The NO/ONOO- Vicious Cycle Mechanism as the Cause of Chronic Fatigue Syndrome/Myalgic Encephalomyelitis

Martin L. Pall*
Professor Emeritus of Biochemistry and Basic Medical Sciences, Washington State University and Research Director, The Tenth Paradigm Research Group

Abstract

Cases of chronic fatigue syndrome/mylagic encephalomyelitis (CFS) are reported to be initiated by nine different short-term stressors, each of which increase levels of nitric oxide in the body. Elevated nitric oxide, acting through its oxidant product, peroxynitrite, initiates a local biochemical vicious cycle, the NO/ONOO- cycle, which is proposed to be the cause CFS and related diseases. Evidence supporting this cycle mechanism in CFS comes from each of the following types of evidence: Case initiation by such stressors, the extensive evidence supporting the existence of individual cycle mechanisms, evidence showing that various cycle elements are elevated in CFS cases, evidence for a basically local mechanism in CFS and related disease, evidence from CFS animal models, genetic evidence from genetic polymorphism studies and evidence from clinical trials of agents predicted to down-regulate the NO/ONOO- cycle. Each of the five principles underlying the NO/ONOO- cycle mechanism is supported by one or more of the above described types of evidence. The cycle involves oxidative stress, excessive nitric oxide synthase (NOS) activity, mitochondrial dysfunction, inflammatory biochemistry, excitotoxicity including excessive NMDA activity and tetrahydrobiopterin depletion. There is evidence, ranging from extensive to modest, supporting roles for each of these in CFS.

Clinical studies of treatment protocols containing 14 or more agents predicted to down-regulate the NO/ONOO- cycle appear to be effective in the treatment of CFS and related diseases. However, none of these have yet been shown to be able to cure substantial numbers of cases of CFS or related illnesses. The author discusses one such protocol and suggests an approach, previously tested only as a single agent treatment, that may strenthen these multi-agent protocols to obtain at least some of the needed cures.

* E-mail: martin_pall@wsu.edu; Tel: 503-232-3883

Introduction

Chronic fatigue syndrome (CFS) and such related multisystem illnesses as multiple chemical sensitivity (MCS), fibromyalgia (FM) and post-traumatic stress disorder (PTSD) are all thought to be caused by a biochemical vicious cycle mechanism now called the NO/ONOO- cycle [1-14]. Cases of these illnesses are initiated by any of several diverse short-term stressors, each of which has the ability to increase nitric oxide levels in the body [1,6,9,13], Table 1.

Cases of CFS are most commonly initiated by viral or bacterial infections, with the viruses including coxsackie, Epstein-Barr, rubella, varicella, parvovirus, Borna and Ross River viruses [1,4]. West Nile virus should probably be added to this list, since substantial numbers of severe infections with that virus are reported to develop chronic CFS-like symptoms [15]. Such initiating stressors as viral, bacterial and protozoan infections and also exposure to ionizing radiation act to increase nitric oxide levels by inducing the inducible nitric oxide synthase, iNOS [1,4,13], whereas each of the stressors that initiate cases of MCS (Table 1) as well as some others [1,10], act via increased NMDA activity which acts, in turn through calcium-dependent stimulation of nNOS and eNOS activity. So the common feature is not the specific nitric oxide synthase (NOS) isozyme involved, let alone the pathway of such NOS stimulation, but rather the consequent increase in nitric oxide. The consequent chronic illness initiated by such largely short-term nitric oxide level increases is thought to be produced by the NO/ONOO- cycle (Fig. 1), a cycle that may be initiated largely via peroxynitrite, a potent oxidant formed by the diffusion-limited reaction of nitric oxide and superoxide ($OO^{\cdot}$-) [1,4,6,10].

Each of the arrows diagrammed in Fig. 1, represents one or more mechanisms by which one of the elements of the cycle produces increases in another such element [1,6]. The combination of these mechanisms produces, therefore, a series of interacting cycles that is known as the NO/ONOO- cycle, based on the structure of nitric oxide (NO) and peroxynitrite (ONOO-), pronounced no, oh no! Other elements of the cycle include superoxide, oxidative stress (an imbalance between oxidants and antioxidants), intracellular calcium levels, NF- B activity (an important transcription factor stimulated by both oxidants (including peroxynitrite and many free radicals) and by intracellular calcium, certain inflammatory cytokines shown in the upper right corner box, iNOS, nNOS and eNOS activity and various mechanisms increasing superoxide levels (Fig. 1) [see ref. 1,6]. In addition, certain receptors occurring in neuronal and also some non-neuronal cells, the vanilloid (TRPV1) receptors and the NMDA receptors are thought to have important roles, as well [1-3,6,10,12]. There are 22 distinct mechanisms proposed to be involved in these arrows, of which 19 are well accepted, established biochemistry and physiology [1]. Of the other three, the actions of nitric oxide and peroxynitrite on the electron transport chain of the mitochondrion and the action of oxidants in increasing TRPV1 activity, these three are now considerably better documented [10] then they were when the Fig. 1 cycle was previously proposed [1,6]. Furthermore, other members of the TRP receptor family, including the TRPA1 receptors and TRPM2 receptors are also stimulated by correlates of oxidative stress [10], suggesting that they may play similar roles, as well.

Consequently, it may be seen from the above, that there is a massive amount of evidence supporting the specific mechanisms of the NO/ONOO- cycle diagrammed in Fig. 1 and the only thing that is truly original is the assumption that these mechanisms fit together in the way one might assume when placing them into juxtaposition with each other.

Despite the complexity of the Fig. 1 cycle diagram, there are two distinct parts of the NO/ONOO- cycle that are not apparent from it [1,2,10,13]:

1. There are several specific mechanisms that produce mitochondrial/energy metabolism dysfunction. These include the attack by peroxynitrite on several important mitochondrial proteins, acting by disrupting iron-sulfur clusters and by nitration of tyrosine residues; stimulation of poly ADP-ribosylation of chromosomal proteins by peroxynitrite-generated single strand nicks in DNA, leading in turn to depletion of NAD/NADH pools; oxidation of cardiolipin molecules in the inner mitochondrial membrane, initiated by elevated superoxide levels in the mitochondrion, leading in turn to lowered complex I, III and IV activity; inhibition of cytochrome oxidase (complex IV) activity by nitric oxide. Each of these will produce lowered oxygen utilization in the tissues, something reported to occur in CFS. Such mitochondrial/energy metabolism dysfunction will act as part of the NO/ONOO- cycle by increasing NMDA activity and probably by increasing intracellular calcium levels, with the latter being a consequence of lowered calcium-ATP activity, an activity essential to the lowering of intracellular calcium levels.
2. Peroxynitrite oxidizes a compound known as tetrahydrobiopterin (BH4), a compound that acts as a cofactor for the nitric oxide synthases and also has a role in the production of catecholamines and serotonin/melatonin. The consequent BH4 deficiency produces a partial uncoupling of the nitric oxide synthases, such that uncoupled enzymes generate superoxide instead of nitric oxide [13]. Such BH4 depletion in tissues with high NOS activity will produce adjacent NOS enzymes generating nitric oxide and superoxide which will react rapidly with each other to form more peroxynitrite. This may serve as an inner vicious cycle within the larger NO/ONOO- cycle, such that increasing BH4 levels and lowering peroxynitrite levels may be essential to effectively treating NO/ONOO- cycle diseases by down-regulating the NO/ONOO- cycle.

The elevated peroxynitrite/BH4 depletion couplet will be expected to lower nitric oxide levels while increasing peroxynitrite levels. It follows that agents that lower this couplet activity, may actually increase nitric oxide levels while producing clinical improvements in the patients [13]. It is even possible that the action of this couplet may produce NO/ONOO- cycle illnesses where the whole body production of nitric oxide may not be elevated in comparison with controls. The two published studies of nitric oxide production in CFS support the view that such production is elevated but that is not necessarily predicted to occur in all NO/ONOO- cycle diseases.

A diagram containing these additional aspects of the cycle is shown in Fig. 2. We have here the reciprocal interactions between peroxynitrite (abbreviated PRN) and BH4 depletion. Also the depletion of ATP pools and its roles in the cycle are diagrammed as well. In the upper left hand corner, it is suggested that not only the TRPV1 (vanilloid) receptor has a role here, but other member of the transfer receptor potential (TRP) family including TRPA1 and TRPM2 may have roles as well [9,10].

Five Principles

There are five principles underlying the NO/ONOO- cycle mechanism, the first two of which have already been discussed [taken from the authors web site and also refs. 1,6,10]:

1. Cases can be initiated by short-term stressors that increase nitric oxide and/or other cycle elements.
2. The chronic phase of illness is produced by the NO/ONOO- cycle. It is predicted, therefore, that the cycle elements will be elevated in the chronic phase of illness.
3. The symptoms and signs of illness must be generated by one or more elements of the cycle.
4. The basic mechanism of the cycle is local and will be localized to different tissues in different individuals. The reason for this primarily local nature is that the three compounds involved, nitric oxide, superoxide and peroxynitrite, have limited half lives in biological tissues. And the mechanisms of the cycle, those various arrows, act at the level of individual cells. This allows for great variations tissue distribution from one patient to another, producing a huge spectrum of illness. The point here is not that there are no systemic changes – clearly antioxidant depletion, neuroendocrine and immune system changes and actions of some inflammatory cytokines will be systemic. But rather this primarily local nature gives much inherent variation due to the tissue localization of the basic mechanism [see Chapter 4 ref.1]. A correlate of the local basic nature of the cycle is that different NO/ONOO- cycle diseases will differ from one another in what tissue or tissues must be impacted by the cycle in order to be diagnosed as a specific cycle-caused disease.
5. NO/ONOO- cycle diseases should be treated by down-regulating the NO/ONOO- cycle biochemistry, rather than by symptomatic relief. In other words, we should treat the cause, rather than the symptoms.

