

**IS GLUTATHIONE DEPLETION
AN IMPORTANT PART OF THE
PATHOGENESIS OF
CHRONIC FATIGUE SYNDROME?**

by

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WHAT IS GLUTATHIONE?

[Refs. 1--5]

- **A tripeptide composed of the amino acids glutamic acid, cysteine, and glycine. Its molecular weight is 307.33 Da.**
- **Found in all cells in the body, in the bile, in the epithelial lining fluid of the lungs, and, at much smaller concentrations, in the blood.**
- **The predominant nonprotein thiol (molecule containing an S-H or sulfhydryl group) in cells.**
- **Its active form is the chemically reduced form, called "GSH."**
- **GSH is compartmentalized, with concentrations ranging from 0.5 to 10 millimolar in various organs and cell types.**
- **GSH serves as a substrate for enzymes, including the glutathione peroxidases and the glutathione-S-transferases.**
- **When oxidized, two glutathione molecules join together via a disulfide bond to form "oxidized glutathione," or "glutathione disulfide," referred to as "GSSG."**
- **Inside cells, the concentration of GSSG is normally maintained at less than 1% of total glutathione by the enzyme glutathione reductase, which is powered by NADPH, produced by the pentose phosphate shunt, part of normal carbohydrate metabolism.**

WHAT ARE SOME OF THE FUNCTIONS OF GLUTATHIONE (GSH)?

[Refs. 1--5]

- **Maintains proper oxidation-reduction (redox) potential inside cells. Redox affects the oxidation state of sulfur in enzymes, and thus affects the rates of biochemical reactions in cells.**
- **Scavenges peroxides and oxidizing free radicals directly and also serves as the basis for the antioxidant network.**
- **Performs Phase II detoxication of heavy metals (such as mercury), organophosphate pesticides, chlorinated hydrocarbon solvents, estradiol, prostaglandins, leukotrienes, acetaminophen, and other foreign and endogenous toxins.**
- **Stores and transports cysteine throughout the body.**
- **Transports amino acids, especially cystine into kidney cells.**
- **Regulates the cell cycle, DNA and protein synthesis and proteolysis, and gene expression.**
- **Regulates signal transduction.**
- **Participates in bile production.**
- **Protects thyroid cells from self-generated hydrogen peroxide.**

In carrying out several of the above functions, GSH plays very important roles in (1) maintaining mitochondrial function and integrity, (2) regulating cell proliferation, and (3) supporting the immune system.

HOW IS GLUTATHIONE (GSH) SYNTHESIZED IN THE BODY?

[Refs. 1--5]

- **GSH is synthesized inside cells by a two-step process. The first step involves the ATP-powered enzyme glutamate cysteine ligase (formerly called gamma-glutamylcysteine synthetase). This step is normally the rate-limiting reaction, and is controlled by the cellular redox state and feedback inhibition, among other factors. The second step makes use of the ATP-powered enzyme glutathione synthetase.**
- **The necessary substrates are cysteine (which is often the rate-limiting substrate when GSH is depleted), glutamic acid (or glutamine) and glycine. Cysteine and glutamic acid are joined together in the first step, and glycine is added in the second step.**
- **The liver is the main producer and exporter of GSH.**
- **A few epithelial cell types can import GSH molecules intact.**
- **Most cell types use the gamma glutamyl (or GSH scavenging) cycle. This cycle makes use of the plasma-membrane-bound exoenzymes gamma-glutamyl transpeptidase and dipeptidase. This cycle disassembles GSH outside the cell and imports the parts for reassembly inside. It also serves as a transport mechanism to bring other amino acids into the cell, cystine (di-cysteine) being favored.**

IS GLUTATHIONE DEPLETED IN CHRONIC FATIGUE SYNDROME?

