The glutathione system: a new drug target in neuro-immune disorders.

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Abstract

Glutathione (GSH) has a crucial role in cellular signaling and antioxidant defenses either by reacting directly with reactive oxygen or nitrogen species or by acting as an essential cofactor for GSH S-transferases and glutathione peroxidases. GSH acting in concert with its dependent enzymes, known as the glutathione system, is responsible for the detoxification of reactive oxygen and nitrogen species (ROS/RNS) and electrophiles produced by xenobiotics. Adequate levels of GSH are essential for the optimal functioning of the immune system in general and T cell activation and differentiation in particular. GSH is an ubiquitous regulator of the cell cycle per se. GSH also has crucial functions in the brain as an antioxidant, neuromodulator, neurotransmitter and enabler of neuron survival. Depletion of GSH leads to exacerbation of damage by oxidative and nitrosative stress, hyper-nitrosylation, increased levels of proinflammatory mediators and inflammatory potential, dysfunctions of intracellular signaling networks, e.g. p53, nuclear factor-κB and Janus kinases, decreased cell proliferation and DNA synthesis, inactivation of complex I of the electron transport chain, activation of cytochrome c and the apoptotic machinery, blockade of the methionine cycle and compromised epigenetic regulation of gene expression. As such, GSH depletion has marked consequences for the homeostatic control of the immune system, O&NS pathways, regulation of energy production and mitochondrial survival as well. GSH depletion and concomitant increase in O&NS and mitochondrial dysfunctions play a role in the pathophysiology of diverse neuro-immune disorders, including depression, Myalgic Encephalomyelitis / chronic fatigue syndrome and Parkinson's disease, suggesting that depleted GSH is an integral part of these diseases. Therapeutical interventions that aim to increase GSH concentrations in vivo include N-acetyl-cysteine, Nrf-2 activation via hyperbaric oxygen therapy, dimethyl fumarate, phytochemicals, including curcumin, resveratrol and cinnamon, and folate supplementation.

Key words: glutathione, oxidative and nitrosative stress, inflammation, cytokines, depression, Myalgic Encephalomyelitis / chronic fatigue, immune

1. Introduction

γ-L-glutamyl-L-cysteinyl-glycine or glutathione is a tripeptide with multiple cellular functions. There are two forms of glutathione; the oxidized form (glutathione disulfide or GSSG) and the reduced form (GSH). Glutathione is a key antioxidant, either by reacting with reactive nitrogen species (RNS), reactive oxygen species (ROS), hypochlorous acid (HOCL), hydroxyl radicals ($HO^{\bullet}$), and other reactive species, or via its role as an indispensable cofactor for numerous enzymes including the glutathione peroxidases and glutathione S-transferases [1].

Glutathione has several key functions in diverse cellular populations. The GSH / GSSG redox couple acts as a vital cellular redox buffer enabling the optimum performance of a myriad of redox sensitive biochemical and biophysical processes [1]. Glutathione is a key component of the antioxidant defenses in the cell and the detoxification of ROS and RNS [2]. S-Glutathionylation, i.e. the posttranscriptional addition of glutathione to protein cysteine, modifies the activity of many proteins, including intracellular signaling molecules, and inhibits the activity of many important enzymes. The glutathione-related redox state of cells plays an important role in the regulation of pro-inflammatory cytokines, including tumor necrosis factor-α (TNFα), interleukin (IL)-1β and IL-6, and the activity of intracellular signaling pathways [3]. Glutathione has antiviral effects and can inhibit the replication of many viruses. Glutathione modulates the function of T cells and natural killer cells and regulates the processes enabling cellular proliferation, division and apoptosis [2]. Glutathione plays a role in DNA synthesis, repair and expression [4]. The function and survival of mitochondria is dependent on the glutathione system. Glutathione has important functions in the brain as an antioxidant and neuromodulator and promoter of neuronal survival. Glutathione acts as a neuromodulator of the glutamate ionotropic receptors, interacts with N-methyl-D-aspartate (NMDA) receptors and protects against glutamate excitotoxicity [5]. These key functions explain why depletion of glutathione may be accompanied by dysfunctions in many organs and cell functions

that are associated with the onset of neuro-immune disorders, including depression, Myalgic Encephalomyelitis / chronic fatigue syndrome (ME/CFS) and Parkinson's disease.

This paper aims to discuss the numerous functions of glutathione in health and disease as well as to review the evidence for disorganization of the glutathione system in depression, ME/CFS and Parkinson's disease, before finally considering treatment approaches for restoring the normal function of the glutathione systems.

2. The glutathione system

2.1. Synthesis and basic biochemistry

The synthesis of glutathione is a two stage process with each stage being dependent on the energy provided by the hydrolysis of ATP [6]. The first and rate limiting step is catalyzed by γ-glutamylcysteine synthase (glutamate cysteine ligase) and involves the formation of a peptide bond between L-glutamic acid and the N terminal of L-cysteine. The second step involves the addition of glycine and is catalyzed by glutathione synthetase. Glutathione is mostly synthesized in the cytosol of the cells where its concentration is some two orders of magnitude higher than in the extracellular environment [7]. Cytosolic glutathione is transported into mitochondria, the endoplasmatic reticulum and the nucleus where it exists in independent pools. The reduced form dominates in most cells or compartments but in the endoplasmatic reticulum the oxidized (GSSG) form is predominant [8,9]. A schematic representation of pathways involved in glutathione synthesis can be seen in **Figure 1**.

2.2. Glutathione peroxidases (GPX)

Glutathione may also serve as a co substrate for glutathione peroxidases which are part of the cellular enzymatic antioxidant network responsible for scavenging organic and inorganic peroxides

[10]. GSSG formed by these reactions (and others) may either be excreted or reduced to glutathione via the action of NAD(P)H-dependent glutathione reductase. For a detailed review of the biochemistry relating to the 6 isoforms of the selenium dependent glutathione peroxidases the reader is referred to the work of Sheehan et al. [11]. Conjugation with electrophiles is a major cause of glutathione depletion as is the export of GSH, GSSG and GSH-conjugates into the intracellular environment. Glycine and glutamate may be salvaged and reabsorbed following hydrolysis [12] but cysteine is lost and glutathione-S conjugates are metabolized to mercapturic acids before being excreted in bile and urine [13].

2.3. Glutathione S-transferases and glutaredoxins

Glutathione S-transferases are enzymes that catalyse the biotransformation of otherwise genotoxic or carcinogenic molecules of endogenous or exogenous origin by conjugation with glutathione [2]. Two families of supergenes encode glutathione S-transferases: sixteen genes code for soluble glutathione S-transferases and six genes code for microsomal glutathione S-transferases [14]. These enzymes are divided into eight classes based on sequence homology: Alpha (GSTA), Theta (GSTT), Mu (GSTM), Omega (GSTO), Kappa (GSTK), Zeta (GSTZ), Sigma (GSTS), and Pi GSTP [15].

Glutaredoxins (GRXs) are glutathione-dependent redox enzymes that utilize glutathione's reducing power to catalyze disulfide reductions in the presence of glutathione reductase [16]. They are highly specific in their actions and enable the displacement of glutathione from mixed disulfides according to the reaction GSH + protein-SSG = GSSG + protein-SH [17]. Glutaredoxins GRX 1 and GRX2 are located in the cytosol [18] and the intermembrane space of mitochondria [19].

3. Glutathione and O&NS detoxification

Glutathione plays a major role in the detoxification of RNS and ROS and as such is an indispensible component of the cellular anti-oxidant defenses [2]. Cellular glutathione is consumed by oxidation conjugation and hydrolysis [7,11,20]. Glutathione can react with ROS/RNS and radical molecules, such as $RO^\bullet$, HOCL and $HO^\bullet$. Glutathione may be directly oxidized by $HO^\bullet$ and peroxinitrite ($ONOO^-$) producing thiyl radicals [21-23]. Glutathione plays a pivotal role in the detoxification of NO and products of ROS-induced lipid peroxidation, such as 4-hydroxynonenal (4HNE) and malondialdehyde (MDA) [24,25]. The GSH / GSSG redox couple together with the NAD(P) / NAD(P)H and FAD / FADH redox couples are responsible for maintaining the redox state of the cell [26,27]. Glutathione in the nucleus is responsible for maintaining the required redox state of thiol proteins involved in DNA synthesis and repair [28].

Glutathione and glutathione S-transferases via their role as electrophile scavengers can act as indirect regulators of biochemical pathways modulated by these molecular entities. One of the pathways so regulated is the stress mediated pathway whose performance is governed by 4HNE levels, a preferred substrate for one of the alpha class glutathione S-transferases [29]. These enzymes thus have a crucial role in protecting cells by enabling the elimination of toxic chemicals, e.g. byproducts of oxidative stress [30,31], including oxidized DNA and catechols. They do so by promoting electrophile conjugates with glutathione and directly inhibiting hydrogen peroxide (H_2O_2) induced lipid peroxidation [31].

Perhaps unsurprisingly polymorphisms in glutathione S-transferases are prime suspects in the cause of many diseases, as is the case with GSTTi and GSTMi where polymorphisms stemming from deletions results in loss of enzyme activity [15,32]. Downregulation or inactivated GSTPi as a result of polymorphisms likely increases genomic damage when an individual with this genotype exposed to carcinogens due to reduced detoxification capacity [15,33]. These polymorphisms are implicated in the development of autoimmune disorders, including systemic lupus erythematosus

stemming from prolonged exposure to toxins in the environment [34], as well as psychiatric disorders such as schizophrenia, autism and bipolar disorder [35-38].

Glutathione and glutathione S-transferases are important players in xenobiotic detoxification which may be usefully considered as consisting of three interrelated phases. The first is biotransformation or bioactivation mediated via cytochrome P450 and other monooxygenases, predictably known as phase 1 enzymes. Six cytochrome p450 enzymes (with CYP3A4 and CYP2D6 being the most important) are responsible for metabolizing ninety percent of prescription drugs and this class of enzymes may be inhibited or induced by xenobiotics leading to adverse reactions or failure of therapy [39]. The first step of the detoxification process can paradoxically generate electrophiles or nucleophiles that are often carcinogenic or toxic [40]. The major metabolic role of glutathione S-transferases is the detoxification of these reactive electrophiles by catalyzing their conjugation (second phase) with glutathione [41,42]. This process usually results in a reduction of electrophile reactivity and increases the water solubility of the compounds favoring their elimination (third phase) thereby greatly reducing the potential of damaging interactions between these chemical entities and nucleic acids and proteins.