These five principles are important in three distinct ways. Firstly, they collectively produce an essentially complete model of any NO/ONOO- cycle disease. Secondly, the fit to each of the five principles for a particular disease or illness provides a distinct type of evidence for the causality of the cycle. Such diverse evidence for causality, such as may be provided by the fit to all five principles, is essential to provide a robust structure of evidence, suggesting that the cycle is the cause of a specific disease. Accordingly, the third important way in which the five principles are important is as follows: The fit to each of the five serves as a criterion for deciding whether a specific disease/illness is a good candidate for inclusion under the NO/ONOO- cycle paradigm. As such, the five principles function for the NO/ONOO- cycle a bit like Koch's postulates function for possible infectious diseases.

CFS and the First Two Principles

We've already discussed the first principle and how it fits well for CFS. Each of the nine short-term stressors implicated in the initiation of cases of CFS (Table 1) can increase the levels of nitric oxide in regions of the body impacted by them. So we have a very good fit for the first principle.

To look for fit to the second principle, we must look for where the various elements of the cycle have been studied in CFS. CFS has been extensively reported in 13 different studies to have elevated markers of oxidative stress [reviewed in 1,14,16] and two additional such studies have been reported more recently [17,18], clearly showing that oxidative stress is one of the most extensively documented properties of CFS. It also has extensive evidence for mitochondrial dysfunction [reviewed in 1,4], leading to lowered oxygen utilization in the tissues [19-21] and lactate accumulation in the cerebrospinal fluid [22].

One specific mechanism described above for the mitochondrial dysfunction, is the generation of cardiolipin oxidation by elevated superoxide levels in the mitochondrion and there is evidence that this occurs in CFS. Hokama and his colleagues [23,24] have reported that a ciguatera epitope occurs in the blood of people with CFS and some other diseases and has also reported that the same epitope occurs in commercial grade cardiolipin [24]. Such commercial grade cardiolipin is almost certainly highly oxidized because cardiolipin contains essentially all highly oxidizable linoleic acid residues, suggesting that the ciguatera epitope is none other than a peroxidation product of cardiolipin. It may be inferred from these observations that CFS patients probably have elevated levels of superoxide in the mitochondria, leading to elevated cardiolipin peroxidation.

There are two studies, one of them my own, reporting that nitric oxide levels are elevated in CFS [25,26]. The importance of this is emphasized by the apparent clinical responses to the potent nitric oxide scavenger, the form of vitamin B_{12} known as hydroxocobalamin [1,11,27].

There have been multiple studies reporting elevated levels of inflammatory cytokines in CFS [reviewed in refs. 1,4,28]. I discuss further evidence, below, for an important causal role for inflammatory responses in CFS from animal model studies, from the effectiveness of an antiinflammatory therapeutic agent and from gene expression studies.

There are clinical observations strongly suggesting that there is excessive excitotoxicity in CFS, including excessive NMDA activity [26,29-31]. In the closely related diseases, FM and MCS, the evidence for excessive NMDA activity is much more extensive [reviewed in 1,4,6,10].

Two recent studies have reported elevated NF- B activity in CFS, while mistakenly calling it NF-kappa beta [32,33]. These two studies ascribed the induction of iNOS and certain inflammatory cytokines to increased NF- B activity, as I had done much earlier [4]. And while TRPV1 activity has not been studied in CFS, it has been shown to be elevated in MCS as well as the comorbid illness irritable bowel syndrome [reviewed in 1,9,10].

And, finally although BH4 depletion has not been studied directly in CFS, there is evidence that will be discussed below that agents known to be able to help restore BH4 pools, are helpful in the therapy of CFS. Furthermore autism patients, which many have argued are similar to CFS patients, have been shown in three clinical trials to be helped by BH4 nutritional supplements [reviewed in chapter 14, ref. 1].

Animal Models

There are limited animal model studies of CFS and these also have shown elevation of certain NO/ONOO- cycle elements.

A mouse model of CFS is initiated to apparent fatigue by treatment with a bacterial extract [34-36] that increases nitric oxide levels [37,38]. It is characterized by elevated inflammatory cytokines [34,35] and also mitochondrial dysfunction in regions of the brain [36]. Other apparent CFS animal models also implicated increased inflammatory cytokines [39,40].

In another mouse model of CFS, chronic fatigue was correlated with elevated markers of oxidative stress [41-43]. A number of antioxidants were found to be useful in treatment [41-43], suggesting that oxidative stress has a substantial causal role.

Thus the available data on these animal models properties appear to be consistent with a NO/ONOO- cycle mechanism.

Genetic Studies

Genes that produce an increased susceptibility for a disease can often provide very useful information regarding possible biological mechanism. Several such studies have been published on CFS, so it is important to look at them to determine whether they are consistent with the NO/ONOO- cycle mechanism. Let’s look at the various examples of genetic roles with this question in mind.

Studies of an Australian family by Torpy and coworkers have shown that a gene for defective cortisol binding protein causes a strong predisposition for developing CFS [44-46]. It was also associated with orthostatic intolerance, a common correlate of CFS and related illnesses. Defective cortisol binding protein is expected to produce a lowered ability to respond to cortisol. A study of genes influencing the hypothalamic-pituitary adrenal axis, which may act through changed cortisol production, were also found to influence the prevalence of unexplained chronic fatigue [47]. Since cortisol and other glucocorticoids are known to lower the induction of iNOS [reviewed in 4], these studies are consistent with a role for nitric oxide in initiating cases of CFS. A role for cortisol is also supported by a recent study of single nucleotide polymorphisms in the glucocorticoid receptor gene [48], where several such alleles were associated with increased risk for CFS.

Alleles of a serotonin transporter gene producing increased serotonin transport and therefore lowered extracellular serotonin was also found to produce increased susceptibility to developing CFS [49]. The authors suggested that this polymorphism may act to produce lowered cortisol production [49]. The serotonin receptor HTR2A was implicated in CFS, because the promoter region polymorphism, -1438G/A was associated with increased prevalence of CFS and also unexplained fatigue [50]. However the interpretation for this is unclear because the data on whether this polymorphism produces increased or decreased promoter activity is mixed [50]. So at this point, it is difficult to interpret the serotonin related genetic activity for CFS.

Vladutiu and Natelson [51] found a polymorphism in the angiotensin converting enzyme (ACE) that was associated with unexplained fatigue in the Gulf War veterans. ACE is known to act by generating angiotensin II. Angiotensin II is known to act to produce tetrahydrobiopterin (BH4) depletion [52-54], leading to partial uncoupling of the nitric oxide synthases and increased superoxide production [52-54]. Thus this study provides some support for a role for two elements of the NO/ONOO- cycle, BH4 depletion and superoxide.

Carlo-Stella et al [55] reported that pro-inflammatory alleles in the TNF- gene and the IFN- gene were associated with CFS. Metzger et al [56] reported that a pro-inflammatory allele in the IL-17 gene was also associated with CFS. Both of these studies, then, implicate inflammatory responses as having causal roles in CFS, and ref.55 implicates two specific inflammatory cytokines suggested to be involved in the NO/ONOO- cycle.

Boles and coworkers [57,58] have shown that maternally inherited mitochondrial mutations can produce CFS or, at least, CFS-like symptoms, along with a much broader spectrum of symptoms. These results suggest that mitochondrial/energy metabolism dysfunction can have a causal role in producing CFS, another observation compatible with the the NO/ONOO- cycle mechanism.

Another observation that may also be compatible with a role of energy metabolism, is that Ehlers-Danlos syndrome may play a causal role in cases of CFS and orthostatic intolerance [59]. Ehlers-Danlos is a genetic defect in the structure of collagen and many cases of Ehlers-Danlos syndrome produce vascular dysfunction and therefore possible tissue hypoxia and deficient oxidative phosphorylation [59-61]. It follows that this observation may possibly be interpreted as being due to a role of normal energy metabolism in preventing cases of CFS.

In summary, then, the various genetic studies provide evidence for an important role of such NO/ONOO- cycle elements as inflammatory biochemistry, mitochondrial/energy metabolism dysfunction and BH4 depletion and consequent increased superoxide production. The role of cortisol can be interpreted as being due to the role of cortisol in limiting iNOS induction and may act in this way to influence another cycle element. So these observations seem to be quite compatible with a NO/ONOO- cycle mechanism, although we clearly need considerably more study of the genetics of CFS.

Generation of Shared Symptoms and Signs

The third principle of the NO/ONOO- cycle is that symptoms or signs of illness must each be generated by one or more elements of the cycle.
Many symptoms and signs of CFS are also shared by other multisystem illnesses proposed to also be NO/ONOO- cycle diseases, notably fibromyalgia, multiple chemical sensitivity and post-traumatic stress disorder. A list of such shared symptoms and signs, along with plausible mechanisms by which they may be generated by cycle elements, is shown in Table 2. One point should be emphasized – these are plausible mechanisms not established mechanisms in CFS.

