

# Lower whole blood glutathione peroxidase (GPX) activity in depression, but not in myalgic encephalomyelitis / chronic fatigue syndrome: another pathway that may be associated with coronary artery disease and neuroprogression in depression

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## Abstract

**BACKGROUND:** Major depression and myalgic encephalomyelitis / chronic fatigue syndrome (ME/CFS) are two disorders accompanied by an upregulation of the inflammatory and oxidative and nitrosative (IO&NS) pathways and a decreased antioxidant status. Moreover, depression is accompanied by disorders in inflammatory and neuroprogressive (IN-PRO) pathways.

**METHODS:** This study examines whole blood glutathione peroxidase (GPX) in depression and in ME/CFS; GPX is an enzyme that reduces hydroperoxides by oxidizing glutathione and consequently protects the cells from oxidative damage. Blood was sampled in 39 patients with depression, 40 patients with ME/CFS and 24 normal volunteers. Whole blood was analysed for GPX activity using the Ransel assay (Randox). Severity of illness was measured by means of the Hamilton Depression Rating Scale (HDRS) and the Fibromyalgia and Chronic Fatigue Syndrome Rating Scale (FF scale).

**RESULTS:** We found that whole blood GPX activity was significantly ( $p=0.001$ ) lower in depressed patients than in normal controls and that there were no significant differences between ME/CFS and controls. In depression and ME/CFS, there were significant and inverse relationships between GPX activity and the FF items, depressed mood and autonomic symptoms. In depression, there were significant and negative correlations between whole blood GPX and the HDRS score and autonomic symptoms.

**DISCUSSION:** The results show that lowered whole blood GPX activity contributes to the lowered antioxidant status in depression. Since GPX activity is a predictor of neuroprogression and coronary artery disease (CAD), lowered GPX activity in depression contributes to the IN-PRO pathways and the comorbidity between depression and CAD. Our results suggest that patients with depression would benefit from Ebselen or a supplementation with glutathione, N-Acetyl-L-Cysteine and selenium.

**Abbreviations:**

- CMS - chronic mild stress
- FF scale - Fibromyalgia and Chronic Fatigue Syndrome Rating Scale
- GPX - glutathione peroxidase
- HDRS - Hamilton Depression Rating Scale
- IN-PRO - inflammatory and neuroprogressive
- IO&NS - inflammatory and O&NS
- LDL - low density lipoproteins
- ME/CFS - myalgic encephalomyelitis / chronic fatigue syndrome
- O&NS - oxidative and nitrosative stress
- PMN - polymorphnuclear
- TRD - Treatment resistant depression

## INTRODUCTION

In aerobic organisms different mechanisms are involved in scavenging free radicals, including enzymes, such as glutathione peroxidase (GPX) (Mill, 1957; Joseph, 1995). The GPX antioxidant enzyme family consists of different isoforms, two of which are present in whole blood, i.e. the cellular GPX1 expressed in red blood cells and the extracellular GPX3 found in plasma and expressed in kidney, lung, heart, breast, placenta, and liver (Chu et al. 1992), but secreted from renal proximal tubular cells and parietal epithelial cells of Bowman's capsule (Avisar et al. 1994; Whitin et al. 2002). GPX1 and GPX3 are immunologically distinct forms which display differences in physical properties and show only a moderate amino acid sequence homology (Chambers et al. 1986; Takahashi et al. 1990). Both GPX1 and GPX3 enzymes contain selenium and are tetramers of four identical subunits each of which presents a selenocysteine in the active site (Flohe, 1978; Margis et al. 2008).

The selenium-dependent GPXs reduce glutathion disulfide (GSSG), the oxidized form of glutathione, into the reduced sufhydryl form, i.e. GSH (Meister, 1988; Mannervil, 1987). The GPXs catalyse the reduction of hydrogen peroxide and lipid peroxides to water or the corresponding alcohols at the expense of GSH, which is used as the ultimate electron donor to regenerate the reduced form of selenocysteine (Meister, 1988; Mannervil, 1987; Ursini and Bindoli, 1987). In resting conditions the selenocysteine site is in a Selenium(-) (Se-) form and will be oxidized by peroxides to SeOH which is then trapped by a GSH molecule to Se-sulfhydryl group (SG) and by another GSH molecule to Se(-) again. Thus, the GPXs are key antioxidant enzymes that scavenge free radicals and as

such have a central role in the control of reactive oxygen species (ROS) (Herbette et al. 2007). This explains why selenium-dependent GPXs protect cells and enzymes from oxidative damage. GPX1 clearly acts as a strong antioxidant with a major protective role in coping with oxidative injury and death, as demonstrated in GPX1 knockout and transgenic mice (Lei, 2002). There is now evidence that abnormal GPX1 and GPX3 expression is relevant to the etiology of different disorders, such as cancer, cardiovascular disease, including arteriosclerosis and stroke, neurodegeneration, or diabetes (Lei, 2002; Voetsch et al. 2007).