There is considerable evidence that it is, at least in a substantial fraction of CFS patients. Here are the results of all the published studies that bear on this question:

- **GSH depletion in CFS was first suggested by Droge and Holm [6].**
- **Cheney [7,8] reported that his CFS clinical patients were almost universally low in GSH.**
- **Richards et al. [9] found that patients could be divided statistically into two distinct groups, one having significantly elevated erythrocyte GSH relative to a healthy control group, and the other having significantly lower values.**
- **Fulle et al. [10] found elevated total (reduced plus oxidized) glutathione in muscle biopsy specimens from PWCs relative to healthy controls, but they did not report values for reduced glutathione alone.**
- **Manuel y Keenoy et al. [11] found that a subgroup of fatigued patients with low magnesium, which did not improve with supplementation, had significantly lower GSH.**
- **Manuel y Keenoy et al. [12] did not find a significant difference between CFS patients and fatigued controls in terms of whole-blood GSH, but they did not compare with a healthy control group.**
- **Kennedy et al. [13] found significantly lower red blood cell GSH in PWCs compared to healthy controls (p=0.05).**
- **Kurup and Kurup [14] found significantly lower red blood cell GSH in myalgic encephalomyelitis patients compared to healthy controls (p<0.01).**

IN THE GENERAL POPULATION, WHAT FACTORS OR CONDITIONS ARE KNOWN TO CAUSE DECREASES IN INTRACELLULAR GLUTATHIONE CONCENTRATIONS?

These factors and conditions can be divided into three groups:

- **The first group is made up of those that (1) lower the rate of GSH synthesis or the rate of reduction of GSSG to GSH, or (2) raise the rate of export of GSH from cells, or (3) lead to loss of GSH from the scavenging pathway. This group includes the following: genetic defects [15], elevated adrenaline secretion [16-20] due to various types of stress, deficient diet [1] or fasting [21], surgical trauma [21,22], burns [23], and morphine [24].**
- **The second group is comprised of toxins that conjugate GSH and remove it from the body [25], such as organophosphate pesticides, halogenated solvents, tung oil (used on furniture), acetaminophen and some types of inhalation anesthesia.**
- **The third group is comprised of conditions that raise the production rates of reactive oxygen species high enough to produce oxidative stress, causing cells to export GSSG. These include strenuous or extended exercise [26], infections (producing leukocyte activation) [21], toxins that produce oxidizing free radicals during Phase I detoxication by cytochrome P450 enzymes [21], ionizing radiation [27], iron overload [28], and ischemia--reperfusion events (such as stroke, cardiac arrest, subarachnoid hemorrhage, and head trauma) [29].**

STRESS, DISTRESS, AND STRESSORS

- **For purposes of this presentation, stressors are defined in the broad sense as events, circumstances or conditions that place demands on a person and tend to move his or her body out of allostatic balance. Allostasis is similar to homeostasis, but allows for changes in the set-point over time to match life circumstances [30]. Stressors can be classified as physical, chemical, biological, or psychological/emotional.**
- **Stress is the state that results from the presentation of such demands. Selye [31] defined stress as "the state manifested by a specific syndrome which consists of all the nonspecifically-induced changes within a biologic system." Although Selye emphasized the nonspecifically-induced responses, the body also exhibits specific responses that depend on the type of stress [32].**
- **Stress can be of a beneficial or a destructive nature. Distress is the destructive type of stress [31].**
- **The perceived stress that people experience depends not only on the stressors to which they are subjected, but also on "their appraisals of the situation and cognitive and emotional responses to it." [33]**
- **A person's history of both the occurrence of stressors and of the degree of perceived stress can be evaluated by structured interviews, and this has been done in a number of studies of CFS risk factors.**

IS THERE EVIDENCE FOR HIGHER OCCURRENCE OF STRESSORS IN CFS PATIENTS PRIOR TO ONSET THAN IN HEALTHY NORMAL CONTROLS?

YES. The types of stressors found to have higher occurrence in one or more CFS risk factor studies [34-45] include the following:

- **Physical:** Aerobic exercise (especially of long duration), physical trauma (especially motor vehicle accidents) and surgery (including anesthesia).
- **Chemical:** Exposure to toxins such as organophosphate pesticides, solvents and ciguatoxin.
- **Biological:** Infections, immunizations, blood transfusions, insect bites, allergic reactions, and eating or sleeping less.
- **Emotional/Psychological:**

Stressful life events, including death of a spouse, close family member or close friend; recent marriage; troubled or failing marriage, separation, or divorce; serious illness in immediate family; job loss, starting new job, or increased responsibility at work; and residential move.