4. Glutathione and post transcriptional modification of proteins

Prolonged elevation of oxidative stress leads to the chemical modification of protein thiols either by the addition of NO motieties in a process described as nitrosylation (protein-SNO or protein-NO) or the transferase enzyme driven addition of glutathione to sulphydril or sulfenic acid groups termed glutathionylation (protein-SSG). Both processes are important examples of post translational modification and have a wide range of effects on protein structure and function as well as conferring resistance to further perhaps irreversible oxidative damage. We now consider these processes in more detail.

4.1. Response of cysteine to elevated O&NS

Production of thiyl (or thiol or mercapto radicals) radicals (RS) may follow exposure of cysteine groups to ROS and RNS. These are very short-lived derivatives that react with protein–SH molecules to form protein–SSG. Exposure of cysteine to ROS and RNS also results in the genesis of sulfenic acids (RSOH), which are highly unstable entities that rapidly undergo further oxidation to sulfinic or dulphonic acids, or react with glutathione to produce protein–SSG [43,44]. Sulfenic acid is readily reduced by a favorable change in the redox state of the cell or via the action of a wide range of reductases [45]. The oxidation of cysteine residues may in some circumstances be beneficial in that the reaction may act as a redox sensor but the normal outcome is the inactivation of a thiol protein especially if the cysteine group is integral to its function.

4.2. Glutathionylation

An alternative to the action of reductases in reversing oxidative damage to protein thiols is the addition of glutathione in a process known as S-glutathionylation [46,47]. The glutathyolation process is enabled by a ROS induced elevation of two forms of glutaredoxins, GRX1 and GRX2 of the pi class of glutathione S-transferases, which catalyze the conjugation of glutathione to cysteine, sulfenic acid or other sulfhydryl moieties [48]. Glutaredoxins catalyze both glutathionylation and deglutathionylation because GRX catalytic mechanisms are bidirectional with resultant direction being decided by the levels of GSG, GSSG, protein-SH and protein–SSG.

The activities of a wide range of proteins may be up or downregulated by S-glutathionylation. Cytoskeletal thiol proteins are particularly susceptible to glutathionylation and other susceptible protein clusters include kinases and other cellular signaling proteins and proteins involved in energy production and calcium homeostasis [49]. S-glutathionylation inhibits the

activity of many enzymes and functional proteins, for example phospho-fructokinase [50], glyceraldehyde-3-phosphate dehydrogenase (GAPDH) [51], creatine kinase [52]; nuclear factor kappa B (NF-κB) [53]; IκB Kinase (IKK) [54]; protein phosphatase 2A [55]; mitochondrial complex I [56]; mitochondrial complex II [57]; protein kinase A [58]; and matrix metalloproteinase [59].

The glutathionylation status of a protein-SH ultimately depends on the ratio of GSH / GSSG concentrations [47]. The presence of chronic oxidative stress leads to the glutathionylation of a wide range of proteins and this reaction may promote or impair their function [60]. However, overall glutathionylation is a mechanism aimed at preventing irreversible damage of thiol proteins as a result of elevated ROS [2]. Glutathionylation of proteins containing cysteine in complex I of the electron transfer chain is a signaling mechanism that may dampen aerobic metabolism during periods of low antioxidant status. This leads to inhibition of complex I which in turn impairs electron flow ultimately decreasing downstream ROS formation [61].

4.3. Nitrosylation

Another process of posttranscriptional modification of thiol proteins is the addition of a NO group, known as nitrosylation [62]. Cysteine sulfhydryl groups located on glutathione and proteins become nitrosylated in physiological and pathological conditions forming protein-GSNO and protein-SNOs, respectively [63,64]. Various reactions between ROS and RNS produce a range of metabolites of which N_2O_3 is the main nitrosylating species [65]. The ready reversibility of any reaction involving NO (nitrosylation and denitrosylation) by virtue of dative bond formation underpins the molecules role as a classical second messenger [66]. The development of GSNO-induced glutathionylation versus nitrosylation is probably dependent on the localized redox environment of the transformed cysteine (Cys) residues [67].

4.4. Glutathionylation versus nitrosylation

Glutathionylated and nitrosylated proteins coexist in an environment of oxidative stress and an exchange of these modifications between proteins occurs with reasonable frequency. There are major differences between proteins in susceptibility to each modification [68]. On some occasions both modifications will impair protein function but on others different affinities of protein thiols for each modification leads to highly specific consequences. Glutathionylation or nitrosylation reactions tend to occur in different cellular compartments with nitrosylation being more dominant where ROS and RNS are created such as in mitochondria and the endoplasmatic reticulum. In fact, mitochondrial ROS generation may be important for the nitrosylation reaction to occur [69].

Transcription factors such as p53 and NF-κB are redox sensitive due to the presence of cysteine residues which are vulnerable to glutathionylation. The effects of this process on the function of these transcription factors are quite subtle and varied. For example, glutathionylation does not impede the activation of NF-κB or its translocation to the nucleus but glutaredoxin-mediated glutathionylation of cysteine residues on the p65 and p50 subunits inhibits their binding to DNA and hence disable the ability to instigate the transcription of survival genes [70,71]. Glutathionylation of p53 and NF-κB has serious consequences as both transcription factors are important components in energy metabolism and immune homeostasis [72,73]. Nitrosylation of the p50 and p65 subunits of NF-κB suppresses the binding of the transcription factor to DNA causing its inactivation [74,75]. Nitrosylation of cysteine groups also impairs multiple steps involved in NF-κB activation [76].

5. Glutathione in immune-inflammatory pathways and cytokine signalling

5.1. Glutathione and cytokines

The redox state of cells plays a cardinal role in the regulation of IL-1β, IL-6, and TNFα – transcription and in the modulation of the signaling pathways activated by these cytokines [77]. MAPK-dependent cytokine expression and signaling is under redox control and glutathione depletion enhances both the transcription and deleterious effects of cytokines and conversely elevated levels of glutathione and cysteine have been shown to suppress transcription of pro-inflammatory cytokines [77,78]. The GSH / GSSG ratio regulates the transcription of IL-6, IL-8, IL-4 and TNFα [79-81]. On the other hand, pro-inflammatory cytokines reduce glutathione synthesis transport and recycling [82,83].

An examination of IL-1β transduction serves to illustrate the pleiotropic effects of glutathione on proinflammatory signaling. IL-1 signalling consists of three phases involving initial complex formation with NF-κB followed by its activation and finally its translocation to the nucleus ultimately provoking gene transcription [76]. A range of thiol-oxidizing compounds have the capability of inhibiting all three phases but their oppressive actions would normally be neutralized by glutathione [84]. However, glutathione is clearly not the sole enabler of NF-κB activation as this transcription factor is also activated by oxidative stress [84]. In a highly oxidative environment, however, NF-κB transcription is enhanced by hyperexpression of glutathione peroxidases, indicating an overall inhibitory role for glutathione. As discussed, glutathione also modulates NF-κB-instigated signaling via glutathionylation thereby impeding DNA binding [84]. All in all, the evidence indicates that the precise redox state is crucial in promoting or inhibiting the activation of NF-κB activation. Glutathione depletion is thus likely to produce a number of different modulating influences on the activation of this transcription factor.

5.2. Glutathione and regulation of the immune response

5.2.1. Glutathione and viral replication

Glutathione, selenium and glutathione-dependent enzymes are all involved in antiviral defenses. Glutathione can inhibit the replication of Lenti retroviruses [85], Delta retroviruses [86], Gamma retroviruses [87], Influenza viruses [88] and Herpes simplex type 1 [89]. Glutathione inhibits HIV by repressing the production of the p24 gag protein and by doing so inhibits budding, infectivity and viral release. Glutathione also inactivates the Gp120 envelope protein, which is very vulnerable to glutathione attack because it is rich in structural disulphide bonds [85,90]. The inhibition of viral envelope proteins is one of the mechanism by which glutathione inhibits the production of Influenza or Sendai virus [85,88,89]. Dengue virus infection directly depletes glutathione and this ability is a conserved evolutionary mechanism favoring its replication. Elevated levels of glutathione inhibit the replication of this virus [91,92].

5.2.2. Glutathione and T cell activation

The activation and proliferation of T cells requires the existence of a strong intracellular and extracellular reducing environment [93,94], which is mainly engineered by antigen-presenting cells, especially dendritic cells [92,95,96]. When dendritic cells are stimulated by T cells they increase their uptake of cystine by deploying x_c^- cystine transporter receptors and extrude cysteine (the reduced form of cystine) into the environment. Although extracellular cystine is abundant, naïve T lymphocytes lack the machinery to transport cystine, with any degree of efficiency and hence depend on cysteine derived from dendritic cells to meet their metabolic needs [97-99]. Cysteine derived from dendritic cells is required by T cells for synthesis of glutathione, ultimately providing the reducing power enabling DNA synthesis [100] and allowing progression from G_1 to the S phase [101,102]. By controlling cystine/cysteine levels, dendritic cells are able to affect intracellular glutathione levels and subsequent redox signaling pathways in T cells [98].

Redox modulation is also part of the immunosuppressive armory deployed by T regulatory (Treg) lymphocytes in mediating their effects when activated. Tregs impair the synthesis of glutathione in dendritic cells by inhibiting the expression of gamma-glutamyl transpeptidase (γGT), the rate limiting enzyme for glutathione synthesis. Tregs also successfully compete for and ultimately oxidize cysteine depriving effector T cells their major source of essential cysteine. Tregs also appear to block the redistribution of glutathione from the nucleus to the cytoplasm in effector T lymphocytes which is an essential step in their proliferation [103].