Lower GPX may also be involved in the pathophysiology of depression. Kodykova et al. (2009) found that women with depression had significantly lowered GPX1 activities. Oczan et al. (2004) detected that GPX activity was significantly lower in patients with affective disorders than in controls. In olfactory-bulbectomized rats, a depression model, GPX activity was decreased (Song et al. 1994). In male Wistar rats, chronic mild stress (CMS)-induced depression was accompanied by lowered brain cortex GPX activity (Eren et al. 2007a; 2007b). Other authors (Srivastana et al. 2002; Andreazza et al. 2009) were unable to detect significant differences in GPX activity in plasma or polymorphnuclear (PMN) leukocytes, between depressed or bipolar patients and controls.

The abovementioned changes in GPX activity take part in an overall decreased TAS in depression, which is characterized by lowered vitamin E, zinc, coenzyme Q10, vitamin C, tryptophan, tyrosine, albumin, glutathione, and catalase activity (Kodykova et al. 2009; Oczan et al. 2004; Maes and Meltzer, 1995; van Hunsel et al. 1996; Maes et al. 1994; 1997; 2000; 2009b; Owen et al. 2005; Khanzode et al. 2003). These decreased defences against oxidative and nitrosative stress (O&NS) play a role in the damage caused by O&NS in depression, which is evidenced by increased levels of malondialdehyde (MDA), 8-hydroxy-2-deoxyguanosine, and IgM responses against phosphatidyl inositol and NO-albumin (Forlenza and Miller, 2006; Sarandol et al. 2007; Maes et al. 2007c; 2008). A lowered antioxidant status plays a role in the activation of inflammatory and O&NS (IO&NS) and neuroprogressive (IN-PRO) pathways in depression (Maes, 2008; Maes et al. 2009c; Berk et al. 2011; Maes et al. 2011). Neuroprogression indicates decreased neurogenesis, neurodegenerative processes and increased neural apoptosis (Berk et al. 2011).

Another medical illness characterized by activated IO&NS pathways is myalgic encephalomyelitis / chronic fatigue syndrome (ME/CFS) (Maes, 2009; Maes et al. 2007a; 2007b). ME/CFS is accompanied by a decreased antioxidant status, as evidenced by lower serum zinc, dehydroepiandrosterone sulfate and plasma COQ10 (Maes et al. 2005; 2006a; 2009a); increased O&NS, as evidenced by higher isoprostane; oxidized low density lipoproteins (LDL); LDL thiobarbituric acid reactive substances (TBARS) and protein carbonyl levels and damage by O&NS to functional proteins and membrane fatty acids (Vecchiet et al. 2003; Kennedy et al. 2005b; Smirnova et al. 2003;

Jammes et al. 2005; Maes et al. 2006b; 2007c; 2008). Fulle et al. (2000) detected an increased GPX activity in the muscles of ME/CFS patients, but to the best of our knowledge no studies have examined the activity of GPX in whole blood of ME/CFS patients. Moreover, there is a strong comorbidity between depression and ME/CFS that is based on shared pathways, such as activated IO&NS pathways, dysfunctional mitochondria; lowered antioxidant levels; lowered omega-3 polyunsaturated fatty acid levels; and increased translocation of gram-negative bacteria (Maes, 2010). Other pathways, however, may discriminate both disorders, e.g. induction of indoleamine 2-3-dioxygenase and neuroprogression that are more specific to depression, and the 2'-5' oligoadenylate synthetase / RNase L pathway that typically occurs in ME/CFS.

The present study has been performed in order to examine GPX activity in whole blood of major depressed and ME/CFS patients and in normal controls under the working hypothesis that GPX is lowered in both depression and ME/CFS.