Difficulties, including ongoing problems with relationships, persistent work problems or financial problems, mental or physical violence, overwork, extreme sustained activity, or "busyness."

Dilemmas "A dilemma is a situation in which a person is challenged to choose between two equally undesirable alternatives." [45] Choosing inaction in response to a dilemma leads to further negative consequences.

Problems in childhood, including significant depression or anxiety, alcohol or other drug abuse, and/or physical violence in parents or other close family members; physical, sexual or verbal abuse, low self-esteem and chronic tension or fighting in the family.

**IS THERE EVIDENCE FOR HIGHER
PERCEIVED STRESS IN CFS PATIENTS
PRIOR TO ONSET, COMPARED TO
HEALTHY CONTROLS?**

YES. Three studies [34, 37, 38] found that CFS patients rated their level of perceived stress prior to onset higher than did healthy, normal controls for a similar period of time.

**IS IT SURPRISING THAT GLUTATHIONE
BECAME DEPLETED IN MANY CFS
PATIENTS?**

NO. In view of the strong correspondence between the results of the CFS risk factor studies and the known GSH depletors, it is not surprising. It appears that the CFS patients who were studied had undergone a variety of factors and conditions that are known to deplete glutathione, and had also experienced high levels of perceived stress as a result.

HOW DOES THE NEUROENDOCRINE SYSTEM RESPOND TO STRESS?

- This system manifests both specifically- and nonspecifically-induced responses to stress [32]. The nonspecifically-induced responses address the combined load of all the various types of stress that are being experienced simultaneously.
- The nonspecific responses are mediated by three parts of this system: (1) the hypothalamus-pituitary-adrenal (HPA) axis, which produces cortisol and other glucocorticoids, (2) the sympathetic-adrenomedullary system, which produces epinephrine (adrenaline), and (3) the sympathoneural system, which produces norepinephrine (noradrenaline) [32].
- Rapid-onset CFS patients report that they had a normal response to stress prior to their onset of CFS. Therefore, it can be surmised that if they experienced a high load of combined long-term stress lasting a few months to several years prior to their onset, they were subject to high levels of both cortisol and adrenaline during this extended period of time.
- Note that depleted rather than elevated cortisol levels are frequently observed clinically in CFS patients (Cleare [46]). However, the decrease in cortisol secretion occurs later in the pathogenesis: "*...the bulk of the data assembled to date is compatible with the view that the disruption in adrenocortical function is a late finding, and that elucidating the status of the central nervous system components which drive the regulation of the HPA axis would be crucial to a more complete understanding of this final event.*" (Demitrack [47])

WHAT ARE THE EFFECTS OF ELEVATED LEVELS OF CORTISOL AND ADRENALINE ON THE IMMUNE SYSTEM AND ON GLUTATHIONE LEVELS?

- Elevation of cortisol is known to suppress the inflammatory response by several mechanisms, including decreasing the expression of cytokines and cell adhesion molecules, and decreasing the production of prostaglandins and leukotrienes [48]. This effect is beneficially used therapeutically in many cases, but it can also have a down side if an infection is present.
- Elevation of cortisol is also known to suppress cell-mediated immunity and to cause a shift to the Th2 type of immune response. Several mechanisms are involved, including suppressing the secretion of IL-1 by macrophages, inhibiting the differentiation of monocytes to macrophages, inhibiting the proliferation of T lymphocytes, and increasing the production of endonucleases, which increases the rate of apoptosis of lymphocytes [33,48].
- Long-term elevation of adrenaline can be expected to deplete GSH, because adrenaline decreases the rate of synthesis of glutathione by the liver (Estrela et al. [18]), increases its rate of export from the liver (Sies and Graf [16]; Haussinger et al. [17]; Estrela et al. [18]), and decreases the rate of reduction (recycling) of oxidized glutathione (Toleikis and Godin [19]).