5.2.3. Glutathione and T cell differentiation patterns

Glutathione levels in antigen-presenting cells govern which pattern of differentiation predominates [104,105]. Glutathione plays a crucial part in enabling the growth-promoting properties of IL-2 [106] by regulating the internalization, binding and degradation, of this cytokine as well as IL-2-driven T-cell proliferation. The weight of evidence suggests that immune responses are optimally at intermediate glutathione concentrations that support efficient DNA synthesis and do not inhibit IL-2 production [107]. For example, IL-2-activated natural killer (NK) cells need a reducing environment, provided by de novo synthesis of glutathione, in order to proliferate and perform their normal functions [108]. Intracellular glutathione concentrations largely govern the replication, differentiation and growth of IL-2-reactive cytotoxic T-lymphocytes [109,110].

5.2.4. Glutathione and cell proliferation

During normal physiology, glutathione becomes localized to the nucleus when cells are actively dividing [111,112]. The activity of nuclear proteins especially telomerase is dependent on GSH / GSSG. Changes in telomerase activity lead to changes in the activity of crucial cell cycle proteins id2 and E2F4 [111]. Glutathione has a key role as a redox sensor at the commencement of

DNA synthesis and glutathione levels play a crucial role in maintaining nuclear architecture by ensuring the requisite redox environment for ensuring DNA integrity prior to replication. Glutathione also influences proteosomal protein degradation in the nucleus and exerts epigenetic control on histone function and chromatin structure prior to replication by reducing the number of disulphide bonds on nuclear proteins and hence generating a reducing environment [111-113]. Glutathionylation and oxidation of nuclear proteins form the basis of reversible mechanisms for regulating cell proliferation repair and state of DNA compaction [112]. Chromatin by virtue of the cysteine motif on histone 3(H3) is redox sensitive. The level of glutathionylation increases during cell division resulting in loss of nucleosomic stability and the formation of a more lax decompacted chromatin structure [114].

In general the cell cycle involves a change from a reduced environment during proliferation and differentiation to a more oxidized milieu when cells undergo apoptoptic cell death [115]. Glutathione has multiple means of influencing cell proliferation. Elevated levels of glutathione lead to the inhibition of p38 MAP and Janus kinases [116]. ROS, mainly in the form of hydrogen peroxide, and glutathione both play an important part in regulating the cell cycle. High hydrogen peroxide levels activate MAP kinases and NF-κB leading to cell necrosis or apoptosis but at a lower dose hydrogen peroxide activates transcriptors such as nuclear factor (erythroid-derived 2)-like 2 (Nfr-2), which is a key regulator of glutathione synthesis. So, when ROS is produced at low concentrations, glutathione is synthesized and cellular proliferation occurs [117]. Given the influence of glutathione and glutathionylation on the activity of immune-inflammatory pathways it is perhaps not surprising that the GSH / GSSG ratio and glutathionylation of proteins are key regulators of apoptosis [118,119].

6. Glutathione and mitochondria

6.1 Glutathione, redox homeostasis and mitochondrial survival

The function and survival of mitochondria is dependent on the glutathione system. Mitochondria are a major site of cellular ROS generation in spite of the presence of a broad wide array detoxifying enzymes and antioxidants. Mitochondria produce over ninety percent of total cellular ROS in some cells [120]. Mitochondria are thus the organelles with the greatest vulnerability to corrosive damage by free radical species such as hydrogen peroxide and superoxide [121,122]. There are other mitochondrial sources of ROS apart from the electron transport chain. These sources include glycerol 3-phosphate dehydrogenase, α-ketoglutarate dehydrogenase, mitochondrial NADPH oxidase (NOX4), monoamine oxidase, etc. [123-128]. Hydrogen peroxide is being continually produced by the electron transport chain. When conditions are in a state of redox balance the amount of hydrogen peroxide exported into the cytoplasm is restrained by the action of matrix peroxidases [129] and glutathione peroxidases [130]. However, both the mitochondrial and cytoplasmic redox environments make an important contribution to maintaining redox homeostasis and the net rate of ROS accumulation within the cell is dependent on the interaction between the GSH / GSSG in the cytoplasm and the regeneration of glutathione in the mitochondrial matrix [130].

The optimum function and indeed the very survival of mitochondria are wholly dependent on the coordinated activity of the glutathione, glutaredoxin and thioredoxin systems which act cooperatively to ensure continual maintenance and repair [131]. Redox sensors located in the cell and mitochondria have the capability of detecting dynamic perturbations in ROS concentrations and act to adjust antioxidant defenses in the face of intracellular or intercellular stressors which threaten to disrupt the redox balance in the mitochondria or cytosol [130-133].

6.2. The glutathione system and other redox couples in mitochondria

The mitochondrial matrix, the inner membrane space and the cytosol contain pools of segregated but interactive redox couples, e.g. NADPH / NADP, NADH / NAD, GSH / GSSG and thioredoxin (Trx) / TrxSS [134,135]. The thioredoxin and glutathione systems are the main hydrogen peroxide scavengers in all mitochondria. The protective properties of the glutathione system are conferred by the actions of glutathione-S-transferase and glutathione peroxidase GPX-4. The concentration of glutathione (GSH + GSSG) is 100- to 1,000-times higher in comparison to any other redox buffering system including thioredoxin so that the intracellular GSH / GSSG pool dominates the redox environment [136]. As such, the glutathione system is the primary protector of mitochondrial membranes against oxidative damage. The glutathione and glutaredoxin systems function cooperatively to reduce protein disulphides formed as a result of increased GSSG formed as a result of a chronic oxidative environment. Glutaredoxin acts to reduce protein disulfides and restore their conformation and function [137]. The thioredoxin system also plays an important part in reducing a range of protein disulphides [138] and also supplies hydrogen atoms used in the reduction of hydrogen peroxides and lipid peroxidases [139]. **Figure 2** depicts the role of the glutathione antioxidant system in protecting mitochondria against the ravages of ROS.

7. Role of glutathione in the central nervous system

7.1. Glutathione, a glutamate receptor modulator

Glutathione is the most plentiful peptide in the brain and spinal cord and is present in glial cells and neurons as well as and in intracellular spaces [140,141]. Glutathione in its oxidized and reduced forms exerts the majority of its effects in the central nervous system (CNS) by interacting directly or indirectly with glutamate receptors. There are two families of glutamate receptors; metabotropic and ionotropic receptors [142]. The latter receptors are classified as N-methyl-D-aspartate (NMDA), 2-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), or kainate

receptors [142,143]. These receptors are gated ion channels which open in response to the binding of a suitable ligand molecule such as the amino acid glutamate to allow passage of sodium or calcium ions. This flow of ions causes depolarisation of the plasma membrane and the genesis of an electrical current. Chronic hyperstimulation of ionotropic receptors induces pathology by mediating the development of excitotoxicity through calcium and O&NS mediated processes [144,145].

Each of the three amino acids contained in glutathione is able to interfere with glutamate-mediated neurotransmission through both direct and indirect receptor modulation. The glutamate group can bind to all types of glutamate receptor. The cysteine based thiol group affects the redox state of those receptors, while glycine is actually an NDMA receptor agonist.

GSH and GSSG at physiologically normal concentrations both inhibit the glutamate binding to synaptic membranes [5,146,147]. Glutathione acts as a neuromodulator by displacing ligands of the glutamate ionotropic receptors from their binding sites and modulating the entry of calcium ions into ionophores regulated by NMDA receptors [148]. Glutathione, at millimolar concentrations, may interact with the redox sensitive site of NMDA receptors [149,150] and such binding likely plays an important role in modulating neuroplasticity [151]. Glutathione displaces glutamate, via its glutamyl moiety, largely from AMPA receptors. GSH and GSSG, depending on their concentrations, can also displace AMPA and kainate from their respective receptors [152,153]. Glutathione appears to confer neuroprotection against exitotoxicity and maintains intracellular Ca^{2+} homeostasis [154,155]. GSH and GSSG ameliorate neuronal damage resulting from chronic NMDA receptor activation [156].

7.2. Glutathione and the cystine / glutamate antiporter system in the brain.

There are two main types of glutamate transports in the CNS. The first is the excitatory amino acid transporter and the second is the x_c- cystine / glutamate antiporter system. We will now discuss these in a little more detail in order to better highlight the adverse effects of low glutathione

on the integrity and functioning of the brain. The excitatory amino acid transporters enable the uptake of glutamate against a concentration gradient [157] and prevent excessive glutamate receptor activation, which can lead to prolonged exitotoxicity and ultimately neuronal cell death. The excitatory amino acid transporters (EAAT1 and EAAT2) are mainly astrocytic transporters responsible for maintaining intracellular glutamate below neurotoxic levels [158]. The excitatory amino acid transporter EAAT2 is held to be responsible for some ninety percent of glutamate uptake under normal conditions [159,160]. Astrocyte excitatory amino acid transporter activity is much higher compared to that of microglia [161]. The x_c- cystine / glutamate antiporter allows the uptake of extracellular cystine (for glutathione synthesis) in exchange of intracellular glutamate [162]. The optimum function of this system is crucial for the homeostatic control of cysteine and glutamate levels [163]. The expression of the x_c- cystine / glutamate antiporters in the CNS is confined to astrocytes [164] and microglia [165]. Through astrocytic glutamate efflux the x_c- antiporter may allow the targeting of glutamate to neuronal sites, allowing glutathione synthesis to modulate neuronal activity. Microglial x_c-antiporter expression is important in the uptake of cystine for glutathione synthesis particularly during activation by lipopolysaccharide (LPS) where the density of x_c-antiporters is increased on the surface of activated microglia [166]. This may damage surrounding neurons by secreting glutamate at the same time as importing cystine to bolster the cells antioxidant defenses.

8. Depleted glutathione: consequences and clinical relevance

8.1. Consequences of depleted glutathione

Given the key roles of glutathione discussed in the previous sections it is not surprising that glutathione depletion causes many perturbations in cell and organ functioning and is associated with the onset of neuro-immune disorders such as depression and ME/CFS. We will now review some of

the effects of glutathione depletion before reviewing the evidence alluding to the existence of O&NS and a dysregulated glutathione system in neuro-immune disorders.