## SUBJECTS AND METHODS

### Subjects

One-hundred and three subjects participated in this study, i.e. 39 major depression patients, 40 ME/CFS patients and 24 normal controls. The patients were all outpatients admitted to the Maes Clinics, Antwerp, Belgium. Major depression was diagnosed according to the DSM-IV-R criteria (APA, 2000), using a semistructured interview. The Severity of depression was measured with the Hamilton Depression Rating Scale (HDRS) (Hamilton, 1960). Staging of treatment resistance was based on prior treatment responsivity according to the criteria of Thase and Rush (1995). Treatment resistant depression (TRD) was diagnosed if the patients showed a non-response to at least two adequate trials with antidepressant agents from different classes, e.g. tricyclics (TCSS) or selective serotonin reuptake inhibitors (SSRIs). ME/CFS was diagnosed by means of the Centres for Disease Control and Prevention (CDC) criteria (Fukuda et al. 1994). The severity of illness was measured using the Fibromyalgia and Chronic Fatigue Syndrome Rating Scale (FF scale) (Zachrisson et al. 2002). This scale measures 12 symptoms, i.e. pain, muscular tension, fatigue, concentration difficulties, failing memory, irritability, sadness, sleep disturbances, autonomic disturbances, irritable bowel, headache, and subjective experience of infection. The total sum on this scale is employed as a measure of the severity of illness. This FF scale was also scored in the depressed patients.

We have excluded the following subjects or patients: a) subjects with medical illnesses, e.g. inflammatory bowel disorders, diabetes type 1 or type 2, hypertension, etc.; b) patients with abnormal blood tests, such as alanine aminotransferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), thyroid stimulating hormone (TSH) and total protein; c) subjects with acute inflammatory or allergic reactions the last 2 months prior to the study; d) patients with life-time diagnoses of psychiatric DSM IV-R disorders, e.g. psychotic, substance use and organic mental disorders; e) ME/CFS patients who (previously) had been diagnosed with depression; f) subjects who had been treated with anti-psychotic drugs, anticonvulsants or mood stabilizers; and g) subjects who had taken dietary supplements with glutathione, N-acetyl-L-cysteine (NAC) and selenium. Patients and controls gave written informed consent after the study protocol was fully explained; the study has been approved by the local ethical committee.

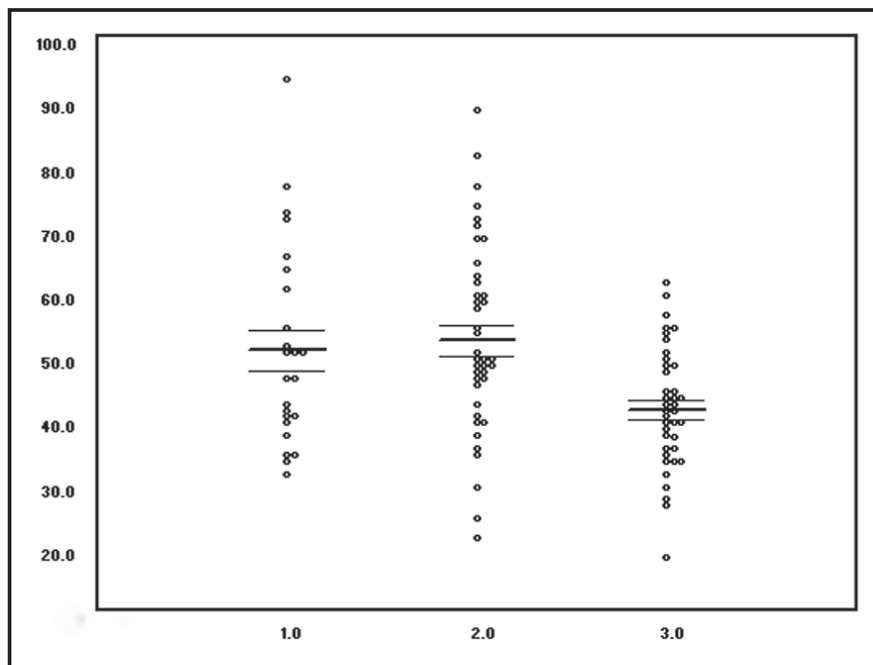
tase (ALP), blood urea nitrogen (BUN), thyroid stimulating hormone (TSH) and total protein; c) subjects with acute inflammatory or allergic reactions the last 2 months prior to the study; d) patients with life-time diagnoses of psychiatric DSM IV-R disorders, e.g. psychotic, substance use and organic mental disorders; e) ME/CFS patients who (previously) had been diagnosed with depression; f) subjects who had been treated with anti-psychotic drugs, anticonvulsants or mood stabilizers; and g) subjects who had taken dietary supplements with glutathione, N-acetyl-L-cysteine (NAC) and selenium. Patients and controls gave written informed consent after the study protocol was fully explained; the study has been approved by the local ethical committee.