Specific Signs and Symptoms for CFS and Where to Look for a Specific Biomarker Test

According to the NO/ONOO- cycle model, the difference between one NO/ONOO- cycle disease/illness and another, lies in the tissue or tissues that must be impacted by the cycle in order to meet the diagnostic criteria for that specific disease. An example of such specific tissue involvement may be found in FM, where it appears likely that the thalamus must be impacted in order to generate the widespread excessive pain that is the most characteristic symptom in FM [1,6].

What is the critical tissue that must be impacted in CFS and what impact does such impact have? I don't know the answer to the first part of this question but I do think I know the answer to the second part. The most characteristic symptom in CFS is thought to be what has been called post-exertional malaise [62-64]. Here, all of the symptoms of CFS are increased following excessive exercise. So the thing that is most characteristic of CFS is the inability to deal effectively with exercise and possibly certain other stressors. This also suggests that in order to develop a specific biomarker test for CFS, we should not look to the various systemic biochemical changes which may be in common with many other diseases, but rather we should look to one or more differences in response to exercise.

When I was discussing this issue with Dr. Paul Cheney, he suggested that the differences he sees with his CFS patients in response to exercise is their cortisol response. When normal people exercise, their cortisol levels go up [65,66]. This is not surprising, given that cortisol is used in the body to adjust to quite a number of stressors. What Cheney told me is that when his CFS patients exercise, cortisol levels either stay the same or drop. Similar cortisol responses to exercise in CFS patients have been reported by Ottenweller et al. [67], suggesting this may be a common, possibly universal feature of CFS patients.

It should be noted that hypothalamic-pituitary-adrenal axis dysfunction occurs in CFS, FM, MCS and PTSD, all proposed NO/ONOO- cycle diseases, as well as in many other chronic inflammatory diseases [Chapter 3 in ref. 1], so some changes in the control of cortisol release are not specific for CFS. These may, rather, reflect some aberration produced by oxidative stress or some other general correlate of these diseases. However, there is some published evidence, that CFS may have a more specific change in cortisol regulation not found in FM patients [68-70].

It may be argued that because of the role of cortisol and other glucocorticoids in lowering iNOS induction, as discussed above, that the failure to up-regulate cortisol levels following exercise may lead to an inappropriate increase in iNOS levels, leading in turn to increased nitric oxide levels and NO/ONOO- cycle activity. In this way, the across the board increase in symptoms in CFS patients following exercise ("post-exertional malaise"), may be explained as a consequence of the up-regulation of the basic mechanism causing CFS.

An up-regulation of the NO/ONOO- cycle biochemistry in response to exercise may explain the observations of Melvin Ramsay in his pioneering observations on CFS. Ramsay reported that those who persist in working after coming down with CFS until they collapse have the poorest prognosis [71]. Somewhat similarly, Harvey et al [72] suggested that "Continuing to be active despite increasing fatigue may be a crucial step in the development of CFS."   A possible causal relationship between hypocortisol responses in CFS and post-exertional malaise was proposed by Baschetti et al [73], who reviewed several studies of cortisol control in CFS. Baschetti et al [73] also noted that many people diagnosed on the Oxford criteria do not have the hypocortisol response to exercise and therefore may not have true chronic fatigue syndrome.

These considerations suggest that a search for specific biomarker tests for CFS, should focus on changes in response to exercise that may be specific for CFS patients, that is not shared either by normals or by others suffering from different diseases. There have been a number of studies showing that in addition to cortisol, CFS patients respond to exercise in ways that are different from the responses of normal controls. Jammes et al [74] reported that oxidative stress markers increased more in CFS patients after exercise than in normal controls, consistent with a NO/ONOO- cycle elevation. La Manca et al [75] found much larger cognitive deficits after exercise in CFS patients than in normal controls. There may be a number of responses to exercise that may be appropriate as specific biomarker tests for CFS. The ones that may be the best, from the standpoint of the NO/ONOO- cycle mechanism, may be cortisol levels or responses of NO/ONOO- cycle elements.

Before leaving this topic, however, there is an additional issue that may be understood as possibly being a consequence of changed cortisol control in CFS.

Peckerman et al [77,78] and Cheney [79] have reported that many CFS patients have cardiac dysfunction including left ventricular function. There is no understanding, to my knowledge, of how these cardiac changes may be produced as a consequence of the CFS pathophysiology. However, there is evidence that lowered cortisol levels can produce cardiac dysfunction, including lowered left ventricular function [80-86]. It seems likely that the need for cortisol in the heart may be expected to be particularly important during and immediately following exercise due to the stresses placed on the heart by exercise. It may be suggested, therefore, that the cardiac dysfunction seen in many CFS patients may be caused by their lowered cortisol production during and following exercise.

Local Nature of the CFS Mechanism

The stunning variation of symptoms seen from one CFS patient to another has created substantial difficulties in coming up with a simple case definition/set of diagnostic criteria for CFS [62,63,76]. It is difficult to expain this variation without either postulating a dozen or more "causes" or, alternatively and much more simply, a single local mechanism with variation in tissue distribution, from one patient to another.

Direct evidence for such variation of impact on local tissues has been seen in brain scan studies of CFS patients, where such tissue variation can be directly observed. I will only discuss two such studies here, both magnetic resonance imaging (MRI) studies from the Natelson group. In one of these [87], CFS patients had much higher frequencies of abnormal scans, but the scan patterns from one patient to another were highly variable. In a second study [88], there were again highly variable scans among patients. Those with the most severe cognitive dysfunction had substantial frontal lobe impact.

Both of these studies provide substantial evidence for a local mechanism, with the second suggesting that specific region impact may be responsible for the generation of specific symptoms. This is not the only suggested role of a specific region of the brain in the generation of specific symptoms in this group of illnesses. Impact on the thalamus is likely to generate the widespread excessive pain in fibromyalgia and the anxiety and panic attack symptoms that are common symptoms with these illnesses may be due to impact on the amygdala [1,6].

Similar variations in brain scans have also been seen in such related illnesses as FM, MCS and PTSD [1].

There is also evidence from variations in gene expression from one CFS patient to another, suggesting variable tissue distribution. The most apparently relevant of such gene expression studies is the recently published study of Kerr et al [89]. In that study, CFS patients were divided into different subtypes based on their gene expression patterns. These also corresponded, at least to some extent to variations is symptomatic patterns. Based on the gene expression patterns [89], different patients differed in terms of gene expression for genes linked to cognitive, musculoskeletal, anxiety/depression, neurological, gastrointestinal and hematological function. Each of these can be interpreted in terms of gene expression relating to different regions of the body. While it is unclear to me why lymphocytes and other white blood cells should have increased gene expression for genes related to, for example, neurological function, it is difficult to avoid the inference that impact on regions of the body must lead to increased gene expression for genes whose functions are linked to those regions of the body. So this study supports the interpretation that there appear to be regional variation among patients of a basically local mechanism, leading, in turn, to classification of different patients to different subtypes.

Gene Expression Studies: Evidence for Role of Cycle Elements

There have been a number of additional gene expression studies of CFS. Several of these provide substantial evidence for the involvement of mechanisms that are part of the NO/ONOO- cycle.

For example, several of these studies provide evidence for changes in gene expression of genes that function on energy metabolism and mitochondrial function [89-92], suggesting an important role of mitochondrial dysfunction in CFS. Several studies also provide evidence for chronic inflammatory responses in CFS [93-95], supporting another aspect of the NO/ONOO- cycle. The data of Kaushik et al provide evidence for a role of neuronal dysfunction including possible excitotoxicity [90].

To my knowledge, there has not been any analysis of the gene expression data to determine whether they provide evidence for elevated NF- B activity or activity of other transcriptional factors that are known to be activated by oxidants and oxidative stress. So we do have evidence from gene expression studies of CFS, supporting roles for elements of the NO/ONOO- cycle but we are still in the early stages of analyzing the available data for consistency with the predictions of the NO/ONOO- cycle.

Therapy: Avoiding Infections, Excessive Exercise, Allergens and Other Stressors

The fifth principle is that NO/ONOO- cycle diseases should be treated by lowering the cycle biochemistry. There are two aspects to this approach: Avoiding stressors that will otherwise up-regulate the NO/ONOO- cycle biochemistry and using agents that lower the cycle biochemistry. Both are important and this section considers the first of these. Among the stressors that are important in exacerbating CFS are infections and there is a large literature reporting that infections can not only initiate cases of CFS, but that infections can exacerbated cases as well. Among the infectious agents that are implicated in chronic infections of CFS cases, are Borrelia (Lyme disease) and other tick-born infectious agents, mycoplasma, Chlamydia and herpes viruses including HHV-6. In some instances, these may have initiated the CFS cases but in others, an opportunistic infectious role may be more plausible.

The general question is whether these can be treated by treatments that help restore normal immune function or whether it is necessary to use antibiotic treatments to effectively treat CFS?

Other stressors that will commonly up-regulate the NO/ONOO- cycle biochemistry include exercise triggering post-exertional malaise, as discussed above and exposure food or other allergens in the many CFS patients that suffer from such allergies.

Stressors that up-regulate the NO/ONOO- cycle biochemistry in related illnesses, such as MCS or PTSD, notably chemical exposure or psychological stress, may also have roles in some CFS cases.

Clearly there is a lot of variation in the impact of various stressors among CFS cases and these lead to variations in the optimal strategy for treatment among cases, as well.