As mentioned above, the glutathione system is the most important protectant in the detoxification of ROS and RNS and as such is a vital component of the cellular anti-oxidant defenses [2,167]. Glutathione reacts directly with different radical molecules and may be directly oxidized by $HO^{\bullet}$ [168,169] and peroxinitrite [170,171]. Glutathione is involved in the detoxification of NO. and products of ROS-induced lipid peroxidation, such as 4HNE and MDA [172,173] and a range of other metabolites produced by oxidative damage [174,175]. A compromised glutathione system therefore leads to the development of chronic O&NS occurring in neuro-immune diseases [167,176,177].

The consequences of glutathione depletion on immune-inflammatory responses heavily depend on the nature of the pro-inflammatory mediators dominant in any given cellular environment. Cytokine regulation and reduced glutathione are intimately interlinked. The GSH / GSSG ratio determines the activation level of the JNK and MAPK pathways and thus the transcription of pro-inflammatory cytokines [178]. The redox state of the cell and the GSH / GSSG ratio govern the transcription of pro-inflammatory cytokines, such as IL-1, IL-6, IL-8 and TNFα also plays a major part in the modulation of the signaling pathways activated by these cytokines [3,79,179]. Thus, glutathione depletion not only enhances the transcription, but also the deleterious effects of these cytokines [78]. The weight of evidence suggests that lowered levels of glutathione may suppress immune responses [180-183].

Depleted glutathione levels dramatically impair proliferation, DNA synthesis and cytotoxic activity of T lymphocytes and may lead to a Th2 polarized immune system [101,111,167,184,185]. Glutathione peroxidase depletion additionally inhibits Th2 and Th17 differentiation of activated naive T cells [186]. Glutathione depletion also contributes to increased blood brain barrier

permeability observed in neuro-immune disorders [187], thereby further increasing central immune-inflammatory processes by potentiating immune cell extravasation over a compromised blood brain barrier.

As described above, many transcription factors are also redox sensitive including inactivation of p53 [188] and NF-κB [189]. NF-κB and p53 are vital players in the regulation of the immune response and energy production [72]. Glutathione levels also modulate signal transduction pathways within T lymphocytes involving AP-1 and tyrosine phosphorylation [190]. Glutathione depletion can also modulate inflammatory cascades by affecting the Janus kinase and STAT3 pathways [191]. In a depleted glutathione environment, glutaredoxin dissociates from signal-regulating kinase resulting in c-Jun N-terminal kinase activation leading to cyclo-oxygenase-2 induction and the production of prostaglandin E_2 [192-194].

The consequences of glutathione depletion reflect how redox mechanisms regulate DNA synthesis in G1 phase and mitosis [195] and how glutathione depletion impairs the proliferation of all cell types [196,197]. Reduced glutathione levels prevent the shift from G1 to the S phase in the cell cycle, thereby decreasing cell proliferation [198]. Glutathione levels are high in cells preparing to divide or entering the mitotic phase but much lower in cells in the Go / G1phase [199].

Glutathione depletion impairs mitochondrial function and ATP production by inhibiting complex I of the electron transport chain [200]. Since glutathione biosynthesis is an energy-dependent process [2] mitochondrial dysfunction significantly depletes glutathione production thereby further exacerbating oxidative damage which further impairs electron transport chain activity. Hence, glutathione depletion is almost certainly a major contributing factor to the progressive nature of mitochondrial diseases [200-202]. Glutathione levels in those with confirmed syndromic and non-syndromic mitochondrial diseases are dramatically lower than those observed in healthy controls [203-205] even to the point of being undetectable [200]. The weight of evidence

indicates that glutathionylation impairs complex I activity compromising energy production [206,207]. Glutathione depletion also impairs the function of proteins regulating calcium homeostasis and energy generation [208].

Glutathione depletion may ultimately drive cytochrome c release and mitochondrial death [209]. In healthy neurons, cytochrome c is held inactive by increased glutathione [210]. ROS-independent glutathione depletion leads to the inhibition of sphingomyelin synthase activity, the activation of caspases and ceramide generation [211,212]. Additionally glutathione loss impairs the canonical NF-κB signaling pathway resulting in the sensitization of cells to apoptosis [213,214]. Glutaredoxins also regulate the function of numerous proteins which play critical roles in the regulation of apoptosis by catalyzing deglutathionylation and glutathionylation, such as NF-κB, procaspase-3, Fas and ASK1. Prolonged elevation of O&NS causes oxidative modifications of crucial Cys residues located in these apoptotic mediators, provoking protein-SSG generation and hence altering protein activity and apoptotic signaling [215]. Mitochondria form the central hub of cellular energetics and apoptotic processes, with the intimate bidirectional relationship between the glutathione system and mitochondria crucial to cellular functioning.

Depletion of glutathione can invoke the apoptotic machinery even in the absence of elevated O&NS [119,216]. In different cell types, glutathione depletion participates in the onset of programmed cell death following numerous apoptotic signals [118,216]. Prolonged glutathione depletion leads to JNK activation and an increase in expression of programmed death receptor 5 [217,218] and Fas mediated increase in mitochondrial membrane permeability [219]. Glutathione depletion may also trigger cell death directly by increasing mitochondrial permeability transition pore formation, leading to cytochrome c release and the activation of death inducing mitochondrial caspases [220,221], even in the absence of programmed death receptor ligation. There is also some evidence that glutathione depletion is an indispensable step in the assembly of the apoptosome

[222]. Decreased glutathione levels lead to the oxidation dependent dimerization and subsequent activation of Bax, and initiation of the intrinsic apoptopic pathway [223]. Glutathione loss leads to Bax translocation, from its bound state in the cytoplasm by 14-3-3 [224], into mitochondria leading to loss of mitochondrial membrane potential delta psi(m), dramatically increasing O&NS and leading to dysregulated calcium ion homeostasis and ultimately capase-3/9 activation and cellular apoptosis [225-227].

The optimum functioning of the NDMA receptor is dependent on the redox environment, with prolonged glutathione depletion compromising synaptic plasticity, memory and learning [228, 229]. Astrocyte glutathione depletion has serious consequences for the integrity and function of neurons [230]. Prolonged conditions of oxidative stress are deleterious in part by reducing the capacity of astrocytes to uptake glutamate [231]. Raised levels of extracellular glutamate are a common observation in many neurological diseases, increasing excitotoxicity or oxidative glutamate toxicity. This state is created by the oxidative stress related inhibition of cystine uptake by the x_c-antiporter system leading to glutathione depletion [232]. Astrocyte glutathione release is well documented and is important to neuronal anti-oxidant defenses [233,234]. As glutathione cannot be directly taken up by neurons, astrocyte released glutathione is co-ordinated with the upregulation of gamma-glutamyl transpeptidase, which cleaves glutathione to products which neurons can uptake, including cysteine [235,236] which is imported by neuronal EAAT3 receptors [237].

Various states, including elevated O&NS, resulting in glutathione depletion have been shown to impair DNA methylation [238-240]. Therefore, elevated O&NS is considered to alter the methylation of DNA, which leads to changes in the gene expression [241]. There are a number of different overlapping mechanisms involved. ROS-induced DNA lesions, such as 8-OHdG, may inhibit the activity DNA methyltransferase at the adjacent cytosine base and subsequently prevent methylation at that site [242]. An unfixed 8-OHdG can lead to the introduction of a G-T transversion

leading to a loss of CpG dinucleotides [243]. In a state of chronic O&NS, homeostatic mechanisms promote an increase in the resynthesis of glutathione in response to glutathione depletion via the methionine cycle. The synthesis of glutathione via the increase in the rate of one carbon metabolism requires S-adenosylmethionine as a substrate for synthesizing the homocysteine ultimately to be used for the glutathione synthesis. This leads to a diminished availability of S-adenosylmethionine for the purpose of DNA methylation [244]. Oxidative stress additionally increases the demand for SAMe but concomitantly decreases its synthesis resulting in sub optimal methylation of DNA [245, 246].

8.2. Glutathione depletion in depression

Glutathione depletion and concomitant increase in O&NS, mitochondrial dysfunctions and impaired DNA methylation play a role in the pathophysiology of neuro-immune disorders, including depression and ME/CFS. There are several reports of a dysregulated glutathione system with lowered glutathione levels and lowered levels of its related enzymes in individuals with depression and ME/CFS. Already in 1934 it was reported that glutathione may be lowered in the blood of schizophrenic patients [247].

Serum glutathione levels in depression have been directly measured. One study reports serum levels from individuals with recurrent depressive disorder. When compared to controls, those with depression had significantly lower glutathione levels. In parallel, decreased levels of glutathione peroxidase-1 are also reported. Interestingly, an increase in glutathione reductase was reported, that may reflect the upregulation of glutathione cycling in response to depletion. Increased markers of oxidative stress were also observed, with increased MDA and hydrogen peroxide levels reported [248]. Maes et al. [249] reported lower glutathione peroxidase activity in whole blood samples taken from depressed patients compared to healthy controls. These authors reported a

significant negative correlation between glutathione peroxidase and the severity of depression and the range and severity of autonomic symptoms. When comparing those considered to be having 'active' depression to those in 'remission', Kaddurah-Daouk et al [250] reported no difference in CSF glutathione levels either in remitted or currently depressed individuals, when compared to controls. However, there was a trend to indicate those with remitted depression had a lower level of CSF glutathione when compared to controls. This is reflected in the change in ratio between methionine and glutathione which is decreased in remitted depressed individuals when compared both the controls and also to those with current depression. It may be postulated that the three week washout out criterion for inclusion into the study, in the currently depressed group, may account for some of the differences with the remitted group, where participants had to have at least 3 months without medication to be included [250].