HOW DO VIRAL INFECTIONS ARISE AT THE ONSET OF CHRONIC FATIGUE SYNDROME?

I propose that glutathione depletion is the trigger for reactivation of endogenous latent viruses in CFS (hypothesis).

Here's the support for this hypothesis:

- Most of the evidence points to reactivation of latent endogenous viruses at the onset of CFS, rather than new, primary infections (Komaroff and Buchwald [49])**
- Infections by members of the Herpes family of viruses, such as Epstein-Barr virus and HHV-6 are commonly found in CFS patients [49].**
- GSH depletion is associated with the activation of several types of viruses [50-53], including Herpes simplex type 1 (HSV-1) [54]. Raising the GSH concentration inhibits replication of HSV-1 by blocking the formation of disulfide bonds in glycoprotein B, a protein that is necessary for proliferation of the virus [54].**
- Glycoprotein B is also found in all other Herpes family viruses studied, including EBV and CMV [55], and very likely is present also in HHV-6 and performs the same vital function there (hypothesis).**

It thus appears very likely that GSH depletion is the trigger for the reactivation of the latent forms of all the Herpes family viruses (hypothesis). Since glutathione likely becomes depleted prior to the onset of CFS, and since infections by these viruses are commonly found in CFS, it seems likely that glutathione depletion initiates the viral infections at the onset of CFS (hypothesis).

CAN ELEVATED CORTISOL AND DEPLETED GLUTATHIONE EXPLAIN THE IMMUNE DYSFUNCTIONS?

YES.

- **The shift to the Th2 immune response, as observed in CFS [56], is a known effect of both elevated cortisol [57] and of depleted GSH [58, 59]. I suggest that elevated cortisol produces the shift initially, and that GSH depletion maintains it later, after the cortisol level drops due to blunting of the HPA axis.**
- **The following dysfunctions seen in CFS [60] are known effects of depleted GSH: lowered natural killer cell and cytotoxic T cell cytotoxicity; inability of T cells to proliferate, as seen in decreased mitogen-induced proliferative response of lymphocytes and decrease in delayed-type hypersensitivity [61].**

In addition, I hypothesize the following:

- **The observed chronic immune activation [60] and the observed continuous activation of the RNase-L pathway in CFS [60] result from the failure of cell-mediated immunity to defeat detected infections, owing to the above effects of GSH depletion.**
- **The observed low molecular weight Rnase-L [62] results from lack of inhibition of caspases because of thiol (GSH) depletion, and they cleave the Rnase-L.**
- **The observed elevated numbers of immune complexes [60] result from the shift to the Th2 response, which produces elevated levels of antibodies.**
- **The observed elevation in antinuclear antibodies [60] results from the observed higher rate of apoptosis [63-66], which is a known consequence of GSH depletion [67].**

HOW DOES PHYSICAL FATIGUE ARISE AT THE ONSET OF CFS?

(HYPOTHESIS)

- When the immune system detects the viral infection, it becomes activated.
- In attempting to proliferate, the lymphocytes draw upon the already depleted supplies of GSH and its precursor, cysteine (or cystine).
- Being in the blood, the lymphocytes have earlier access to GSH and cysteine than do the skeletal muscles.
- Competition in CFS between the immune system and the skeletal muscles for these substances has already been hypothesized by Bounous and Molson [68], and I agree with their hypothesis.
- The skeletal muscles become more depleted in GSH.
- This produces a rise in their concentration of peroxynitrite. (Peroxynitrite forms from superoxide and nitric oxide. Superoxide becomes elevated because the depletion of GSH causes a rise in hydrogen peroxide, and this exerts product inhibition on the superoxide dismutase reaction, causing superoxide levels to rise.)
- As Pall [69] has stated, "*Peroxynitrite reacts with and inactivates several of the enzymes in mitochondria so that mitochondrial and energy metabolism dysfunction is one of the most important consequences of elevated peroxynitrite.*"
- The resulting partial blockades in the Krebs cycles and the respiratory chains in the red, slow-twitch skeletal muscle cells decrease their rate of production of ATP. Since ATP is what powers muscle contractions, the lack of it produces physical fatigue. It becomes chronic because GSH remains depleted.