Therapy: Agents that Lower NO/ONOO- Cycle Biochemistry

In Chapter 15, ref.1, there were 30 different agents/classes of agents listed and available currently that were predicted to lower different aspects of the NO/ONOO- cycle biochemistry and others have become apparent since that chapter was written. Of these agents/classes of agents, we have clinical trial studies on sixteen of them in CFS and/or FM and all 16 have been reported to be helpful in treatment (Table 3). These studies not only provide evidence supporting the NO/ONOO- cycle mechanism as a whole, but also provide evidence for a substantial causal role for several specific aspects of the cycle mechanism.

As can be seen from Table 3, of these 16 classes of agents, at least four have antioxidant properties, providing evidence that oxidative stress has an important causal role in generating these illnesses. Some of these agents either act as NMDA antagonists, or act indirectly to lower NMDA activity, thus providing strong evidence for a causal role of excessive NMDA activity. Carnitine/acetyl carnitine, coenzyme Q10 and possibly hyperbaric oxygen are likely to act to help improve mitochondrial function, thus providing evidence for a substantial causal role of mitochondrial/energy metabolism dysfunction.

The potent nitric oxide scavenger, hydroxocobalamin is a form of vitamin B_{12}, raising the question of whether it is acting here primarily as a nitric oxide scavenger or to allay a B_{12} deficiency. In the published clinical trial study [27], there was no correlation between initial B_{12} levels and the clinical response. Furthermore, much higher doses are needed to get good clinical responses here than are needed to treat a B_{12} deficiency [Chapter 6, ref.1]. It seems unlikely, therefore, that hydroxocobalamin is acting to allay a B_{12} deficiency. The potent action of hydroxocobalamin as a nitric oxide scavenger is well established, such that hydroxocobalamin has been used in experimental settings to establish a role for nitric oxide in biological processes [11, Chapter 6, ref.1].

There is also weaker evidence for two other aspects of the NO/ONOO- having a causal role. The long chain omega-3 fatty acids in fish oil are well established to have antiinflammatory aspects, so that their reported efficacy provides some evidence for an inflammatory causal role. High dose vitamin C and high dose folate supplements help restore BH4 levels, suggesting a causal role of BH4 depletion, but again, there are other possible interpretations for their action, so the evidence for BH4 depletion being causal in CFS, from clinical trial data alone, must be viewed as relatively weak. Interestingly in autism, which some view as being similar to CFS, there are three clinical trial studies all reporting that BH4 supplements produce statistically significant improvements [Chapter 14, ref.1].

The evidence for roles of oxidative stress, mitochondrial dysfunction and excitotoxicity including excessive NMDA activity from clinical trial data are relatively strong. We have weaker but still suggestive data for roles of excessive nitric oxide, chronic inflammatory biochemistry and BH4 depletion from such clinical trial data. It is difficult to see how these various cycle elements can be implicated from clinical trial data alone, unless the NO/ONOO- cycle or something very similar to it is the central causal mechanism in CFS and FM. When you add the various other types of evidence, supporting roles for each of these cycle elements in CFS that were reviewed above, the case for the NO/ONOO- cycle being the central causal mechanism in CFS becomes very substantial.

Treatment Protocols with Multiple Agents Lowering NO/ONOO- Cycle Biochemistry

In Chapter 15, ref.1, five treatment protocols are discussed that include at least 14 agents/classes of agents and have been tried with CFS, FM and/or MCS. Of these, only two, those of Teitelbaum and Nicolson, have been tested in clinical trials and found to be effective. The other three, Cheney's, Petrovic's and the one I worked on with Dr. Grace Ziem only have clinical observations of apparent effectiveness. Nevertheless, it appears that these are considerably more effective than are the individual agents that make them up, suggesting that an approach using multiple agents may be an attractive one for the treatment of presumed NO/ONOO- cycle diseases. The reader can get more information on those protocols from Chapter 15, ref.1.

I will discuss here, an additional protocol, this one containing 22 different agents predicted to down-regulate various aspects of the NO/ONOO- cycle, with each of these agents being nutritional supplements available to persons in the U.S., Canada and the EU. This nutritional support protocol was designed by me for the Allergy Research Group, using four supplement combinations that are newly formulated and three others that were already being sold by the Allergy Research Group. The components of this protocol, and indeed the entire nutritional support protocol, is not sold to treat or cure any disease.

Of the 22 different types of supplements included, 11 are listed in Table 3 and are follows:

- Trimethylglycine (betaine)
- Coenzyme Q10
- RNA
- Folic acid (folate)
- Hydroxocobalamin (B_{12})
- Ecklonia cava extract
- Acetyl-L-carnitine
- Flavonoids
- Fish oil
- Magnesium
- Vitamin C

The other 11 are described in Table 4.

The reader needs to be skeptical about my descriptions of apparent responses to the nutritional support protocol provided below for three distinct reasons:
1. These descriptions are all based on feedback from physicians and other health care providers as well as from anecdotal reports from individuals with these multisystem illnesses who have tried this protocol.
2. There have been <u>no</u> clinical trials of this protocol.
3. I receive a small royalty for the design of four of these supplement combinations, and <u>so I have a conflict of interest here</u>. The reader needs to keep that in mind.

Suggested dosage for Allergy Research Group nutritional support protocol, follows. My suggestion is that people interested in taking this protocol, introduce one combination at a time, for three days before introducing the next. The idea here is that if any of the seven supplement combinations are not well tolerated, it can be dropped by the individual. Some individuals, particularly in the MCS group, do not tolerate individual supplements and there is at least one type of person who does not tolerate most of them.

1. #75930 CoQ-Gamma E with Tocotrienols & Carotenoids: one capsule per day in the morning. Those with body weights over 100 lbs should add a second capsule at mid-day.
2. #75780 FlaviNox: one capsule, four times per day, three preferably with or after meals. Those with body weights over 120 lbs, should add a second capsule with each of three meals.
3. #75940 MVM-A Antioxidant Protocol, multivitamin mineral supplement with added acetyl L-carnitine: one capsule, four times per day, three preferably with or after meals. Those with body weights over 120 lbs, should add a second capsule, with breakfast and with dinner.
4. #75960 NAC Enhanced Antioxidant Formula: one each twice per day, with or after breakfast and supper.

5. #71250 or #73870 Super EPA (fish oil): one per day in the morning after breakfast. Those with body weights over 100 lbs, should add a second capsule at mid-day, taken with or after lunch.
6. #75910 FibroBoost (*Ecklonia cava* extract): one each twice per day, with or after breakfast and supper.
7. #70010 Buffered Vitamin C: one capsule, four times per day, preferably three with or after meals.

It is suggesting that the three products that are to be taken four times per day, be taken at the same times, with three being taken with or after the three meals of the day and the fourth taken at bedtime.

You can get much more information about these products from the Allergy Research Group web site:
www.allergyresearchgroup.com/

Description of responses (and again, skepticism should be maintained):

Something like 80 to 85% of individuals trying this protocol report distinct improvements, with roughly similar percentages for CFS, FM and MCS sufferers. This is somewhat surprising given that most medical care providers feel that the MCS cases are the most difficult to treat and there are reports in the literature that perhaps 10% of CFSand FM patients see a full recovery but there are no similar reports for the MCS group. In most cases a wide variety of symptoms show improvement. Improvements where seen are maintained—that is relapses among those staying on the protocol are rare. However, stressors do cause a distinct worsening of symptoms but that worsening is not sustained when the stressor is no longer present.

In some cases, there are remarkable improvements, even among those who have been ill for two decades or more. And such remarkable improvements even occur in those who have been severely ill. Where people respond well to this nutritional support protocol, they typically respond within a month or less, although I suggest that people who tolerate it well, stay on it for three months even when they do not respond in less than a month, as some take longer to respond. However, others show much more modest improvements and there are some who show no clear-cut improvement.

A low percentage do not tolerate most of the protocol. Some, perhaps all of these have high levels of mercury. The probable reason for this inability to tolerate is that -lipoic acid is present in four of the seven components, and -lipoic acid is capable of mobilizing mercury in the body. It seems likely, therefore, that those with high levels of mercury will have to undergo extensive mercury detoxification in order to go back on the protocol.

In addition to the a low percentage with mercury toxicity problems, there are others (perhaps 10-15%) who tolerate most of the protocol but do not respond in any distinct fashion to it, either positively or negatively. I speculate that these may be people with major issues caused by intransigent chronic infections or possibly chemically sensitive individuals who are continually exposed to chemicals. Still others may have problems continual exposure to environmental molds or with food allergens. However, this is speculation that must be questioned.

In general, most individuals with CFS, MCS or FM respond well to the protocol, even those who have been ill for two or more decades and those who have been severely ill. The fact that all three of these respond roughly equally well to the protocol suggests a that the NO/ONOO- cycle is a common mechanism for all three. However, with some having been on the protocol for a year or more at this point, we are not seeing the cures that might have been anticipated. This nutritional support protocol is limited to agents that can be sold over-the-counter and limited to nutritional supplements, so there may be ways of improving it, a number of which are discussed in Chapter 15, ref.1.

How Can We Obtain Substantial Numbers of Cures?

The simple answer to this question is that I don't know yet, but there is an approach that is feasible and plausible that should be tried, in my judgment. First let's ask why we are not seeing substantial numbers of cures?

