. CA1-2 transition is in the dorsal medial region of the hippocampus and consists mostly of CA2. However, due to the landmarks used for labeling it, its volume is influenced by the thickness of the dorsal CA1. To reflect this fact, the sector is called CA1-2 transition rather than CA2 (please see supplementary material for more information). The volume of the total hippocampus was determined from the T1 image using the hippocampal masks provided by the FreeSurfer subcortical parcellation routine (Fischl et al., 2002). All maps were visually checked for accuracy by different, specially trained raters who were blinded to the diagnosis and manually corrected by overlaying the label generated in FreeSurfer onto the T1 image in review. This procedure generated a map of comparable accuracy as obtained by a manual marking scheme (ICC for manual

correction of the Freesurfer labels: 0.9). After excluding significant left/right differences, the volumes from the left and right hemisphere were combined, that is added, to provide a single measure from each subfield to be used in the analysis. ICV was determined from the T2-weighted image, which was skull-stripped using the BET program (FMRIB Image Analysis Group, Oxford University, www.fmrib.ox.ac.uk/fsl). To correct for volume differences due to different head sizes, all volumes were normalized to the ICV using the following formula: normalized volume = raw volume × 1,000 ccm/ICV ccm.

2.6. Statistics

The Statistical Package for the Social Sciences (SPSS) for Mac was used for statistical calculations. All tests were 2-tailed with an alpha = 0.05. Variables that were non-normally distributed (GSH, GSSG, CA1-CA2 transition zone) were transformed into their natural logarithms prior to statistical analyses with parametric methods. In order to minimize the number of statistical comparisons, a "total net antioxidant score," using standardized scores of normally distributed raw data of all patients, was calculated by the following formula: [(GSH+Gpx+Vitamin C)-GSSG]. Univariate associations between two continuous variables were analyzed using the Pearson's r. For group-wise comparisons, we used Student's T-test. Multiple linear regressions, with sex and age as covariates, were used to test the relationship between this score and total HC and subfield volumes. Our main hypothesis was that total net antioxidant score would be positively correlated with total HC volume. We also did exploratory analyses, investigating associations between HC subfield volumes and total net antioxidant score, based on experimental data pertaining to this. Since these analyses were exploratory, we did not correct for multiple comparisons (Bender and Lange, 2001).

3. Results

3.1. Associations between demographic variables and hippocampal volume and oxidative stress

Across groups, age was negatively associated with total net antioxidant score at a trend level (Pearson's r=-0.29, 2-tailed, n=35 P=0.095), but there were no significant associations between age and total HC volume or HC subfield volumes (Pearson's r, 2-tailed, n=35, all P>0.49). Body mass index was not significantly associated with total net antioxidant score or HC total or subfield volumes (Pearson's r, 2-tailed, n=35, all P>0.36). Women had larger mean CA1, CA1-CA2 transition zone and CA3&DG subfield volumes than men (Student's T-test, 2-tailed, n=35, all P<0.05). Based on these bivariate associations, we controlled for age and gender in the multiple linear regressions.

3.2. Group comparisons on each variable

There were no significant between-group differences in demographics (Table 1a), biochemical measures (Table 1b) or hippocampal volumes (Table 1c).

3.3. Associations between hippocampal volumes and oxidative stress

Although we were interested in assessing between-group relationships between oxidative stress and HC volume, we recognized that our sample sizes provided limited statistical power to assess those. Therefore, since there was no *a priori* basis for assuming different

relationships between oxidative stress and HC volume in MDD subjects versus controls, we first assessed, as our primary analysis, these relationships in the combined group of subjects. We subsequently explored these relationships in each group separately as secondary, exploratory analyses.