### Methods

Blood for the assay of GPX activity was taken in the morning hours (8.30-11.30) after an overnight fast. We assayed GPX in whole blood by employing the RANSEL kit (RANDOX, Randox Laboratories Ltd, 55 Diamond Road, Crumlin, Co Antrim, United Kingdom, BT29 4QY). The RANSEL method is based on the method developed by Paglia and Valentine (1967). GPX catalyses the oxidation of glutathione by cumene hydroperoxide; in the presence of glutathione reductase and NADPH the oxidized glutathione is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP<sup>+</sup>. The reaction is performed on an automated chemistry analyzer (Dayona from RANDOX) and the decrease in absorbance at 340 nm is measured. The GPX concentration may be calculated as U/L of hemolysate = 8412 x  $\Delta A$  340 nm/minute. Under our assay conditions, one unit of GPX is defined as the amount of enzyme that catalyses the transformation of 1  $\mu$ mol of NADPH per minute. The activity of GPX is expressed in U/g Hb and is calculated as the activity of GPx in U/L divided by the concentrations of Hb in g/L. The precision of our assays is checked with the controls provided by RANSEL. Our assay can be used to measure all of the glutathione-dependent peroxidases in whole blood. The inter-assay coefficient of variation is < 7%.

### Statistics

Differences between treatment means were analyzed by analysis of variance (ANOVA) or covariance (ANCOVA). Relationships between variables were ascertained by means of Pearson's product-moment correlation coefficients, regression analyses and multiple regression analyses with an *p*-to-enter of *p*=0.05. The independence of classification systems was checked by means of analysis of contingency Tables ( $\chi^2$ -test) and Fisher's exact probability test. In order to assess the symptom profiles of different groups we used a stepwise discriminant multiple ANOVA (MANOVA) with an *F*-to-enter of *p*=0.05. The diagnostic performance of plasma CoQ10 for depression and TRD was checked by means of ROC (receiver operating characteristics) analysis with computation of the area under the ROC



**Figure 1.** Scatter plot of whole blood glutathione peroxidase (GPX) activity (expressed in U/g Hb) in normal controls (1.0), patients with myalgic encephalomyelitis / chronic fatigue syndrome (2.0) and major depression (3.0).

curve, sensitivity, specificity and predictive value of a positive test result (PV+) and with kappa statistics (Zweig and Campbell, 1993). The significance was set at  $\alpha=0.05$  (two tailed).

## RESULTS

Figure 1 shows that there are significant differences in whole blood GPX activity between the three study groups ( $F=7.2$ ,  $df=2/100$ ,  $p=0.001$ ). The Dunn-Scheffe test showed that major depressed patients had significantly lower GPX values than normal controls ( $t=2.66$ ,  $p=0.008$ ) and ME/CFS patients ( $t=3.59$ ,  $p=0.0008$ ), while there were no significant differences in whole blood GPX between normal controls and ME/CFS patients ( $t=0.44$ ,  $p=0.7$ ). Patients with ME/CFS (mean age= $37.5 \pm 14.5$  years) were somewhat younger ( $F=4.4$ ,  $df=2/100$ ,  $p=0.01$ ) than normal controls (mean age= $45.5 \pm 9.9$  years;  $t=2.47$ ,  $p=0.015$ ) and depressed patients (mean age= $44.6 \pm 11.8$  years;  $t=2.55$ ,  $p=0.01$ ), while there were no significant differences in age between depressed patients and controls ( $t=0.24$ ,  $p=0.8$ ). The male / female ratio was somewhat different ( $\chi^2=7.9$ ,  $df=2$ ,  $p=0.02$ ) between depressed patients (17/22), ME/CFS patients (5/35) and controls (7/17), with the only intergroup difference being a lower male/female ratio in ME/CFS than in depressed patients ( $\chi^2=8.0$ ,  $df=1$ ,  $p=0.005$ ). However, covarying for age and sex in an ANCOVA did not change the abovementioned results on the differences in GPX among the groups ( $F=6.4$ ,  $df=2/98$ ,  $p=0.003$ ). Age ( $F=0.2$ ,  $p=0.7$ )

and gender ( $F=0.00$ ,  $p=0.9$ ) were not significant in this ANCOVA. Lower GPX yielded a significant albeit very weak diagnostic performance for major depression versus controls and ME/CFS patients: at  $GPX < 40$  U/g Hb we found a sensitivity = 51.3% and a specificity of 71.9% ( $\kappa=0.23$ ,  $t=2.31$ ,  $p=0.02$ ).