In congruence with previous reports, serum superoxide dismutase and glutathione peroxidase levels are decreased and lipid peroxidation increased in those with depression compared to controls [251]. Moreover, when comparing those with first episode depression to those with chronic depression, superoxide dismutase levels were reduced in both groups, but further reduced in the recurrent group. This pattern was also seen in glutathione peroxidase and MDA levels with respective decreased and increased levels reported in the recurrent group compared to the first-episode group. In a study of matched controls, fibroblast cultures were investigated to compare oxidative stress and glutathione levels in those with major depression to healthy controls. Results showed increased protein carbonylation, while no changes in fibroblast glutathione levels were reported [252]. Similar to the work of Kaddurah-Douk et al. [250], these authors also found an increase in glutathione reductase. When investigating postmortem tissue, studies have found decreased levels of both oxidized and reduced glutathione when compared to controls in the anterior cingulate cortex. Glutathione reductase and glutathione peroxidase levels were not found to be

statistically altered, however there was an indication that they were decreased in the depression group [253]. In post-mortem prefrontal cortex, glutathione S-transferase levels (Mu isoform) were significantly lower in depressed and bipolar patients [254]. Reduced glutathione in postmortem brain has also been reported in autism [255].

The use of ^{1}H magnetic resonance spectroscopy in treatment free patients demonstrated lower glutathione levels compared to non-depressed controls [256]. Do et al. [257] reported lowered glutathione measured both by spectroscopy and in CSF in patients with schizophrenia. There is a possible correlation between reduced glutathione and negative symptoms of schizophrenia, a signal that is reinforced by the results of clinical trials of glutathione replenishment strategies with N-acetyl cysteine, discussed later [258]. Biomarker studies such as those outlined above are further supported by genetic studies also showing perturbations in the glutathione pathway. In a study taken from one of the largest epidemiological samples available, the HUNT study, researchers have found increased polymorphisms in both the CGLM (3 SNPs) and GCLC (9 SNPs) and a trinucleotide repeat [259]. These enzymes are responsible for the production of glutathione.

The pathophysiology of depression also appears to involve disturbed erythron and iron homeostasis likely as a result of inflammation and depleted glutathione. Changes in the erythron and iron metabolism are reported, such as decreased serum iron, number of erythrocytes, hemoglobin and hematocrit and increased serum ferritin [260] and depleted polyunsaturated fatty acid levels in the erythrocyte membrane [261, 262].

Erythrocytes play a major role as free radical scavengers [263]. They are continuously exposed to ROS in the systemic circulation and the autoxidation of hemoglobin in the cytosol [264]. Erythrocytes are at high risk of oxidative stress due to high ferrous iron load largely stemming from ferryl-hemoglobin formation and hydroxyl radical production via the Fenton reaction between H_2O_2 and Fe^{2+} [265]. The plasma membranes of erythrocytes are very sensitive to damage by oxidative

stress because of the very high percentage of unsaturated lipids which underlie their considerable flexibility [263]. Progressively increasing oxidative stress causes changes in the primary structure and functions of hemoglobin, which may lead to hemolysis [266].

Glutathione is the primary defense against oxidative stress in erythrocytes and adequate levels are essential for maintaining the natural conformation of hemoglobin [266]. Optimum erythrocyte glutathione levels are therefore critical in minimizing the damaging effects of ROS and autoxidation of hemoglobin in the cytosol [265]. In conditions of chronic oxidative stress, GSSH is expelled from the cell by virtue of membrane transporters and increased membrane permeability [2, 266]. Hence in this context glutathione is quite literally a life enabling molecule. A visual representation of the relationship between iron, hemoglobin and glutathione in erythrocytes may be viewed in **Figure 3**. Given the role of erythrocytes as ROS scavengers, depleted numbers of these cells could contribute to the elevated levels of ROS seen in major depression [263].

8.3. Glutathione depletion in ME/CFS

The plethora of different unvalidated diagnostic criteria for ME and CFS makes comparison between research findings produced by different authors sometimes difficult [267]. Using proton NMR spectroscopy, Shungu et al. [268] reported depleted levels of glutathione in the CSF and cerebral cortex of people with ME/CFS which correlated inversely with several measures of physical function. This was in contrast with earlier work which did not detect any abnormalities in the brain or CFS of patients albeit using inferior technology [269]. These authors cited the small sample size as the likely reason that the depletion in glutathione did not reach statistical significance. Kennedy et al. [270] reported low glutathione levels in erythrocytes in patients with ME/CFS echoing an earlier finding [271]. Increased glutathione peroxidase activity in skeletal muscle extracted from patients with this illness has also been reported in people carrying the same

diagnosis [272]. Logan and Wong [273] reported an improvement in disability levels in patients with ME/CFS following supplementation with glutathione or N-acetyl cysteine. Other workers have suggested the presence of low glutathione in patients with ME/CFS and it has been suggested that low glutathione and the consequent increase in blood brain barrier permeability may be a critical element in the etiopathology of ME/CFS [274,275]. A number of authors have reported depleted glutathione levels in CFS and an improvement in fatigue following supplementation with ginsing or moxibustion [276,277]. Several research teams using rat models of ME/CFS have reported depleted levels of glutathione peroxidase in their animals [278,279]. This may complement the finding reported by Maes et al [249] who found that serum glutathione peroxidase levels were not altered in people with ME/CFS. Several authors have reported findings of low glutathione in studies using mouse models of ME/CFS where fatigue has been induced mechanically or by immunological means and relieved by the use of certain dietary supplements or acupuncture [280-285].

8.4. Implication of the glutathione system in the pathophysiology of depression and ME/CFS

Recently, we have reviewed that depression and ME/CFS are accompanied by similarly dysregulated immune-inflammatory and O&NS pathways, including increased levels of pro-inflammatory cytokines, intracellular signaling pathways, oxidative damage of fatty acids and lipids (as indicated by increased 4-HNE and MDA), oxidative damage to DNA (as demonstrated by increased 8-OHdG levels), autoimmune reactions directed towards neoepitopes produced by the corrosive actions of ROS on fatty acids located in cellular membranes), and autoimmune reactions against oxidative specific epitopes and nitrosatively modified proteins [249,286]. We also reviewed that mitochondrial dysfunctions are heavily involved in the pathophysiology of these three disorders, including lowered ATP production, impaired oxidative phosphorylation and dysfunctions in the mitochondrial respiratory chain [259,286,287]. Epigenetic changes are now acknowledged to take

part in the pathophysiology of depression and a chronic fatigue-related condition, such as fibromyalgia [288-290]. Induction of apoptotic pathways and alterations in glutamate metabolism are observed in depression and ME/CFS [72,276,288,291-293]. It is therefore not difficult to appreciate that depletion of glutathione and its related enzymes may contribute to the abovementioned pathways in depression and ME/CFS. Treatments targeting the glutathione system in depression and ME/CFS further underscore the important role of glutathione and related enzymes in the pathophysiology of these neuro-immune disorders (see section 9).

8.5. Glutathione depletion in Parkinson's disease

The interplay between depleted levels of glutathione, mitochondrial dysfunction, elevated oxidative stress and apoptosis is also evidenced in the pathophysiology of Parkinson's disease [294]. Depletion of glutathione (GSH + GSSH) in the substantia nigra of the brain is an early biochemical occurrence in the development of this illness and is detected prior to the inhibition of complex I activity [295]. Complex I deficiency is seen in the substantia nigra and the frontal cortex in patients with Parkinson's disease but it is also evident in peripheral mononuclear blood cells, platelets and skeletal muscle, indicating that impaired mitochondrial complex I activity is a global phenomenon in Parkinson's disease [294]. Glutathione depletion leads to impaired development of iron sulphur proteins and leads to iron buildup in cells [296]. Hence glutathione depletion is likely driver of the elevated iron levels seen in the substantia nigra in Parkinson's disease patients [297]. Impaired iron homeostasis is a source of cellular toxicity via the generation of superoxide and hydroxyl radicals via the Fenton reaction and these species are the effector molecules by which excess iron levels contribute to the pathogenesis of this disease [298, 299]. In these conditions the damage caused by hydroxyl radicals is exacerbated as the loss of glutathione inactivates the main mechanism enabling their clearance from the cell [2]. While some authors argue that inhibition of mitochondrial complex

I and subsequent mitochondrial dysfunction in Parkinson disease substantia nigra cells is mediated by glutathionylation, the loss of GSH and GSSH argues for inhibition by nitric oxide in this case and not GSSH [297, 295]. As previously discussed, glutathione biosynthesis is an energy-dependent process [2] and mitochondrial dysfunction significantly depletes glutathione production thereby further exacerbating oxidative damage which further impairs electron transport chain activity which in turn leads to elevated levels of ROS and RNS [300].

In the case of Parkinson's disease, mitochondrial dysfunction driven by spiraling increases in O&NS and glutathione depletion may become self-sustaining via a feedback loop leading to futher compromised electron transport chain inhibition at complex III, exacerbated macromolecule damage, failure of the ubiquitin proteome system and, ultimately, neuronal apoptosis involving all the mechanisms discussed above [294].

9. Treatments targeting the glutathione system

9.1. Glutathione

Glutathione supplementation would obviously be an attractive option but its absorption and effectiveness in blood is low [301]. Oral glutathione is readily hydrolyzed by dipeptidases in the gastrointestinal tract and hence this form of administration is not a suitable vehicle for raising glutathione levels in cells. Glutathione administered as an infusion also suffers from serious defects. It is rapidly eliminated by gamma-glutamyl transpeptidase in the circulation and has a fleetingly short half life of 7 to 10 minutes [302]. Glutathione supplementation in any shape or form cannot replenish diminished levels of glutathione in the central nervous system as the molecule does not cross the blood brain barrier to any significant extent [303,304]. One study demonstrated that only 0.5% of glutathione injected into the carotid space of rats could later be detected in brain tissue [302].

9.2. N-acetyl Cysteine

Administration of the cysteine prodrug N-acetyl-cysteine restores intracellular glutathione levels. N-acetyl-cysteine increases glutathione levels in vivo and vitro [305-307]. N-acetyl-cysteine is a well-tolerated treatment for glutathione deficiency with an excellent safety record and has been used successfully to increase glutathione levels in a wide range of metabolic and neuro-immune illnesses as well as in infections, including HIV. In excess of sixty five % of forty six placebo-controlled clinical trials with N-acetyl-cysteine have reported benefits measured empirically or by self reported improvement in quality of life parameters [308]. For a detailed review of its chemistry and biological activity see Samuni et al. [309].