SINCE GLUTATHIONE IS AT THE BASIS OF THE BODY'S ANTIOXIDANT SYSTEM, ITS DEPLETION CAN BE EXPECTED TO PRODUCE OXIDATIVE STRESS. HAS THIS BEEN OBSERVED IN CFS?

YES. Oxidative stress is now well-established in CFS.

The following researchers have presented evidence for oxidative stress in CFS:

- **Ali [70,71]**
- **Cheney [7,8]**
- **Richards et al. [9,72]**
- **Fulle et al. [10]**
- **Manuel y Keenoy et al. [11,12]**
- **Vecchiet et al. [73]**
- **Kennedy et al. [13]**
- **Smirnova and Pall [74]**

WHAT EFFECTS DO ELEVATED CORTISOL AND DEPLETED GLUTATHIONE HAVE ON BRAIN FUNCTION, AND ARE THEY OBSERVED IN CFS?

- **Long-term cortisol elevation is known to damage the hippocampus, and GSH depletion is involved [75].**
- **Additional depletion of GSH would likely exacerbate the effects of elevated cortisol on the hippocampus.**
- **The hippocampus is involved with memory, sleep, and control of the HPA axis.**
- **Deficits in all these areas are seen in CFS.**
- **Examination of the hippocampus in CFS by magnetic resonance spectroscopy suggested significantly lower metabolism in the right hippocampus [76].**
- **It seems likely that elevated cortisol and depleted GSH account for at least some of the CFS brain function deficits.**

SINCE GLUTATHIONE NORMALLY REMOVES MERCURY FROM THE BODY, ITS DEPLETION CAN BE EXPECTED TO ALLOW BUILDUP OF MERCURY IN CFS PATIENTS. IS THIS OBSERVED?

YES. While there are no published controlled studies of mercury level testing in CFS patients, several clinicians who specialize in treating CFS have reported that many of their patients have high mercury levels:

- Ali [77]
- Godfrey [78]
- Conley [79]
- Poesnecker [80]
- Teitelbaum [81]
- Corsello [82]
- Goldberg [83]

In addition, immune testing has shown significantly elevated hypersensitivity to mercury in many CFS patients (Stejskal et al., [84]; Sterzl et al., [85]; and Marcusson, [86]). This suggests that the immune system has responded to elevated mercury levels.

(Note that there have been epidemiological studies that showed no evidence that dental amalgams are associated with CFS as a causal factor [87,88]. However, this does not constitute evidence that amalgams do not give rise to elevated mercury levels after CFS onset in people who have amalgams and who may have developed CFS as a result of other causes.)

CAN GLUTATHIONE DEPLETION EXPLAIN AUTOIMMUNE THYROIDITIS IN CHRONIC FATIGUE SYNDROME?

YES.

- **It is known that thyroid cells normally produce hydrogen peroxide to oxidize iodide ions as part of the pathway for producing thyroid hormones. Normally, this oxidation occurs outside the cell membrane, and the interior of the cell is protected from the hydrogen peroxide by intracellular GSH [89].**
- **It has been shown by Duthoit et al., [90] that if hydrogen peroxide is allowed to enter thyroid cells, it will attack and cleave thyroglobulin, producing C-terminal fragments that can diffuse into other cells and are recognized by autoantibodies from patients with autoimmune thyroid disease. This suggests that hydrogen peroxide entry into thyroid cells may be the cause of this disease.**
- **It has been shown by Wikland et al. [91], using fine needle aspiration cytology, that about 40% of patients suffering from chronic fatigue show evidence of chronic autoimmune thyroiditis, even though TSH levels were in the normal range in many of them.**

HYPOTHESIS: It seems likely that GSH depletion accounts for this high prevalence.

WHY IS CFS MORE PREVALENT IN WOMEN THAN IN MEN?