Across both groups, the total net antioxidant score was directly correlated with total HC volume (*beta*=0.36, *P*=0.040, *df*=34, adjusting for age and sex) and with CA3&DG volume (*beta*=0.40, *P*=0.018, *df*=34, adjusting for age and sex), as hypothesized (Figures 2A and 2B). The total net antioxidant score was not significantly associated with CA1 (*beta*=0.12, *P*=0.452, *df*=34, adjusting for age and sex), CA1-2 transition zone (*beta*=0.18, *P*=0.283, *df*=34, adjusting for age and sex), or subiculum (*beta*=0.05, *P*=0.773, *df*=34, adjusting for age and sex) subfield volumes. Post-hoc analyses of individual oxidative stress markers showed that GPX correlated significantly with total HC, CA1, and CA3&DG volumes (all p-values <0.05, adjusting for age and gender). The relationship between HC volume and many of the other oxidative stress markers reached trend level significance but did not reach statistical significance (all p-values>0.05).

When the MDD and control groups were analyzed separately, the correlation between total net antioxidant score and CA3&DG volume remained statistically significant in the MDD group alone (*beta*=0.62, *P*=0.015, *df*=15, adjusting for age and sex). The remaining correlations within the individual groups were in the same direction and of the same magnitude as in the combined groups (See Figures 2A and 2B) but failed to meet statistical significance, likely due to insufficient power. Specifically, the r^2 values for the relationship between the antioxidant score and total HC volume are 0.22, 0.20 and 0.33 for the combined sample, the healthy control group and the MDD group, respectively. Similarly, the r^2 values for the relationship between the antioxidant score and CA3&DG volume are 0.32, 0.36 and 0.47 for the combined sample, the healthy control group and the MDD group, respectively. There were no significant between-group differences in the relationships between the total net antioxidant score and any of the HC volume measures (Fisher r-to-z Test, not significant), although, again, power was very limited for that determination.

4. Discussion

4.1. Summary

The objective of the present study was to assess the relationship between peripheral oxidative stress markers and HC volume in a group of MDD subjects and controls. Due to the small sample size, we combined the groups for our primary analysis. This was consistent with the null hypothesis that the relationships between HC volume and oxidative stress would not differ in MDD subjects versus controls. As hypothesized, we found significant positive associations between the total net antioxidant score and total HC volume in the combined group of subjects. In line with previous animal studies, the hippocampal region most robustly associated with peripheral oxidative stress was the CA3&DG subfield. Within each individual group, the relationships between total net antioxidant score and HC volume were similar to the combined group data, but generally failed to meet statistical significance, likely due to type II errors. The one exception was the robust relationship between the total net antioxidant score and CA3&DG volume, which remained significant in the MDD group

alone. To the best of our knowledge, this is the first clinical study in MDD subjects or in healthy controls testing the relationship between oxidative stress markers in the periphery and HC volume.

4.2. Our results in relation to other studies

We are aware of only one previous clinical study that has investigated associations between peripheral markers of oxidative stress and HC volume. In a cross-sectional small sample study of subjects with Alzheimer's disease (n=7) who were administered vitamin C, increased plasma and cerebrospinal fluid (CSF) levels of antioxidant Vitamin C were significantly associated with larger HC volume (Quinn et al., 2003). Fraguas et al showed, in a sample of subjects with first-episode psychosis, that low blood GSH levels at baseline were associated with greater loss of cortical gray matter over a two-year period, although HC volume was not assessed in this study (Fraguas et al., 2012). Despite the scarcity of clinical studies, there are some experimental studies supporting our findings of a link between increased oxidative stress and the HC. In a post-mortem study, Che et al. found that oxidative damage to nucleic acids in neurons was significantly increased in CA1, CA3 and DG of brain tissue from subjects with schizophrenia, bipolar disorder and MDD (Che et al., 2010). Moreover, Chang et al. showed that administration of Vitamin C could ameliorate lead-induced oxidative damage in the developing rat hippocampus (Chang et al., 2012). In addition, another animal study found that a post-natal deficiency in Vitamin C was associated with a lower total number of neurons in the HC (Tveden-Nyborg et al., 2009). The results from the present study are generally consistent with, and add to, these preclinical findings in a clinical setting.