There is evidence that N-acetyl-cysteine may boost immune function directly [310] and that N-acetyl-cysteine possesses heavy metal chelating capacity [311]. The studies repost a range of results which appear to vary in accordance with the dose and duration of N-acetyl-cysteine treatment. De Quay et al. [312] reported that markedly depleted glutathione and cysteine levels in plasma and T-lymphocytes normalized following a single dose of N-acetyl-cysteine at 30 mg/kg, whereas no changes were reported using 800 mg a day [313]. Herzenberg et al. [314] reported that in HIV patients N-acetyl-cysteine at a maximum dose of 8 g a day for 8 weeks increased glutathione levels by 113% and that the N-acetyl-cysteine group had a higher chance of survival after 24 months than the control group. Chen et al. [315] noted that N-acetyl cysteine at a concentration of 10^{-4} Moles/L significantly increased glutathione levels and the GSH / GSSG ratio in HIV positive patients whereas N-acetyl-cysteine at 10^{-7} Moles/L did not.

N-acetyl-cysteine has been used with some efficacy as an adjunctive treatment for depression. Randomized controlled trials of N-acetyl cysteine have shown benefit following 6 months of treatment at 2000 mg/day in people with bipolar disorder [316]. When specifically

investigating depression, the researchers have shown that those who were currently experiencing a depressive episode at the commencement of the study had significant reductions in depressive symptoms at the 6 month timepoint [317]. In a larger, maintenance designed trial, participants were reported to have large decreases in depression severity scores following 8 weeks of open-label N-acetyl cysteine treatment [318]. Following this, participants were randomized to continued N-acetyl cysteine treatment or placebo. Results showed no statistical differences between N-acetyl cysteine and placebo groups at the six-month time point [318]. In a RCT investigating 2000 mg/day of NAC (compared with placebo and in addition to treatment as usual) for major depressive disorder, results showed no improvement at the 12-week treatment cut-off between N-acetyl cysteine and placebo groups. However, when exploring the effects of including the post-discontinuation visit, depressive scores were reduced in the N-acetyl-cysteine group. There was a suggestion of efficacy in individuals with more severe depression, defined as a Montomery Asberg Depression Rating score above 25, but with no efficacy evident below that cut-off. Other functional outcomes were found to be significant at week 12. Taken together, these data provides some support for the use of N-acetyl cysteine as an adjunctive antidepressant (Berk et al., in press).

In a study of N-acetyl cysteine in schizophrenia, beneficial effects were seen across symptom domains including akathisia, but with highest effect sizes in negative symptoms [316,318]. This pattern mirrors the spectroscopy finding that lowered glutathione appears linked to negative symptoms of schizophrenia. This finding has recently been replicated [319]. N-acetyl cysteine has also shown efficacy in two studies in autism, particularly in symptoms of irritability [320].

9.3. Folate, lipoic acid and coenzyme Q10

Oral folate supplementation is a proven approach for increasing the levels of glutathione [321], glutathione S-transferase, glutathione peroxidase and glutathione reductase [322].

Methylfolate is the only form of folate capable of crossing the blood brain barrier due to the presence of a methylfolate active transport system within that structure [323,324].

A dose of 15mg of methylfolate a day has proven to be an effective treatment for people with depression who are resistant to antidepressants [323]. Folate supplementation is an effective adjuvant to SSRIs in treatment resistant depression even when folate levels are normal [325,326]. The lowest effective dose of methylfolate capable of augmenting antidepressants is 7.5 mg, which is approximately equivalent to 52 mg of folic acid [327]. By binding to folate transport receptors, methylfolate precursors may compete with folate derived methylfolate for uptake into the central nervous system. This obviously limits the amount of methylfolate that can enter the brain [328]. Thus, high doses of methylfolate which don't involve the synthesis of precursor molecules can provide significantly more active methylfolate than high doses of folic acid [328].

Alpha-lipoic acid is a mitochondrial nutrient that improves age-associated mitochondrial and cognitive dysfunction, which is at least partly mediated by its antioxidant effects [329]. In non-alcoholic steatosis, alpha-lipoic acid affords protection by doubling glutathione peroxidase levels with associated increases in sirtuins, further enhancing mitochondrial functioning [330].

Coenzyme Q10 is a robust anti-oxidant and mitochondria regulator that affords protection against O&NS [331]. Coenzyme Q10 is significantly decreased in depression and ME/CFS and in many other conditions, where it often associates with fatigue [332,286]. Coenzyme Q10, in a concentration dependent manner, prevents stress induced decreases in glutathione peroxidise and glutathione [333]. Coenzyme Q10 shows possible benefits on depressive symptoms in an elderly depressed population [334].

Supplementation with garlic extracts from fresh raw garlic, aged black garlic and aged red garlic could be also as a protection against the loss of glutathione as the extracts reduce ROS production and increase glutathione levels [335]. Exposition to dietary acrylamide is very frequent

during lifetime, and there is evidence that acrylamide reduces glutathione concentrations. The effect is counteracted by procyanidin B_2 and cacao polyphenolic extract [336]. An important strategy to increase glutathione is the use of glutathione-esters as the esters can restore mitochondrial levels of glutathione. For example, central administration with one of these glutathione esters, monoethyl (GEE), results in increased glutathione, providing neuroprotection against O&NS or mitochondrial impairment.

9.4. Treatments targeting Nrf-2

Nrf-2 is redox-sensitive and orchestrates cellular cytoprotective responses to increased oxidative stress by regulating cellular antioxidant defenses [337]. It moderates a plethora of genes by interacting with the antioxidant response element (ARE) [338]. Nrf-2 is normally confined to the cytoplasm by being bound to an inhibitor molecule, i.e. Keap-1 [337]. The latter molecule likely acts as a redox sensor [337] and oxidative stress leads to the dissociation of the molecular complex releasing Nrf-2 which consequently translocates to the nucleus [339]. The dissociation of the Nrf-2 / Keap-1 complex largely follows the modification of Keap-1 cysteine residues via direct oxidation or conjugation [339,340]. Numerous studies have demonstrated that Nrf-2 is critical for the activation of the cellular glutathione system and maintaining the redox state [341]. Nrf-2 regulates the de novo glutathione synthesis, glutathione peroxidase (GPX2), glutathione *S*-transferases, glutathione reductase, the synthesis of enzymes that mediate glutathione synthesis, i.e. glutathione cysteine ligase modifier subunit and glutathione cysteine ligase catalytic subunit, and the x_c-cysteine / glutamate antiporter system and consequently determines cellular glutathione levels [341]. For example, Nrf-2 activation restores glutathione levels in neutrophils taken form patients with chronic peridontitis [342,343]. In the next sections we discuss different treatment modalities that may upregulate Nrf-2.

9.4.1. Hyperbaric Oxygen Therapy (HBOT)

The Nrf-2-mediated oxidative stress response is activated by hyperbaric oxygen therapy (HBOT) [344]. HBOT induces significant changes in Nrf-2-induced antioxidant pathways, while only minimal changes were observed following treatment with 100% O_2 [345]. At normal pressure, HBOT does not appear to increase the transcription of Nrf-2, but likely acts to free Nrf-2 and enable its translocation to the nucleus [344, 346]. A number of studies report increased glutathione following HBOT [347-349]. HBOT has also been shown to provoke transcription of the cytoprotective protein heme oxygenase-1, another mechanism by which HBOT expression may protect cells against the corrosive effects of O&NS [350-352].

9.4.2. Phytochemicals

Many phytochemicals upregulate the Nrf-2 / Keap1 system [353] and have been demonstrated to upregulate glutathione levels [354,355]. Curcumin, for example, upregulates the transcription of genes coding for antioxidant and phase II enzymes [356-358]. This induced transcription is modulated via ROS-induced activation of Nrf-2, which proceeds through phosphorylation of MAP kinases and PKC [359,360]. The efficacy of curcumin as a treatment has been demonstrated in a number of studies involving animal models of depression [361-363]. In a recent randomized trial, curcumin, 1000 mg/day, was shown to be as effective as fluoxetine, 20 mg/day [361]. Gupta et al. [363] demonstrated the efficacy of curcumin in ameliorating fatigue and oxidative stress in mice with immunologically-induced fatigue, which is perhaps the closest animal model of ME/CFS.

Carnosol [364], resveratrol [365] and cinnamaldehyde [366] stimulate antioxidant enzymes via the same mechanism. Reservatrol has demonstrated efficacy in mouse models of ME/CFS at

least as far as reducing hippocampal atrophy is concerned [367]. However, as with direct glutathione intake, the efficacy of curcumin and resveratrol are limited by their metabolism in the gastro-intestinal tract.

Green tea's epigallocatechin gallate (EGCG) is another compound with ubiquitous benefits, including affording protection against depression [368]. This is mediated partly via EGCG improving mitochondrial functioning, but also by EGCG increasing Nrf-2 and endogenous anti-oxidants, including glutathione and glutathione peroxidase [369]. In addition, glutathione levels are dose-dependently increased after use of sulforaphane, followed by lipoic acid, resveratrol and Polygonum multiflorum. All four compounds potently induce glutathione levels via activation of the Nrf2-ARE pathway [370]. As such the efficacy of many phytochemicals across a range of conditions is intimately associated with the regulation and availability of glutathione.