- **It has been found recently that the monthly menstrual cycle in women presents an additional demand on GSH that does not occur in men. 17-beta estradiol is elevated in women from the late follicular phase through the early luteal phase of the cycle. This hormone stimulates the activity of the enzyme glutathione peroxidase [92].**
- **Perhaps this occurs to protect against elevated production of reactive oxygen species generated during the rapid growth of the endometrium.**
- **The resulting reactions depress the endometrial GSH level during the time the estradiol level is high [92].**

HYPOTHESIS: I propose that this additional demand for GSH in women exacerbates the GSH depletion that occurs as a result of other causes, and that this makes women more vulnerable to developing CFS, accounting for the higher observed prevalence of CFS in women than in men.

WHAT APPROACHES HAVE BEEN USED TO BUILD GLUTATHIONE?

- **Diet high in sulfur-containing amino acids (as in animal-based protein, such as milk, eggs and meat) and antioxidants (as in fresh fruits and vegetables) [93].**
- **Diet high in GSH, e.g. fresh fruits and vegetables and meats [94].**
- **Curcumin [95].**
- **N-acetylcysteine together with glutamic acid or glutamine and glycine [96], or NAC together with dietary protein [97].**
- **Non-denatured whey protein [98]**
- **Oral reduced glutathione [4]**
- **Intravenous reduced glutathione [99]**
- **Intramuscular reduced glutathione [100]**
- **Transdermal reduced glutathione skin cream or lotion [101]**
- **Sublingual reduced glutathione troches [102]**
- **Reduced glutathione rectal suppositories [103]**
- **Reduced glutathione aerosol [104]**
- **Reduced glutathione nasal spray [105]**

HAS GLUTATHIONE REPLETION BEEN USED CLINICALLY IN CFS, AND IF SO, WHAT HAVE BEEN THE RESULTS?

YES.

Patricia Salvato, M.D. [100] has used intramuscular injections of GSH combined with ATP clinically for several years. In 1998 she reported on a study of 276 CFS patients, using 100 mg of GSH and 1 mg of ATP weekly. After 6 months of treatment, 82% experienced improvement in fatigue, 71% experienced improvement in memory and concentration, and 62% experienced improvement in levels of pain.

Paul Cheney, M.D. reported in 1999 [7,8] on his clinical use of oral undenatured whey protein in CFS patients. The dosage varied with different patients, up to 40 grams per day. He reported that several of his patients improved on this treatment, and some who had had active infections with herpes family viruses, mycoplasma, or chlamydia were cleared of them by this treatment.

John S. Foster, M.D. and his colleagues reported in 2002 [99] on their use of GSH in an intravenous fast push (over 2 to 3 minutes). Dosage ranged up to 2,500 mg, 1 or 2 times weekly, as part of a detoxification protocol used on a variety of patients, including some with CFS. They reported that the treatment *"has been promising in addressing neurodegenerative and neurotoxic disorders."*

CONCLUSION

Glutathione depletion indeed appears to be an important aspect of the pathogenesis of chronic fatigue syndrome for at least a substantial fraction of patients.

Is repletion of glutathione likely to be the complete answer for treating CFS?

No. GSH depletion occurs near the beginning of the complex pathogenesis of CFS. There are likely to be many interactions and vicious circles as the pathogenesis develops into the pathophysiology, and there may also be damage that is difficult to correct. The mediators of such damage would likely be infections, toxins and reactive oxygen species, all of which are able to build up because of the depletion of GSH. It is likely that a multifaceted treatment protocol will be necessary.

There are also some cautions that should be exercised:

- When GSH repletion is begun in patients who have been GSH-depleted for extended periods of time, their immune and detoxication systems can begin to function at higher levels of performance. If their bodies have accumulated elevated levels of toxins (especially mercury) and infections, glutathione repletion can cause significant Herxheimer-type reactions as pathogens are killed and toxins are mobilized. Care should be taken to proceed slowly and cautiously in such cases in order to avoid moving toxins into the central nervous system or exacerbating symptoms to a level that is intolerable to the patient.**
- Plasma cysteine level should be monitored periodically when repleting glutathione, to ensure that it does not rise to levels that could be neurotoxic [106].**

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