In the combined patient group, we detected significant inverse correlations between whole blood GPX and the FF symptoms depressed mood ( $r=-0.38$ ,  $p=0.0008$ ) and autonomic symptoms ( $r=-0.24$ ,  $p=0.03$ ). We found that 19.8% of the variance in GPX was explained ( $F=9.5$ ,  $df=2/76$ ,  $p=0.0004$ ) by the regression on depressed mood ( $F=13.2$ ,  $p=0.0008$ ) and autonomic symptoms ( $F=4.89$ ,  $p=0.003$ ). Discriminant MANOVA showed that two FF items significantly ( $F=8.8$ ,  $df=1/77$ ,  $p=0.004$ ) discriminated patients with lower ( $< 40$  U/g Hb) versus higher ( $> 40$  U/g Hb) GPX activity, e.g. depression and autonomic symptoms (loadings on the discriminant score were 0.82 and 0.60, respectively).

In depressed patients we found a significant and negative correlation between whole blood GPX and the HDRS score ( $r=-0.36$ ,  $p=0.02$ ), but not with the FF score ( $r=0.28$ ,  $p=0.08$ ), although there was a significant and positive correlation between the HDRS and the FF score ( $r=0.58$ ,  $p=0.00003$ ). We found significant and negative correlations between whole blood GPX and two FF symptoms, i.e. autonomic symptoms ( $r=-0.42$ ,  $p=0.007$ ) and irritability ( $r=-0.35$ ,  $p=0.03$ ). GPX activity was not significantly different between TRD and non-TRD patients ( $F=0.00$ ,  $df=1/37$ ,  $p=0.9$ ), nor between depressed patients who had been taking antidepressants and those who did not ( $F=0.00$ ,  $df=1/37$ ,  $p=0.9$ ;

17 with versus 22 without). Thus, any possible effects of use of antidepressants on the results can be disregarded.

## DISCUSSION

This study found that whole blood GPX activity was significantly lower in major depressed patients as compared with controls and ME/CFS patients and that there were no significant differences in GPX activity between patients with ME/CFS and controls.

The first major finding of this study is that depression is characterized by a lowered whole blood GPX activity and that it is significantly and inversely correlated to the severity of illness, as measured with the HDRS score and symptoms, such as depressed mood and irritability, another characteristic of depression. These findings corroborate those of Kodydková et al. (2009) and Oczan et al. (2004) who found a lowered GPX activity in depressed patients. In some other studies no significant differences could be found in GPX activity between depressed patients and controls (Srivastava et al. 2002; Andreatza et al. 2009). However, Andreatza et al. (2009) examined bipolar disorder, while we examined major depressed patients in the acute phase of their illness. Srivastava et al. (2002) assayed GPX activity in PMNs, whereas we determined whole blood GPX activity. In any case, the findings on lower GPX activity are in agreement with translational studies which show lowered GPX activity in the bulbectomized rat and in Wistar rats with CMS-induced depression (Song et al. 1994; Eren et al. 2007a; 2007b). In addition, reduced concentrations of glutathione have been detected in animal models of stress-induced depression (Khazode et al. 2003; Pal and Dandiya, 1994). Lamotrigine and escitalopram, but not venlafaxine, significantly increased GPX activity in the brain of CMS-induced rats (Eren et al. 2007a). In another human study, it was found that GPX activity normalized during treatment (Oczan et al. 2004). Taken together, those results show that lower GPX activity plays a role in the pathophysiology of depression.

The findings on lower whole blood GPX activity extent previous findings that the antioxidant capacity in the blood of depressed patients is diminished as has been evidenced by lowered vitamin E and C, serum zinc, tryptophan, tyrosine, albumin, glutathione, CoQ10 and catalase activity (see Introduction). The abovementioned results are also in agreement with other studies showing a decrease in the TAS in the blood of depressed patients (Galecki et al. 2009) and depressed patients with leukemia (Zhou et al. 2006).