9.4.3. Dimethyl fumarate

Dimethyl fumarate is approved as a treatment for psoriasis in some European countries. Dimethyl fumarate has antiproliferative effects on lymphocytes, diminishes inflammatory gene expression (e.g. microglial and astroglial IL-1, IL-6 and TNFα), increases anti-inflammatory gene expression (e.g. IL-10), suppresses NF-κB nuclear transport and dependent transcription (in astroglia) and consequently reduces NO synthase gene expression, and has antioxidative and neuroprotective effects [371]. Dimethyl fumarate activates the Nrf-2 pathway leading to a significant stimulation of the glutathione system [371,372]. Thus, in astrocytes, dimethyl fumarate increases Nrf-2 levels (after an initial decrease), reverses LPS-induced decreases in mRNA of glutathione reductase, c-glutamylcysteine and glutathione synthetase, reduces nitrite levels and increases mRNA of the anti-inflammatory haem oxygenase-1 [371]. Dimethyl fumarate is also used as a treatment of multiple sclerosis following the demonstration of the disease modifying ability in

those patients and in experimental autoimmune encephalomyelitis [373, 374]. A Phase 2 trial with dimethyl fumarate in relapsing remitting multiple sclerosis showed significant reductions in new gadolinium enhancing lesions [375].

9.5. Psychiatric Medications

It should be noted that antidepressants, mood stabilizers and many antipsychotic medications increase glutathione and Nrf-2 levels [338]. As such, many of their multi-targeted effects, which seem necessary for their efficacy [293], may be mediated via the important cellular and intercellular functions of glutathione and its interactions with, and regulation of, factors known to be altered in psychosis and mood disorders [376].

Conclusions

The glutathione-related redox state of cells plays an important role in the regulation of pro-inflammatory cytokines, including TNFα, IL-1β and IL-6, and the activity of intracellular signaling pathways. Glutathione depletion enhances the transcription and damaging effects of these cytokines and predisposes to a pro inflammatory environment. Glutathione also has antiviral effects and can inhibit the replication of many viruses, hence depletion of glutathione can have serious consequences and compromise an effective immune response against invading viruses. Glutathione modulates the function of T cells and NKCs with depletion in glutathione levels leading to compromised NK function, T cell activation and proliferation and a predisposition to a Th2 biased immune response.

Glutathione is a crucial component of cellular antioxidant defenses and hence a vital player in detoxification of ROS and RNS and their toxic metabolites such as lipid peroxides. The glutathione system as a whole enables the detoxification of xenobiotics and polymorphisms in

glutathione enzymes are a major source of pathology and drug resistance. S-Glutathionylation, i.e. the posttranscriptional addition of glutathione to protein cysteine, modifies the activity of many proteins include kinases and other cellular signaling proteins and proteins involved in energy production and calcium homeostasis and inhibits the activity of many important enzymes such as phospho-fructokinase and creatine kinase. The activities of a wide range of other proteins may be up or downregulated by S-glutathionylation.

The GSH / GSSH ratio and the level of glutathionylation regulate the mechanisms enabling cellular proliferation, DNA synthesis and repair, division and apoptosis with a depletion of GSH leading to impaired cellular division and proliferation and increased levels of apoptosis. Mitochondrial survival and function is dependent on the glutathione system. Glutathione depletion and increased glutathionylation leads to inhibition of the electron transport chain diminishing ATP production increasing the production of ROS and inhibiting the de novo synthesis of glutathione from its amino acid precursors. This situation ultimately leads to loss of mitochondrial membrane potential, the release of cytochrome c and mitochondrial apoptosis.

Glutathione has important functions in the brain as an antioxidant and neuromodulator and promoter of neuronal survival. Glutathione synthesis acts as a neuromodulator of the glutamate ionotropic receptors, interacts with NMDA receptors and protects against glutamate excitotoxicity. The optimum functioning of the NDMA receptor is dependent on the redox environment, with prolonged glutathione depletion compromising synaptic plasticity, memory and learning. Glutathione may also be a neurotransmitter in its own right and its depletion impairs the activity of the methionine cycle leading to impaired DNA methylation and epigenetic regulation of gene expression.

Lowered activity of the glutathione system is observed in neuro-immune disorders, including depression, ME/CFS and Parkinson’s disease. The abovementioned functions of the glutathione

system and the consequences of glutathione depletion explain that lowered activity of the glutathione system plays a role in the immune-inflammatory and O&NS pathophysiology of these diseases. A number of approaches show promise in raising glutathione levels and combatting the elevated levels of O&NS which has a causative role in the development of many neuro-immune diseases. Although direct glutathione supplementation appears to be ineffective, the use of N-acetyl cysteine, phytochemicals, HBOT and dimethyl fumarate shows considerable promise.

Competing interests

No specific funding was obtained for this specific review.

The authors declare that they have no competing interests.

Authors' contributions

GM and MM participated in the design of this review, while all authors helped to write the paper and approved the final version.

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Figure 1

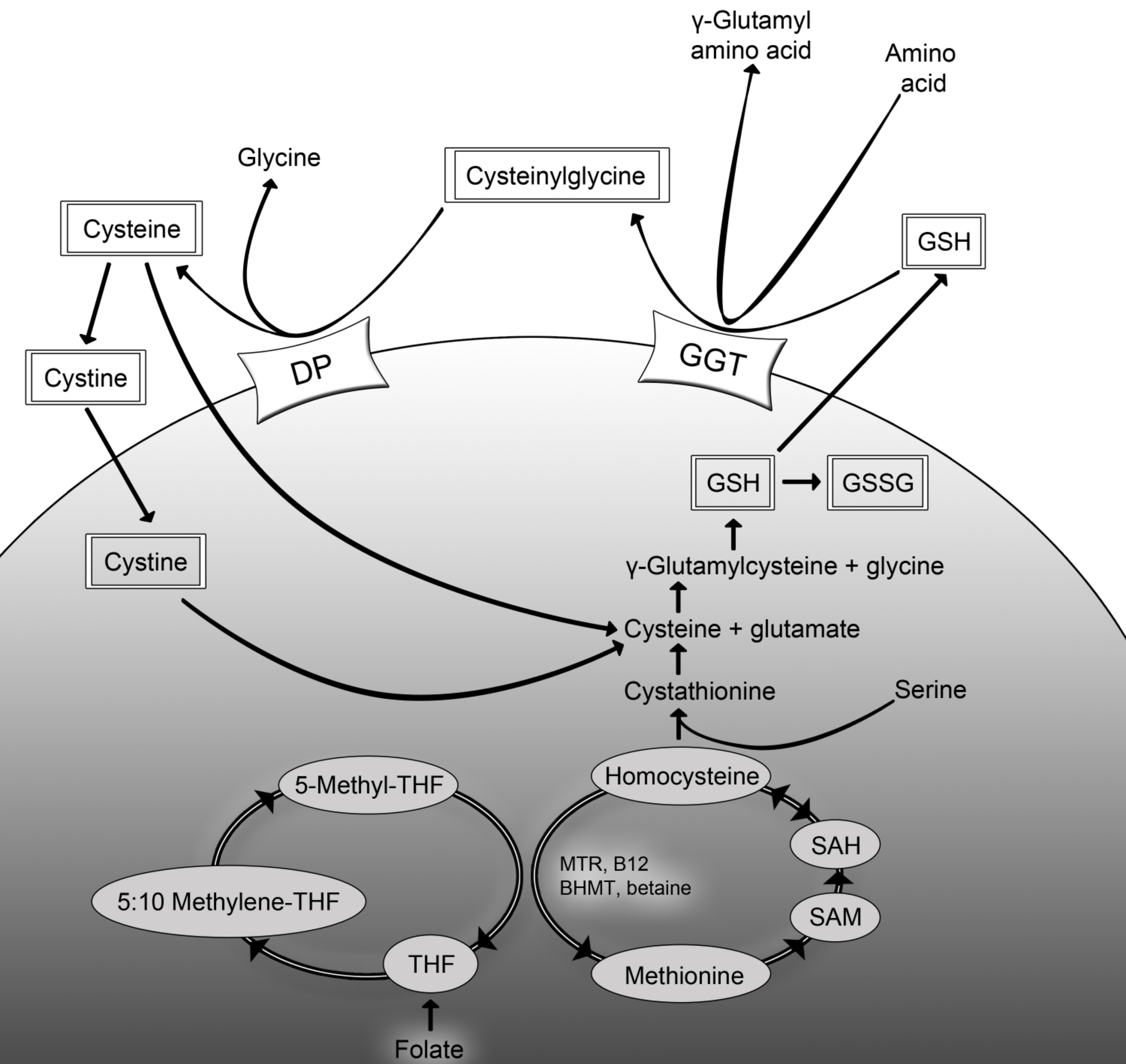
Glutathione (GSH) is normally produced de novo via a two-step reaction. In the first step γ-glutamylcysteine is the product of a reaction between cysteine and glutamate enabled by the enzyme glutamate cysteine ligase (GCL). Glycine is then added to produce GSH through glutathione synthetase. GCL activity and cysteine levels are the two rate-limiting factors in GSH synthesis. The activity and expression of GCL is regulated by GSH or more accurately the GSH/GSSG ratio via a number of redox sensitive pathways in response to a GSH shortage or oxidative stress. Other mechanisms exist to maximize the regeneration of GSH and minimize the concentration of GSSG. Such mechanisms involve the regulation of cysteine importation and the exportation of GSH. Another mechanism involves the up-regulation of γ-glutamyl transpeptidase (GGT). This enzyme located on the outer membrane of cells transfers the glutamate from GSH to an amino acid acceptor molecule. The synthesis of GSH is also associated with the trans-sulfuration pathway, which synthesizes cystathionine from homocysteine and serine and leads to the formation of cysteine. The S-adenosylmethionine (SAM) / S-adenosylhomocysteine (SAH) ratio acts as sensor mechanism which results in the diversion of homocysteine into the trans-sulfuration pathway resulting in increased GSH synthesis. The reaction from 5-methyltetrahydrofolate (5-methyl-THF) to tetrahydrofolate (THF) transfers a methyl group to homocysteine theraby forming methionine.

Figure 2

Glutathione (GSH) and antioxidant enzymes, including superoxide dismutase (SOD), glutathione peroxidase, catalase may reduce the reactive oxygen species (ROS), which are produced through activity of the respiratory chain in the mitochondria. Loss of glutathione in mitochondria predisposes to pathology as the glutathione peroxidase enzymes require GSH as a cofactor. GSH is also unique in its ability to detoxify hydroxyl radicals. In an environment of low GSH, ROS levels rise and the loss of glutathione peroxidase activity is relatively more important in mitochondria which normally lack catalase. Ultimately, the excessive production of hydrogen peroxide and hydroxyl radicals in mitochondria will lead to damaged mitochondrial DNA, lipids and proteins compromising mitochondrial function and ultimately necrotic cell death.