4.3. Certain HC subfields are more strongly associated with oxidative stress

While the total HC volume was significantly correlated with the antioxidant score, we found that the hippocampal region most robustly associated with oxidative stress was the CA3&DG subfield. In support of our findings, preclinical data suggest that this HC subfield may be especially sensitive to oxidative stress (Chang et al., 2012; Uysal et al., 2012). Cruz-Sanchez et al. suggested, in a postmortem study in Alzheimer's disease, that an oxidative stress pathway starts in the CA3 subfield, progresses to CA1, and then to other brain regions, building a model for Alzheimer's disease progression (Cruz-Sanchez et al., 2010). Moreover, Steullet et al. demonstrated, in an animal model, that glutathione deficiency results in a more pronounced impairment of the structural and functional integrity of fast spiking interneurons, that are important for information processing, in CA3&DG compared to CA1 (Steullet et al., 2010), suggesting that the former hippocampal region is of particular importance in the interaction between oxidative stress and brain dysfunction. These latter findings may help to explain our significant association between oxidative stress and CA3&DG volume but not CA1 volume. Interestingly, the DG is an important area for stem cell proliferation, a process than may be suppressed by oxidative stress (Huang et al., 2012). In line with our hypothesis, the CA1-2 transition was not significantly correlated with total net antioxidant score. Indeed, CA2 has been considered a more resistant subfield because it tends to be spared from pathology (Sadowski et al., 1999). In support of this notion, an animal model of epilepsy suggested that CA2 had better defenses against seizure-induced oxidative stress than other HC subfields (Sashindranath et al., 2010). However, due to our

small sample size, it is possible that the lack of significant correlations between oxidative stress and CA1 and CA1-2 transition zone volumes might represent type II statistical errors.

4.4. No significant between-group differences in oxidative stress or HC volumes

In the present study, we did not find significant differences in peripheral oxidative stress markers or in HC volumes between MDD subjects and healthy controls. This could be due to our small sample size with limited power to detect significant differences, although other explanations are possible. As reviewed by Ng et al., findings of altered antioxidant enzyme levels in MDD patients have been mixed, with studies showing increased, decreased or unchanged levels (Ng et al., 2008). Our experimental design was maximized to detect differences by excluding individuals with somatic illnesses or those taking medications (including antidepressants) that could potentially affect oxidative stress markers. This restriction may have contributed to differences in our results from certain other studies that found differences in oxidative stress markers between depressed and control individuals. We also did not find any significant differences in HC volume between MDD patients and controls. This result is in line with some (Posener et al., 2003), but not all (Hickie et al., 2005), studies. Although most studies report diminished HC volume in MDD subjects, there is variability in this finding for reasons that have not yet been fully explained (McKinnon et al., 2009).

4.5. Selection of oxidative stress markers

We assessed levels of GSH, Gpx, Vitamin C, and GSSG as indicators of oxidative stress. Experimental studies have shown that Vitamin C is an important antioxidant that additionally plays a significant role in neuronal differentiation and maturation (Lee et al., 2003; Qiu et al., 2007). Gpx is an intracellular antioxidant enzyme that reduces hydrogen peroxide to water in order to limit its damaging effects and thereby mitigates the susceptibility for cell death (Lubos et al., 2011). Gpx also reduces lipid and non-lipid hydroperoxides at the expense of reduced GSH which is, in turn, oxidized, forming GSSG (Flohe, 1971). As reviewed by Ghosh et al., GSH and Gpx have powerful antioxidant properties, and deficiency in these compounds may be involved in the pathophysiology of neurodegenerative disorders such as Alzheimer's and Parkinson's disease (Ghosh et al., 2011). GSH and GSSG can be released locally in the brain, mainly from astrocytes, and perturbations in these systems may lead to increased oxidative stress, impaired neuronal function, and progression of neurodegeneration (Dringen and Hirrlinger, 2003). However, the relationship between glutathione concentrations in the brain and in the blood is not well-described.