In principle, there are three possible answers to this, assuming that the NO/ONOO- cycle mechanism is central to the etiology of these diseases:

1. The first is that there are aspects of the cycle that we do not yet understand and that therefore cannot effectively treat. This is certainly possible since the cycle has become much more complex since I first proposed it [4]. Additional aspects of the cycle have been added as a function of important observations on these multisystem diseases. They have also been added as I have researched the basic chemistry, biochemistry and physiology of various potential cycle elements. Thus new mechanisms, the arrows in Figs 1 and 2, have been added as it has been possible to document them. Obviously if we are missing something essential in our understanding of the cycle, we don't know how to deal with it.
2. The second possibility is that many of the sufferers have to deal with stressors that are up-regulating the cycle and are not adequately avoiding these stressors. The three of these that may be most important for the CFS group are chronic infections, allergens including food allergens and exercise leading to post-exertional malaise.
3. The third possibility is that there is one (or perhaps more) aspect of the cycle that we are not effectively down-regulating, that may be so important to the cycle that it is preventing our obtaining such cures. It is this third possibility that is the focus of the rest of this section.

I have argued above, that <u>the</u> most central part of the cycle is the central couplet, the reciprocal interactions between peroxynitrite and BH4. Recall that peroxynitrite oxidizes BH4, leading to BH4 depletion and partial uncoupling of the nitric oxide synthases, causing them to generate both superoxide and nitric oxide, which react with each other to form more peroxynitrite (see Fig.2). This central couplet, acting as a vicious cycle within the larger NO/ONOO- cycle may well be the core of the cycle and therefore the most important part of the cycle to down-regulate. We do have agents in the Allergy Research Group protocol predicted to down-regulate both ends of this couplet, but it is not clear that they are effective *in vivo* at the doses one normally uses.

My candidate for an agent to down-regulate this central couplet is high dose, intravenous (IV) ascorbate (vitamin C). This so-called megadose therapy approach is attractive for four different reasons:

1. It has been reported to be effective in clinical trials for both CFS [103-106] and MCS [107]. I am also aware of physicians who have used it in their treatment of both of these diseases, and reported very substantial apparent effectiveness. My suggestion is to use it with a wide ranging protocol designed to down-regulate various aspects of the NO/ONOO- cycle, rather than on its own, but the apparent effectiveness of it as a single agent is certainly encouraging.

2. Ascorbate scavenges peroxynitrite and its breakdown products, although it is not very effective at the usual blood levels obtained from the use of oral supplements [108-112]. Because IV ascorbate can generate blood levels on the order of 30 times or more compared with those typically obtained from oral supplements [113,114], it is expected to be much more effective at these high levels.

3. When BH4 is oxidized by peroxynitrite it is first converted to the one electron oxidation product, BH3 [111,112], which is itself unstable. However BH3 can be reduced back to BH4 by ascorbate [111,112] and with the high levels generated by high dose IV ascorbate, may be expected to be fairly efficiently reduced before it is converted to other, higher level oxidation products. Thus high dose, IV ascorbate may be expected to allow recovery of much of the BH4 that was oxidized by peroxynitrite.

4. High dose ascorbate produced by such IV infusion leads to the generation of substantial amounts of hydrogen peroxide in the body [115,116] and hydrogen peroxide has been shown to induced the enzyme GTP cyclohydolase I [117-119]. GTP cyclohydrolase I is the first and rate limiting enzyme in the *de novo* pathway for the synthesis of BH4. Thus high dose IV ascorbate will be expected to increase the availability of BH4 by stimulating its synthesis via the *de novo* pathway.

As one might expect from a combination of these three mechanisms, there is evidence that high dose ascorbate increases availability of BH4 [120,121].

In summary, there are three mechanisms by which high dose IV ascorbate is predicted to down-regulate the couplet that appears to be most central to the NO/ONOO- cycle. This same treatment appears to be effective in clinical trial studies in the treatment of CFS and MCS. It is, consequently, my best candidate for an addition to protocols containing wide-ranging agents down-regulating various aspects of the NO/ONOO- cycle, to hopefully obtain substantial numbers of cures of NO/ONOO- cycle diseases.

Summary: CFS as a NO/ONOO- Cycle Disease

There are a number of types of evidence that provide support for a NO/ONOO- cycle mechanism for CFS:

1. It is supported by a pattern of evidence where nine diverse short-term stressors reported to initiate cases of CFS can all act to increase nitric oxide levels.
2. It is supported by the large amount of data supporting the existence of and biological impact of specific cycle mechanisms in animals and humans.
3. It is supported by plausible mechanisms by which cycle elements can generate symptoms and signs often found in cases of CFS.
4. The basic local nature is supported by the stunning variation in symptoms seen among different CFS patients, the variations in brain tissue distribution seen in brain scan studies and the variations in gene expression reported in different CFS patients.
5. It is supported by animal model data.
6. It is supported by the fact that other, related illnesses, including FM and MCS also can be explained through a NO/ONOO- cycle mechanism.
7. It is supported by studies of genes that help determine susceptibility to CFS.
8. It is supported by clinical trial and clinical observation studies of agents that are reported to be helpful in the CFS and/or FM illnesses.

The last two types of evidence (7 and 8), provide evidence for a role for several different aspects of the NO/ONOO- cycle mechanism. Notably, oxidative stress, mitochondrial/energy metabolism dysfunction, elevated nitric oxide levels, inflammatory biochemistry, excitotoxicity including excessive NMDA activity and BH4 depletion. It is difficult to see how this group of mechanisms could be involved in CFS, unless the NO/ONOO- cycle or something very similar to it is the central cause of CFS.

It is my opinion that the NO/ONOO- cycle mechanism has already led to approaches that can produce substantial improvements in the large majority of cases of CFS and other related multisystem illnesses and that modification of these approaches, will probably lead to cures for many such sufferers. But then, I have always been an optimist when it comes to these diseases.

References

1. Pall M. L. (2007) Explaining “Unexplained Illnesses”: Disease Paradigm for Chronic Fatigue Syndrome, Multiple Chemical Sensitivity, Fibromyalgia, Post-Traumatic Stress Disorder, Gulf War Syndrome and Others. Harrington Park (Haworth) Press, Bighamton, NY.
2. Pall M. L. (2002) NMDA sensitization and stimulation by peroxynitrite, nitric oxide and organic solvents at the mechanism of chemical sensitivity in multiple chemical sensitivity. *FASEB J* 16,1407-1417.
3. Hill H.-U., Huber W., Müller K. (2008) Multiple Chemikalien Sensibilität. Shaker-Verlag, Aachen.
4. Pall M. L. (2000) Elevated, sustained peroxynitrite levels as the cause of chronic fatigue syndrome. *Med Hypotheses* 54,115-125.
5. Pall M. L. (2001) Common etiology of posttraumatic stress disorder, fibromyalgia, chronic fatigue syndrome and multiple chemical sensitivity via elevated nitric oxide/peroxynitrite. *Med Hypotheses* 57,139-145.
6. Pall M. L. (2006) The NO/ONOO- cycle as the cause of fibromyalgia and related illnesses: Etiology, explanation and effective therapy. In: New Research in Fibromyalgia, John A. Pederson, Ed., pp 39-59, Nova Science Publishers, Inc., Hauppauge, NY.
7. Pall M.L. (2007) Nitric oxide synthase partial uncoupling as a key switching mechanism for the NO/ONOO- cycle. *Med Hypotheses* 69,821-825.
8. Pall M. L., Satterlee J. D. (2001) Elevated nitric oxide/peroxynitrite mechanism for the common etiology of multiple chemical sensitivity, chronic fatigue syndrome, and posttraumatic stress disorder. *Ann N Y Acad Sci* 933,323-329.
9. Pall M. L., Anderson J. H. (2004) The vanilloid receptor as a putative target of diverse chemicals in multiple chemical sensitivity. *Arch Environ Health* 59,363-372.
10. Pall M. L. (2009) Multiple chemical sensitivity: toxicological questions and mechanisms. General and Applied Toxicology, 3rd Edition, Bryan Ballantyne, Timothy C. Marrs, Tore Syversen, Eds., Wiley & Sons, New York, in press.
11. Pall M. L. (2001) Cobalamin used in chronic fatigue syndrome therapy is a nitric oxide scavenger. *J Chronic Fatigue Syndr* 8,39-45.
12. Pall M. L. (2003) Elevated nitric oxide/peroxynitrite theory of multiple chemical sensitivity: central role of N-methyl-D-aspartate receptors in the sensitivity mechanism. *Environ Health Perspect* 111,1461-1464.
13. Pall M. L. (2008) Post-radiation syndrome as a NO/ONOO(-) cycle, chronic fatigue syndrome-like disease. *Med Hypotheses* 71,537-541.
14. Smirnova I. V., Pall M. L. (2003) Elevated levels of protein carbonyls in sera of chronic fatigue syndrome patients.
Mol Cell Biochem 248,93-95.
15. Carson P. J., Konweko P., Wold P., et al. (2006) Long-term clinical and neuropsychological outcomes of West Nile virus infection. *Clin Infect Dis* 43,723-730.
16. Kennedy G., Spence V. A., McLaren M., Hill A., Underwood C., Belch J. J. (2005) Oxidative stress levels are raised in chronic fatigue syndrome and are associated with clinical symptoms.