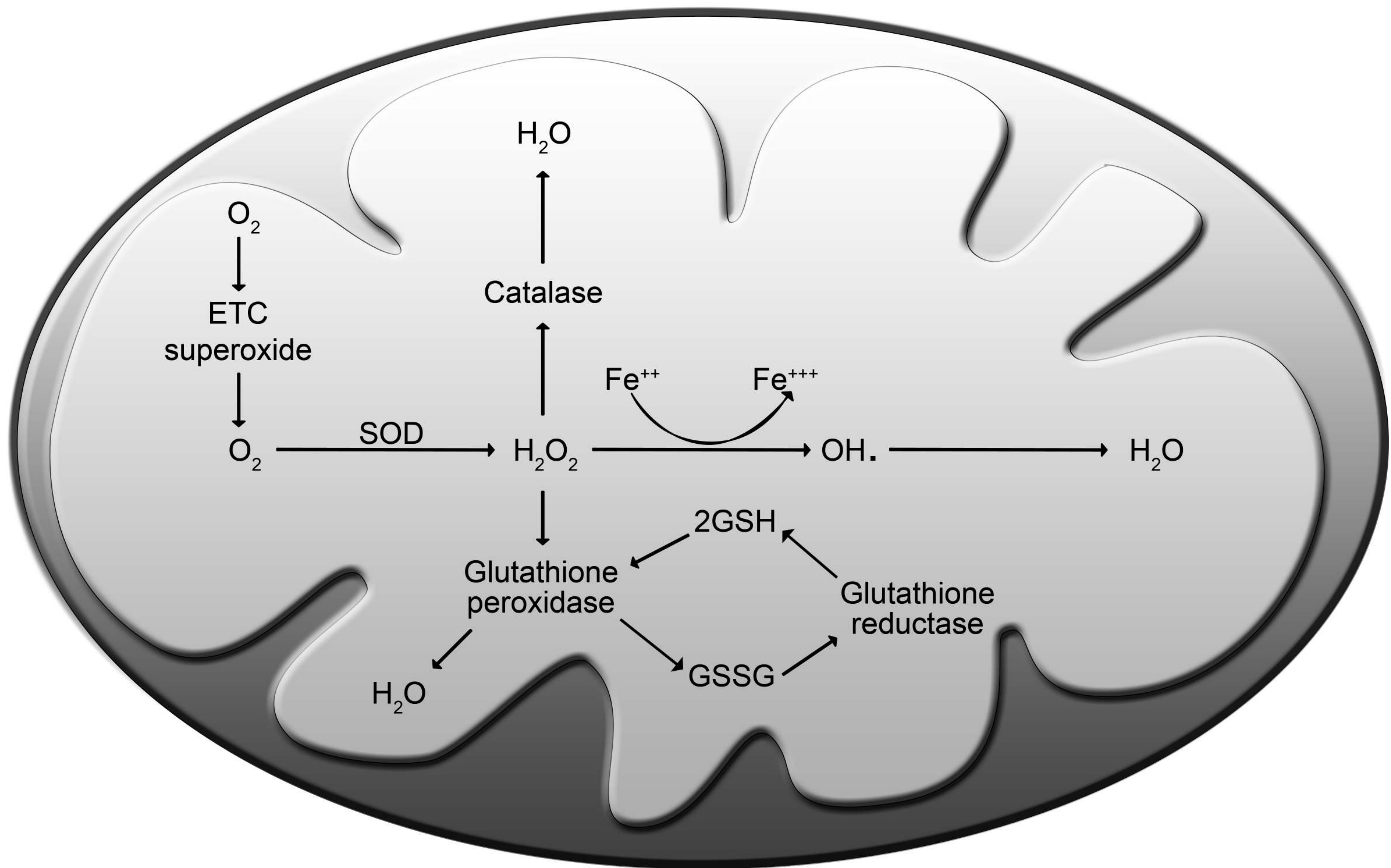
Figure 3.

Methemoglobin (MetHb) is a conformationally abnormal hemoglobin (Hb) continually produced in the body where the iron molecule is in the higher oxidation ferric state, rather than the normal ferrous state, which inhibits its oxygen-binding properties. Red blood cells can combat the development of oxidative stress, which would otherwise lead to inactivation of hemoglobin and disruption of red blood cell membranes by a number of mechanisms. The most important is reliant on nicotinamide adenine dinucleotide phosphate (NADPH), which is produced by the pathway often called the hexose monophosphate shunt. This NADPH reduces oxidized glutathione to glutathione (GSH) maintaining high levels of GSH even when ROS concentrations are high. GSH acts as an essential cofactor for glutathione peroxidase, resulting in the reduction or elimination of ROS and minimizing the formation of methemoglobin.



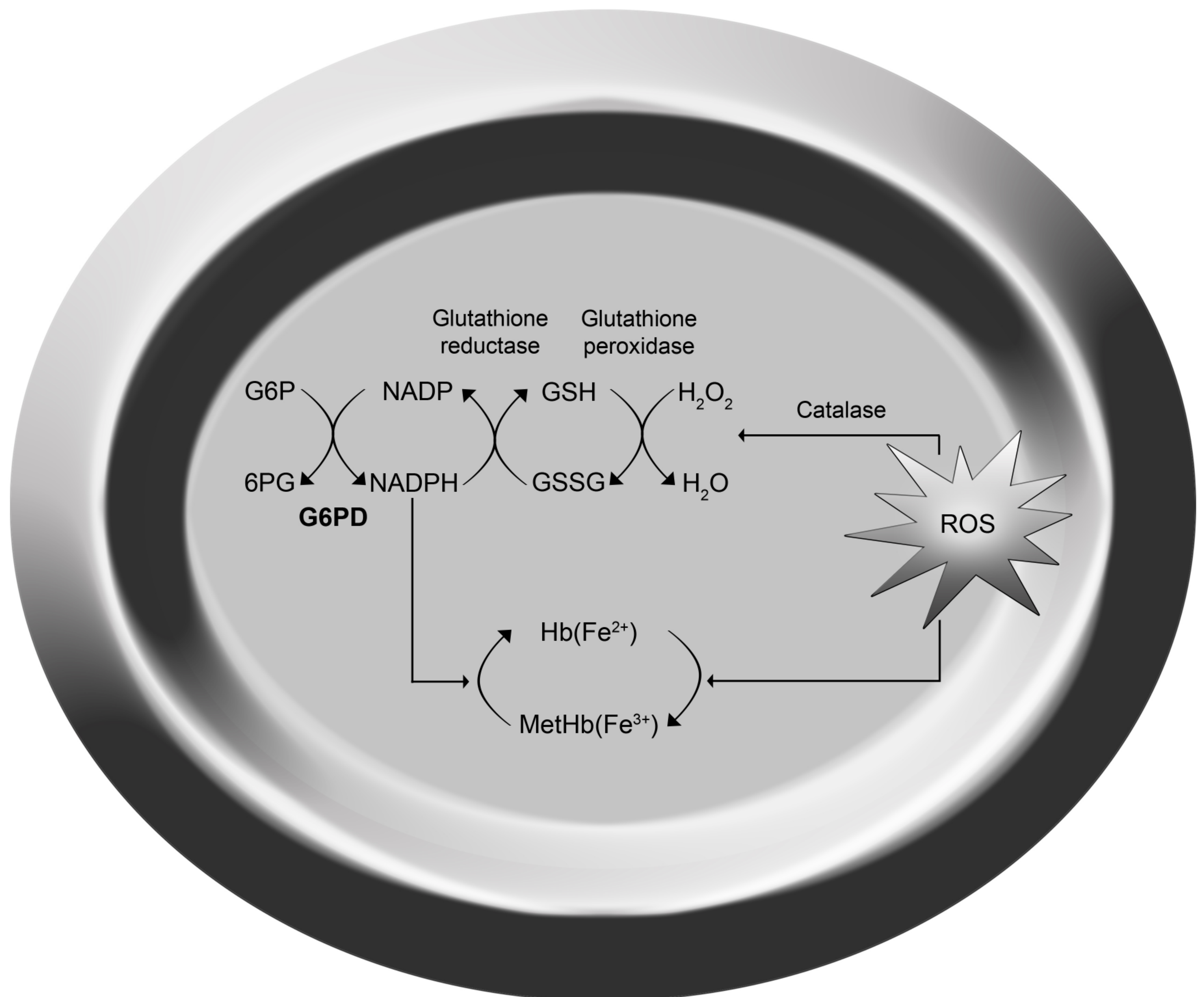
Abbreviations.

B12: vitamin B12.
BHMT: betaine homocysteine methyltransferase.
DMA: dimethylarsinic acid.
DP: dipeptidase.
GGT: γ-glutamyltransferase.
GSH: glutathione.
GSSG: oxidized glutathione.
InAs: inorganic As.
MMA: monomethylarsonic acid.
MTR: methionine synthetase.
SAM: S-adenosylmethionine.
SAH: S-adenosylhomocysteine.
THF: Tetrahydrofolate.



Abbreviations.

ETC superoxide: electron transport chain superoxide.
Fe: Iron.
GSH: glutathione.
GSSG: oxidized glutathione.
H_2O: water
H_2O_2: hydrogen peroxide.
SOD: superoxide dismutase.



Abbreviations.

6PG: 6-Phosphogluconic acid.
G6P: Glucose 6-phosphate.
G6PD: Glucose-6-phosphate dehydrogenase
GSH: glutathione.
GSSG: oxidized glutathione.
H_2O: water
H_2O_2: hydrogen peroxide.
.

$Hb(Fe^{2+})$: hemoglobin.
$MetHb(Fe^{3+})$: methemoglobin.
NADP: nicotinamide adenine dinucleotide phosphate.
NADPH: nicotinamide adenine dinucleotide phosphate-oxidase.
ROS: reactive oxygen species.