4.6. Strengths and limitations of the present study

To the best of our knowledge, the study by Quinn et al., in subjects with Alzheimer's Disease, is the only previous clinical study showing associations between HC volume and peripheral, as well as central, indicators of oxidative stress (Quinn et al., 2003). In that study, approximately half of the study participants were taking a daily Vitamin C supplement at doses ranging from 500-2000 mg per day. Thus, a specific advantage of the present study was that none of the subjects was receiving exogenous antioxidant supplementation. Moreover, all subjects in our study were medically healthy, and did not

take any potentially interfering medications (including antidepressants) for at least six weeks before the study.

Another strength of the study is our use of high resolution MRI with in-house developed, reliable mapping protocols and tracing methods that allowed separate volume estimation of individual HC subfields. Moreover, we calculated a total net antioxidant score using standardized levels of oxidative stress markers in order to limit the number of statistical calculations and to simultaneously consider multiple sources of antioxidant potency. This approach of combining variables into a single score has also been advocated in order to preserve degrees of freedom and to avoid over-fitting in regression models (Babyak, 2004), and may thus be seen as an additional advantage of our study.

A limitation in the present study is the small sample size; therefore, our results should be considered preliminary, and future replications are warranted. As previously discussed, our study may have been underpowered in some aspects, which particularly limits interpretation of data from the two individual groups of subjects. Even though we had specific hypotheses based on experimental studies, and even though the subfield analyses were construed as exploratory, we did not correct for multiple comparisons in the subfield analyses, and this may be regarded as an additional weakness of the study. An important caveat in interpreting our data is that, while significantly correlated with HC volume, our oxidative stress markers are derived from plasma, and their relationship to brain oxidative stress processes is unknown. Therefore we cannot infer causality between increased oxidative stress in the periphery and smaller HC volume. However, results from a previous study suggest that peripheral oxidative stress markers are indeed associated with brain oxidative stress. Quinn et al. reported a strong association between plasma and CSF levels of Vitamin C (Quinn et al., 2003), suggesting that peripheral markers of at least this antioxidant molecule are reflected in the brain, which supports the findings of the present study. Moreover, Quinn et al. found that HC volume was inversely correlated with the CSF-to-plasma ratio of Vitamin C, suggesting that peripheral Vitamin C may be an even more robust correlate of HC volume than central levels.

4.7. Conclusion

This is among the first clinical studies to demonstrate a relationship between HC volume and peripheral oxidative stress markers. Although the relationship between these peripheral markers and brain oxidative stress is unknown, these preliminary data are consistent with models of oxidative stress being associated with diminished HC volume, and of the CA3&DG possibly being more strongly associated with oxidative stress. These findings suggest that peripheral markers of oxidative stress may be helpful to monitor in aging and various neuropsychiatric diseases as an index of hippocampal involvement. Moreover, peripheral oxidative stress markers might find increasing use as peripheral biomarkers of CNS pathology and as probes of oxidative stress involvement in CNS illnesses. It would be important in future clinical trials to assess how different pharmacological or behavioral therapies might alter a specific barometer of oxidative status.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