Free Radic Biol Med 39,584-589.
17. Richards R. S., Wang L., Jelinek H. (2007) Erythrocyte oxidative damage in chronic fatigue syndrome. Arch Med Res 38,94-98.
18. Miwa K., Fujita M. (2008) Increased oxidative stress suggested by low serum vitamin E concentrations in patients with chronic fatigue syndrome. Int J Cardiol [Epub ahead of print].
19. McCully K. K., Natelson B. H., Iotti S., Sisto S., Leigh J. S. Jr. (1996) Reduced oxidative muscle metabolism in chronic fatigue syndrome. *Muscle Nerve* 19,621-625.
20. McCully K. K., Natelson B. H. (1999) Impaired oxygen delivery to muscle in chronic fatigue syndrome. *Clin Sci (Lond)* 97,603-608.
21. Farquhar WB, Hunt BE, Taylor JA, Darling SE, Freeman R. (2002) Blood volume and its relation to peak O(2) consumption and physical activity in patients with chronic fatigue. *Am J Physiol Heart Circ Physiol* 282,H66-H71.
22. Mathew S. J., Mao X., Keegan K. A., Levine S. M., Smith E. L., Heier L. A., Otcheretko V., Coplan J. D., Shungu D. C. (2008) Ventricular cerebrospinal fluid lactate is increased in chronic fatigue syndrome compared with generalized anxiety disorder: an in vivo 3.0 T (1)H MRS imaging study. NMR Biomed. 2008 Oct 21. [Epub ahead of print].
23. Hokama Y., Uto G. A., Palafox N. A., Enlander D., Jordan E., Cocchetto A. (2003) Chronic phase lipids in sera of chronic fatigue syndrome (CFS), chronic ciguatera fish poisoning (CCFP), hepatitis B, and cancer with antigenic epitope resembling ciguatoxin, as assessed with MAb-CTX. *J Clin Lab Anal* 17,132-139.
24. Hokama Y., Empey-Campora C., Hara C., Higa N., Siu N., Lau R., Kuribayashi T., Yabusaki K. (2008) Acute phase phospholipids related to the cardiolipin of mitochondria in the sera of patients with chronic fatigue syndrome (CFS), chronic Ciguatera fish poisoning (CCFP), and other diseases attributed to chemicals, Gulf War, and marine toxins. *J Clin Lab Anal* 22,99-105.
25. Pall M. L. (2002) Levels of nitric oxide synthase product citrulline are elevated in sera of chronic fatigue syndrome patients. *J Chronic Fatigue Syndr* 10(3/4),37-41.
26. Kurup R. K., Kurup P. A. (2003) Hypothalamic digoxin, cerebral chemical dominance and myalgic encephalomyelitis. *Int J Neurosci* 113,683-701.
27. Ellis F. R., Nasser S. (1973) A pilot study of vitamin B12 in the treatment of tiredness. *Br J Nutr* 30,277-283.
28. Spence V. A., Kennedy G., Belch J. J., Hill A., Khan F. (2007) Low grade inflammation and arterial wave reflection in patients with chronic fatigue syndrome. *Clin Sci (Lond)* 114,561-566.
29. Carpman V. (1995) CFIDS treatment: the Cheney clinics strategic approach. *CFIDS Chronicle* Spring 1995,38-45.
30. Hoh D. (1998) Treatment at the Cheney clinic. *CFIDS Chronicle* 1998 11,13-14.
31. Goldstein J. A. (2004) Tuning the Brain: Principles and Practice of Neurosomatic Medicine. Haworth Medical Press, Binghamton, NY.
32. Maes M., Mihaylova I., Kubera M., Bosmans E. (2007) Not in the mind but in the cell: increased production of cyclo-oxygenase-2 and inducible NO synthase in chronic fatigue syndrome. *Neuro Endocrinol Lett* 28,463-469.

33. Maes M., Mihaylova I., Bosmans E. (2007) Not in the mind of neurasthenic lazybones but in the cell nucleus: patients with chronic fatigue syndrome have increased production of nuclear factor kappa beta. *Neuro Endocrinol Lett* 28,456-462.
34. Chao C. C., DeLaHunt M., Hu S., Close K., Peterson P. K. (1992) Immunologically mediated fatigue: a murine model. *Immunopathol* Clin Immunol 64,161-165.
35. Sheng W. S., Hu S., Lamkin A., Peterson P. K., Chao C. C. (1996) Susceptibility to immunologically mediated fatigue in C57BL/6 versus Balb/c mice. *Clin Immunol Immunopathol* 81,161-167.
36. Sheng W. S., Lin J. C., Apple F., Hu S., Peterson P. K., Chao C. C. (1999) Brain energy stores in C57BL/6 mice after C. parvum injection. Neuroreport 10,177-181.
37. Smith S. R., Manfra D., Davies L., Terminelli C., Denhardt G., Donkin J. (1997) Elevated levels of NO in both unchallenged and LPS-challenged C. parvum-primed mice are attributable to the activity of a cytokine-inducible isoform of iNOS. *J Leukoc Biol* 61,24-32.
38. Rees D. D., Cunha F. Q., Assreuy J., Herman A. G., Moncada S. (1995) Sequential induction of nitric oxide synthase by Corynebacterium parvum in different organs of the mouse. *Br J Pharmacol* 114,689-693.
39. Katafuchi T., Kondo T., Take S., Yoshimura M. (2006) Brain cytokines and the 5-HT system during poly I:C-induced fatigue. *Ann N Y Acad Sci* 1088:230-237.
40. Dantzer R., Kelley K. W. (2007) Twenty years of research on cytokine-induced sickness behavior. *Brain Behav Immun* 21,153-160.
41. Singh A., Naidu P. S., Gupta S., Kulkarni S. K. (2002) Effect of natural and synthetic antioxidants in a mouse model of chronic fatigue syndrome. *J Med Food* 2002 5,211-220.
42. Singh A., Garg V., Gupta S., Kulkarni S. K. (2002) Role of antioxidants in chronic fatigue syndrome in mice. Indian J Exp Biol. 40,1240-1244.
43. Singal A., Kaur S., Tirkey N., Chopra K. (2005) Green tea extract and catechin ameliorate chronic fatigue-induced oxidative stress in mice. *J Med Food* 8,47-52.
44. Torpy D. J., Bachmann A. W., Grice J. E., Fitzgerald S. P., Phillips P. J., Whitworth J. A., Jackson R. V. (2001) Familial corticosteroid-binding globulin deficiency due to a novel null mutation: association with fatigue and relative hypotension. J Clin Endocrinol Metab 86,3692-3700.
45. Torpy D. J., Bachmann A. W., Gartside M., Grice J. E., et al. (2004) Association between chronic fatigue syndrome and the corticosteroid-binding globulin gene ALA SER224 polymorphism.
Endocr Res 30,417-429.
46. Torpy D. J., Ho J. T. (2007) Corticosteroid-binding globulin gene polymorphisms: clinical implications and links to idiopathic chronic fatigue disorders. *Clin Endocrinol (Oxf)* 67,161-167.
47. Smith A. K., White P. D., Aslakson E., Vollmer-Conna U., Rajeevan M. S. (2006) Polymorphisms in genes regulating the HPA axis associated with empirically delineated classes of unexplained chronic fatigue. *Pharmacogenomics* 7,387-394.
48. Rajeevan M. S., Smith A. K., Dimulescu I., Unger E. R., Vernon S. D., Heim C., Reeves W. C. (2007) Glucocorticoid receptor polymorphisms and haplotypes associated with chronic fatigue syndrome. *Genes Brain Behav* 6,167-176.

49. Narita M, Nishigami N., Narita N., et al. (2003) Association between serotonin transporter gene polymorphism and chronic fatigue syndrome. *Biochem Biophys Res Commun* 311,264-266.
50. Smith A. K., Dimulescu I., Falkenberg V. R., et al. (2008) Genetic evaluation of the serotonergic system in chronic fatigue syndrome. *Psychoneuroendocrinology* 33,188-197.
51. Vladutiu G. D., Natelson B. H. (2004) Association of medically unexplained fatigue with ACE insertion/deletion polymorphism in Gulf War veterans. *Muscle Nerve* 30,38-43.
52. Kase H., Hashikabe Y., Uchida K., Nakashini N., Hattori Y. (2005) Supplementation with tetrahydrobiopterin prevents the cardiovascular effects of angiotensin II-induced oxidative and nitrosative stress. *J Hypertens* 23,1375-1382.
53. Oak J. H., Cai H. (2007) Attenuation of angiotensin II signalling recouples eNOS and inhibits nonendothelial NOX activity in diabetic mice. *Diabetes* 56,118-126.
54. Yamamoto E., Kataoka K., Shintaku H., et al. (2007) Novel mechanism and role of angiotensin II induced vascular endothelial injury in hypertensive diastolic heart failure. *Arterioscler Thromb Vasc Biol* 27,2569-2575.
55. Carlo-Stella N., Badulli C., De Silvestri A., et al. (2006) A first cytokine polymorphism in CFS: Positive association of TNF-857 and IFNgamma 874 rare alleles. *Clin Exp Rheumatol* 24,179-182.
56. Metzger K., Frémont M., Roelant C., De Meirleir K. (2008) Lower frequency of IL-17F sequence variant (His161Arg) in chronic fatigue syndrome patients. *Biochem Biophys Res Commun* 376,231-233.
57. Boles R. G., Burnett B. B., Gleditsch K., et al. (2005) A high predisposition to depression and anxiety in mothers and other matrilineal relatives of children with presumed maternally inherited mitochondrial disorders. *Am J Med Genet B Neuropsychiatr Genet* 137B,20-24.
58. Higashimoto T., Baldwin E. E., Gold J. I., Boles R. G. (2008) Reflex sympathetic dystrophy: complex regional pain syndrome type I in children with mitochondrial disease and maternal inheritance. *Arch Dis Child* 93,390-397.
59. Rowe P. C., Barron D. F., Calkins H., Maumenee I. H., Tong P. Y., Geraghty M. T. (1999) Orthostatic intolerance and chronic fatigue syndrome associated with Ehlers-Danlos syndrome. *J Pediatr* 135,494-499.
60. Germain D. P. (2006) The vascular Ehlers-Danlos syndrome. *Curr Treat Options Cardiovasc Med* 8,121-127.
61. Pepin M., Schwarze U., Superti-Furga A., Byers P. H. (2000) Clinical and genetic features of Ehlers-Danlos syndrome type IV, the vascular type. *N Engl J Med* 342,673-680.
62. Jason L. A., Torres-Harding S. R., Jurgens A., Helgerson J. (2004) Comparing the Fukuda et al. Criteria and the Canadian Case Definition for Chronic Fatigue Syndrome. J Chronic Fatigue Syndr 12(1),37-52.
63. Carruthers B. M., Jain K. L., De Meirleir D. L., et al. (2003) Myalgic Encephalomyelitis/ Chronic Fatigue Syndrome: Clinical Working Case Definition, Diagnostic and Treatment Protocols. J Chronic Fatigue Syndr 11(1),7-115.