The lower GPX activity and impaired antioxidative protection arguably predispose towards an induction of the IO&NS pathways and damage to membrane fatty acids and functional proteins and, by inference, to the neurotoxic damage that occurs in depression (Flohe, 1978). Moreover, GPX has neuroprotective properties on its own. This may be deduced from studies using Ebselen (2-phenyl-1,2-benziselenazol-3[2H]-one),

which mimics the activity of glutathione peroxidase (Muller et al. 1984) and which displays potent antioxidant and neuroprotective effects in vitro and in vivo (Posser et al. 2009). Ebselen displays neuroprotective properties against a number of different injuries, such as oxidative stress and oxidative damage of DNA that is involved in the delayed neuronal death in the brain regions; trimethyltin hydroxide-induced hippocampal injury; and neuronal damage and death caused by ischemic attacks and coronary artery occlusion (Satoh et al. 2004; Jean Harry et al. 2003; Seo et al. 2009; Li and Cao, 2002; He et al. 2007). Ebselen prevents impaired neurogenesis within the dentate gyrus of the hippocampus as shown in a rat model of alcoholism (Johnsen-Soriano et al. 2007). The neuroprotective effects of Ebselen are obtained through different mechanisms, which are all relevant to major depression (Flohe, 1978), such as increases in glutathione, ROS-scavenging activity; glutamate-related mechanisms; a decreased expression of nuclear factor kappa beta and inducible nitric oxide synthase (iNOS); decreases in pro-inflammatory cytokines, such as interleukin-1 and tumor necrosis factor alpha; and inhibition of indoleamine 2,3-dioxygenase (Porciúncula et al. 2003; 2004; Yoshizumi et al. 2004; József and Filep, 2003).

Our results that GPX is decreased in depression suggest that those patients would benefit from a treatment with antioxidants that modulate the GPX-glutathione pathways. Toward this end, not only Ebselen (see this Discussion), but also a combination of supplements with NAC, glutathione, cysteine and selenium is useful (Maes, 2011). Recently, it has been shown that NAC is an effective augmentation strategy to treat depressive symptoms in bipolar disorder (Berk et al. 2008). Relevant to the IN-PRO pathophysiology of depression is the finding that the neuroprotective activity of Ebselen is augmented by NAC (Arakawa et al. 2007). Moreover, Ebselen exhibits antidepressant effects. In the rodent forced swimming test, Ebselen produces an antidepressant effect that seems to be dependent on its interaction with the noradrenergic and dopaminergic systems (Posser et al. 2009). In mouse, Ebselen prevents the biochemical effects of prolonged immobilization stress, including increased interleukin-1, cyclooxygenase-2 and nicotinamide adenine dinucleotide phosphate-oxidase in the brain, which eventually induce neuronal cell death in the cerebral cortex (Lee et al. 2006).

Another finding of this study is that lowered GPX activity is significantly correlated to the presence of autonomic symptoms, which occur in depression (Maes et al. 1993). There is ample evidence that glutathione and GPX are involved in the function of the autonomic system. For example, GSH has an inhibitory whereas GSSH has a stimulatory effect on sympathetic nerve activity (Murakami et al. 1987). GSH decreases blood pressure (Kennedy et al. 2005a). Systemic depletion of GSH is found to be related to dysfunctions of the cardiac vagal system in hepatitis C patients (Barbaro et al. 1997). In stress-exposed rats, reduced glutathione levels correlate with increased

sympathetic activity (Mercanoglu et al. 2008). The latter is the major contributing factor for the induction and prognosis of myocardial infarction. As such, lowered GPX and GSH may contribute to the comorbidity between depression and cardiovascular disorders which we have described in detail somewhere else (Maes et al. 2010).

In our study we were unable to detect any differences in whole blood GPX between ME/CFS patients and normal controls. Previously, increased GPX activity was detected in the muscles of patients with ME/CFS, indicating increased oxidative stress in muscles with consequently induced antioxidant enzyme activities (Fulle et al. 2000). The findings of our study, however, do not provide further evidence, either for disorders in GPX in the whole blood of patients with ME/CFS, or for a role of GPX activity in the IO&NS pathophysiology of ME/CFS.

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