1. We included 16 unmedicated MDD subjects and 19 healthy controls

2. We measured hippocampal (HC) volume and plasma levels of oxidative stress markers

3. Total net antioxidant score was positively correlated with HC volume

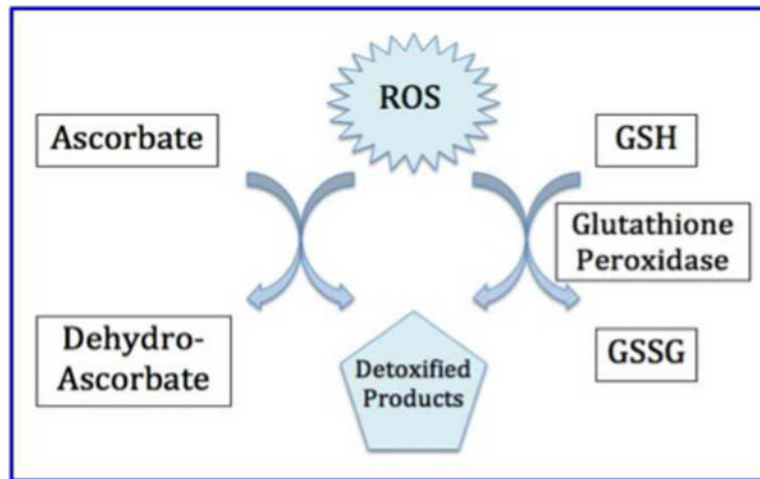4. The HC subfield most strongly associated with oxidative stress was CA3&DG