64. Jason L. A., Corradi K., Gress S., Williams S., Torres-Harding S. (2006) Causes of death among patients with chronic fatigue syndrome. Health Care Women Int 27,615-626.
65. Mastorakos G., Pavlatou M. (2005) Exercise as a stress model and the interplay between the hypothalamus-pituitary-adrenal and the hypothalamus-pituitary-thyroid axes. *Horm Metab Res* 37,577-584.
66. McMurray R. G., Hackney A. C. (2005) Interactions of metabolic hormones, adipose tissue and exercise. *Sports Med* 35,393-412.
67. Ottenweller J. E., Sisto S. A., McCarty R. C., Natelson B. H. (2001) Hormonal responses to exercise in chronic fatigue syndrome. *Neuropsychobiology* 43,34-41.
68. Demitrack M. A., Crofford L. J. (1998) Evidence for and pathophysiologic implications of hypothalamic-pituitary-adrenal axis dysregulation in fibromyalgia and chronic fatigue syndrome.
Ann N Y Acad Sci 840:684-697.
69. Crofford L. J., Young E. A., Engleberg N. C., et al. (2004) Basal circadian and pulsatile ACTH and cortisol secretion in patients with fibromyalgia and/or chronic fatigue syndrome. *Brain Behav Immun* 18,314-325.
70. Adler G. K., Mansfredsdottir V. F., Rackow R. M. (2002) Hypothalamic-pituitary-adrenal axis function in fibromyalgia an chronic fatigue syndrome. The Endrocrinologist 12,513-522.
71. Ramsay M. A. (1988) Myalgic Encephalomyelitis and Postviral Fatigue State: The Saga of Royal Free Disease, 2nd Edition. London, Gower.
72. Harvey S. B., Wadsworth M., Wessely S., Hotopf M. (2008) Etiology of chronic fatigue syndrome: testing popular hypotheses using a national birth cohort study. *Psychosom Med* 70,488-495.
73. Baschetti R. (2005) Chronic fatigue syndrome, exercise, cortisol and lymphadenopathy. *J Intern Med* 258,291-292.
74. Jammes Y., Steinberg J. G., Mambrini O., Brégeon F., Delliaux S. (2005) Chronic fatigue syndrome: assessment of increased oxidative stress and altered muscle excitability in response to incremental exercise. *J Intern Med* 257,299-310.
75. LaManca J. J., Peckerman A., Sisto S. A., DeLuca J., Cook S., Natelson B. H. (2001) Cardiovascular responses of women with chronic fatigue syndrome to stressful cognitive testing before and after strenuous exercise. *Psychosom Med* 63,756-764.
76. Fukuda K., Straus S. E., Hickie I., Sharpe M. C., Dobbins J. G., Komaroff A. (1994). The chronic fatigue syndrome: a comprehensive approach to its definition and study. International Chronic Fatigue Syndrome Study Group. *Ann Intern Med* 121,953-959.
77. Peckerman A., LaManca J. J., Dahl K. A., et al. (2003) Abnormal impedance cardiography predicts symptom severity in chronic fatigue syndrome. *Am J Med* 326,55-60.
78. Peckerman A., LaManca J. J., Qureshi B., et al. (2003) Baroreceptive reflex and integrative stress responses in chronic fatigue syndrome. *Psychosom Med* 65,889-895.
79. http://phoenix-cfs.org/Cardio%20IVa%20Superoxide.htm

80. Aoyama T., Matsui T., Novikov M., et al. (2005) Serum and glucocorticoid-responsive kinase-1 regulates cardiomyocyte survival and hypertrophic response. *Circulation* 111,16523-1659.
81. Narayanan N., Yang C., Xur A. (2004) Dexamethasone treatment improves sarcoplasmic reticulum function and contractile performance in aged myocardium. *Mol Cell Biochem* 266:31-36.
82. Chiu CZ, Nakatani S, Zhang G, et al. (2005) Prevention of left ventricular remodeling by long-term corticosteroid therapy in patients with cardiac sarcoidosis. *Am J Cardiol* 95:143-146.
83. Iga K., Hori K., Gen H. (1992) Deep negative T waves associated with reversible left ventricular dysfunction in acute adrenal crisis. Heart Vessels 7,107-111.
84. Allolio B., Ehses W., Steffen H. M., Muller R. (1994) Reduced lymphocyte beta 2-adreoreceptor density and impaired diastolic left ventricular function in patients with glucocorticoid deficiency. *Clin Endocrinol* 40,769-775.
85. Boachour G., Tirot P., Varache N., et al. (1994) Hemodynamic changes in acute adrenal insufficiency. *Intensive Care Med* 20,138-141.
86. Emir M., Ozisik K., Cagli K., et al. (2005) Beneficial effect of methylprednisone on cardiac myocytes in a rat model of severe brain injury. *Tohoku J Exp Med* 207,119-124.
87. Natelson B. H., Cohen J. M., Brassloff I., Lee H. J. (1993) A controlled study of brain magnetic resonance imaging in patients with the chronic fatigue syndrome. *J Neurolo Sci* 15,213-217.
88. Lange G., DeLuca J., Maldjian J. A., Lee H., Tiersky L. A., Natelson B. H. (1999) Brain MRI abnormalities exist in a subset of patients with chronic fatigue syndrome. *J Neurol Sci* 171,3-7.
89. Whistler T., Unger E. R., Nisenbaum R., Vernon S. D. (2003) Integration of gene expression, clinical and epidemiological data to characterize chronic fatigue syndrome. J Transl Med 1(1),10.
90. Kaushik N., Fear D., Richards S. C., et al. (2005) Gene expression in peripheral blood mononuclear cells from patients with chronic fatigue syndrome. *J Clin Pathol* 58,826-832.
91. Saiki T., Kawai T., Morita K., Ohta M., Saito T., Rokutan K., Ban N. (2008) Identification of marker genes for differential diagnosis of chronic fatigue syndrome. *Mol Med* 14,599-607.
92. Whistler T., Taylor R., Craddock R. C., Broderick G., Klimas N., Unger E. R. (2006) Gene expression correlates of unexplained fatigue. *Pharmacogenomics* 7,395-405.
93. Aspler A. L , Bolshin C., Vernon S. D., Broderick G. (2008) Evidence of inflammatory immune signaling in chronic fatigue syndrome: A pilot study of gene expression in peripheral blood. *Behav Brain Funct* Sep 26,4:44.
94. Fuite J., Vernon S. D., Broderick G. (2008) Neuroendocrine and immune network re-modeling in chronic fatigue syndrome: An exploratory analysis. *Genomics* Sep 30. [Epub ahead of print].
95. Fang H., Xie Q., Boneva R., Fostel J., Perkins R., Tong W. (2006) Gene expression profile exploration of a large dataset on chronic fatigue syndrome. *Pharmacogenomics* 7,429-440.