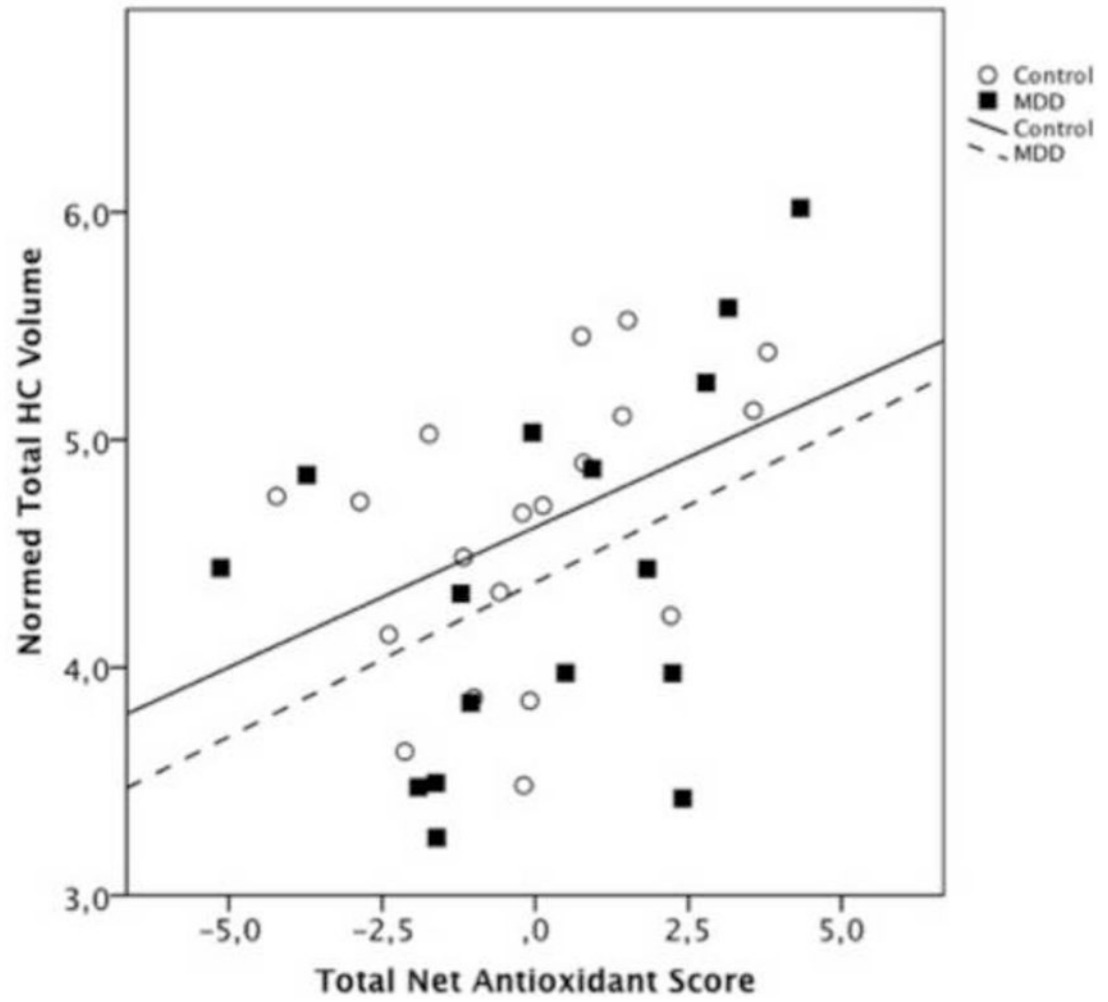
Figure 1.
Reactive oxygen species (ROS) are detoxified by reduced glutathione (GSH), which is oxidized to oxidized glutathione (GSSG) by the antioxidant enzyme, Glutathione Peroxidase. Similarly, the antioxidant Ascorbate (Vit. C) detoxifies ROS in its conversion to Dehydro-ascorbate.
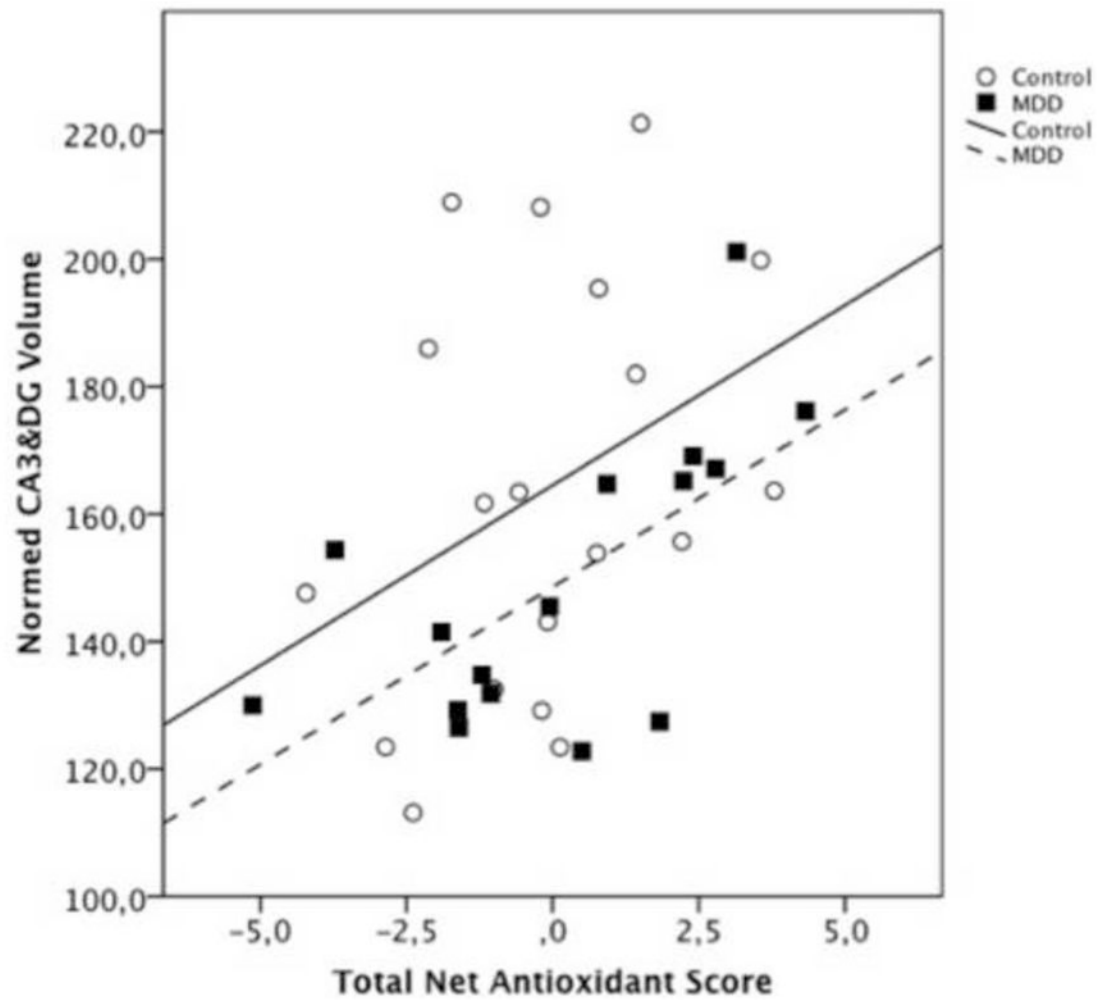
2A

2B

Figure 2.
A. Total hippocampal (HC) volume plotted against total net antioxidant score in MDD subjects and controls. The association in the two groups combined was significant after controlling for age and gender (*beta*=0.36, *P*=0.040).
B. CA3 & Dentate Gyrus (DG) subfield volume plotted against total net antioxidant score in MDD subjects and controls. The association in the two groups combined was significant after controlling for age and gender (*beta*=0.40, *P*=0.018)

Table 1A

Demographics.

Non-normally distributed variables were log-transformed (Ln) into normality before analyses. Raw values are presented here, since they are easier to interpret.

	Control	MDD	Statistical test
Number	19	16	
Sex	63% Female	63% Female	Pearson chi-square= 0.00, *P*= 0.97
Age (Yrs, mean + SD)	36.5 + 12.1	33.6 + 7.2	Student's T-test, *t*= 0.86, *P*= 0.40
Ethnicity	14 Caucasians 2 African-Americans 1 Asians 2 Others	11 Caucasians 2 African-Americans 1 Asian 2 Other	Pearson chi-square= 0.10, *P*= 0.99
Body Mass Index (mean + SD)	24.56 + 3.79	24.24 + 3.75	Student's T-test, *t*= 0.26, *P*= 0.79
17-item Hamilton Depression Rating Scale score (mean + SD)	N/A	19.05 + 3.58	N/A

Table 1B. Mean (+ SD) levels of oxidative stress markers in MDD patients and controls. Total net antioxidant score (Mean + SD) was calculated with standardized scores of normally distributed raw data using the following formula: (GSH+Glut Perox+Vitamn C)-GSSG. Thus, positive or negative scores are possible.

	Control (n=19)	MDD (n=16)	Statistical test (Student's T-test)
Total net antioxidant score	-0.13 + 2.10	0.12 + 2.62	*t*= -0.30, *P*=0 .76
Vitamin C (µg/mL)	8.13 + 6.09	7.05 + 6.23	*t*= 0.68, *P*=0 50
GSH (µM)	1127.42 + 448.17	1234.44 + 731.38	*t*= -0.14, *P*= 0.89
GSSG (µM)	90.77 + 41.94	71.96 + 38.03	*t*= 1.53, *P*= 0.14
Glutathione Peroxidase (U/L)	261.21 + 31.65	258.81 + 34.82	*t*= 0.21, *P*= 0.83

Table 1C. Mean (+ SD) Total hippocampal (HC) volume and HC subfields, normed to total intracranial volume.

Values are sums of both sides.

	Control (n=19)	MDD (n=16)	Statistical test (Student's T-test)
Total Hippocampus	4.60 + 0.61	4.39 + 0.82	*t*= 0.87, *P*= 0.39
CA1 Subfield	233.15 + 36.70	222.61 + 33.32	*t*= 0.88, *P*= 0.38
CA1-CA2 transition zone Subfield	11.35 + 1.71	10.67 + 1.23	*t*= 1.28, *P*= 0.21
CA3&DG Subfield	163.80 + 32.75	149.20 + 22.66	*t*= 1.50, *P*= 0.14
Subiculum Subfield	114.66 + 23.31	116.21 + 16.82	*t*= -0.23, *P*= 0.82