96. Crofford L. J., Rowbotham M. C., Mease P. J., Russell I. J,, Dworkin R. H., Corbin AE, et al. (2005) Pregabalin for the treatment of fibromyalgia syndrome: results of a randomized, double-blind, placebo-controlled trial. *Arthritis Rheum* 52,1264-1273.
97. Bierman R. (2008) Ecklonia Cava Extract: Superior Polyphenol and Super-Antioxidant for Our Time. *Townsend Lett Doctors Patients*, Jan 2008.
98. Gilula M. F. (2007) Cranial electrotherapy stimulation and fibromyalgia. *Expert Rev Med Devices* 4,489-495.
99. In Focus (2007) http://www.nutricology.com/In-Focus-April-2007-Ecklonia-Cava-sp-52.html
100. Teitelbaum J. E., Johnson C., St Cyr J. (2006) The use of D-ribose in chronic fatigue syndrome and fibromyalgia: a pilot study. *J Altern Complement Med* 12,857-862.
101. Malaguarnera M., Gargante M. P., Cristaldi E., et al. (2008) Acetyl L-carnitine (ALC) treatment in elderly patients with fatigue. *Arch Gerontol Geriatr* 46,181-190.
102. Gebhart B., Jorgenson J. A. (2004) Benefit of ribose in a patient with fibromyalgia. *Pharmacotherapy* 24,1646-1648.
103. Kodama M., Kodama T. (2006) Four problems with the clinical control of interstitial pneumonia, or chronic fatigue syndrome, using the megadose vitamin C infusion system with dehydroepiandrosterone-cortisol annex. *In Vivo* 20,285-291.
104. Kodama M., Kodama T. (2005) The clinical course of interstitial pneumonia alias chronic fatigue syndrome under the control of megadose vitamin C infusion system with dehydroepiandrosterone-cortisol annex. *Int J Mol Med* 15,109-116.
105. Kodama M., Kodama T., Murakami M. (1996) The value of the dehydroepiandrosterone-annexed vitamin C infusion treatment in the clinical control of chronic fatigue syndrome (CFS). II. Characterization of CFS patients with special reference to their response to a new vitamin C infusion treatment. *In Vivo* 10,585-
106. Kodama M., Kodama T., Murakami M. (1996) The value of the dehydroepiandrosterone-annexed vitamin C infusion treatment in the clinical control of chronic fatigue syndrome (CFS). I. A Pilot study of the new vitamin C infusion treatment with a volunteer CFS patient. *In Vivo* 10,575-584.
107. Heuser G., Vojdani A. (1997) Enhancement of natural killer cell activity and T and B cell function by buffered vitamin C in patients exposed to toxic chemicals: the role of protein kinase-C. *Immunopharmacol Immunotoxicol* 19,291-312.
108. Ferroni F., Maccaglia A., Pietraforte D., Turco L., Minetti M. (2004) Phenolic antioxidants and the protection of low density lipoprotein from peroxynitrite-mediated oxidations at physiologic CO2. *J Agric Food Chem* 52,2866-2874.
109. Sakihama Y., Tamaki R., Shimoji H., Ichiba T., Fukushi Y., Tahara S., Yamasaki H. (2003) Enzymatic nitration of phytophenolics: evidence for peroxynitrite-independent nitration of plant secondary metabolites. *FEBS Lett* 553,377-380.
110. Whiteman M., Ketsawatsakul U., Halliwell B. (2002) A reassessment of the peroxynitrite scavenging activity of uric acid. *Ann N Y Acad Sci* 962,242-259.
111. Patel K. B., Stratford M. R., Wardman P., Everett S. A. (2002) Oxidation of tetrahydrobiopterin by biological radicals and scavenging of the trihydrobiopterin radical by ascorbate. *Free Radic Biol Med* 32,203-211.

112. Kuzkaya N., Weissmann N., Harrison D. G. Dikalov S. (2003) Interactions of peroxynitrite, tetrahydrobiopterin, ascorbic acid, and thiols: implications for uncoupling endothelial nitric-oxide synthase. *J Biol Chem* 278,22546-22554.
113. Padayatty S. J., Sun H., Wang Y., Riordan H. D., Hewitt S. M., Katz A., Wesley R. A., Levine M. (2004) Vitamin C pharmacokinetics: implications for oral and intravenous use. *Ann Intern Med* 140,533-537.
114. Duconge J., Miranda-Massari J.R., Gonzalez M. J., Jackson J. A., Warnock W., Riordan N. H. (2008) Pharmacokinetics of vitamin C: insights into the oral and intravenous administration of ascorbate. *P R Health Sci J* 27,7-19.
115. Chen Q., Espey M. G., Krishna M. C., Mitchell J. B., Corpe C. P., Buettner G. R., Shacter E., Levine M. (2005) Pharmacologic ascorbic acid concentrations selectively kill cancer cells: action as a pro-drug to deliver hydrogen peroxide to tissues. *Proc Natl Acad Sci U S A* 102,13604-13609.
116. Clément M. V., Ramalingam J., Long L. H., Halliwell B. (2001) The in vitro cytotoxicity of ascorbate depends on the culture medium used to perform the assay and involves hydrogen peroxide.
Antioxid Redox Signal 3,157-163.
117. Shimizu S., Shiota K., Yamamoto S., Miyasaka Y., Ishii M., et al. (2003) Hydrogen peroxide stimulates tetrahydrobiopterin synthesis through the induction of GTP-cyclohydrolase I and increases nitric oxide synthase activity in vascular endothelial cells. *Free Radic Biol Med* 34,1343-1352.
118. Ishii M., Shimizu S., Wajima T., Hagiwara T., Negoro T., Miyazaki A., Tobe T., Kiuchi Y. (2005) Reduction of GTP cyclohydrolase I feedback regulating protein expression by hydrogen peroxide in vascular endothelial cells. *J Pharmacol Sci* 97,299-302.
119. Kalivendi S., Hatakeyama K., Whitsett J., Konorev E., Kalyanaraman B., Vásquez-Vivar J. (2005) Changes in tetrahydrobiopterin levels in endothelial cells and adult cardiomyocytes induced by LPS and hydrogen peroxide--a role for GFRP? *Free Radic Biol Med* 38,481-491.

120. Baker T. A., Milstien S., Katusic Z. S. (2001) Effect of vitamin C on the availability of tetrahydrobiopterin in human endothelial cells.
J Cardiovasc Pharmacol 37,333-338.
121. d'Uscio L. V., Milstien S., Richardson D., Smith L., Katusic Z. S. (2003) Long-term vitamin C treatment increases vascular tetrahydrobiopterin levels and nitric oxide synthase activity. *Circ Res* 92,88-95.

Table 1: The stressors implicated in the initiation of these illnesses are summarized.

Illness	Stressors Implicated in Initiation of Illness
Chronic fatigue syndrome	**Viral infection, bacterial infection, organophosphorus pesticide exposure**, carbon monoxide exposure, ciguatoxin poisoning, physical trauma, severe psychological stress, toxoplasmosis (protozoan) infection, ionizing radiation exposure
Multiple chemical sensitivity	**Volatile organic solvent exposure, organophosphorus/carbamate pesticide exposure**, organochlorine pesticide exposure, pyrethroid exposure; hydrogen sulfide; carbon monoxide; mercury
Fibromyalgia	**Physical trauma (particularly head and neck trauma), viral infection**, bacterial infection, severe psychological stress, pre-existing autoimmune disease
Post-traumatic stress disorder	**Severe psychological stress**, physical (head) trauma

The stressors indicated in bold are the ones most commonly implicated for that specific disease/illness. It should be noted that the majority of such stressors are implicated in the initiation of more than one illness. Modified from the author's web site, with permission.

Table 2 Explanations for Symptoms and Signs

Symptom / Sign	Explanation based on elevated nitric oxide / peroxynitrite theory
energy metabolism /mitochondrial dysfunction	Inactivation of several proteins in the mitochondrion by peroxynitrite; inhibition of some mitochondrial enzymes by nitric oxide and superoxide; NAD/NADH depletion; cardiolipin oxidation
oxidative stress	Peroxynitrite, superoxide and other oxidants
PET scan changes	Energy metabolism dysfunction leading to change transport of probe; changes in perfusion by nitric oxide, peroxynitrite and isoprostanes; increased neuronal activity in short-term response to chemical exposure
SPECT scan changes	Depletion of reduced glutathione by oxidative stress; perfusion changes as under PET scan changes
Low NK cell function	Superoxide and other oxidants acting to lower NK cell function
Other immune dysfunction	Sensitivity to oxidative stress; chronic inflammatory cytokine elevation
Elevated cytokines	NF- B stimulating of the activity of inflammatory cytokine genes
Anxiety	Excessive NMDA activity in the amygdala
Depression	Elevated nitric oxide leading to depression; cytokines and NMDA increases acting in part or in whole via nitric oxide.
Rage	Excessive NMDA activity in the periaqueductal gray region of the midbrain
Cognitive/learn-ing and memory dysfunction	Lowered energy metabolism in the brain, which is very susceptible to such changes; excessive NMDA activity and nitric oxide levels and their effects of learning and memory
Multiorgan pain	All components of cycle have a role, acting in part through nitric oxide and cyclic GMP elevation
Fatigue	Energy metabolism dysfunction
Sleep disturbance	Sleep impacted by inflammatory cytokines, NF- B activity and nitric oxide
Orthostatic intolerance	Two mechanisms: Nitric oxide-mediated vasodilation leading to blood pooling in the lower body; nitric oxide-mediated sympathetic nervous system dysfunction
Irritable bowel syndrome	Sensitivity and other changes produced by excessive vanilloid and NMDA activity, increased nitric oxide
Intestinal permeabilization leading to food allergies	Permeabilization produced by excessive nitric oxide, inflammatory cytokines, NF- B activity and peroxynitrite; peroxynitrite acts in part by stimulating poly(ADP)-ribose polymerase activity

Taken from the author's web site with permission. It should be noted that while each of these are plausible mechanisms and, in most cases well-documented mechanisms under some pathophysiological circumstances, in most cases their role in generating these symptoms in these multisystem illnesses is not established.

Table 3

Agent or class	Mechanism	Comments
Vitamin C (ascorbic acid)	Chain breaking antioxidant; lowers NF-kappa B activity; reported to scavenge peroxynitrite and also help restore tetrahydrobiopterin (BH4) levels by reducing an oxidized derivative of BH4	May require high doses to be effective with the latter two mechanisms; this may be the basis of so called “megadose therapy” for vitamin C; clinical trials on CFS and MCS used high dose IV ascorbate
Magnesium	Lowers NMDA activity and may be useful in improving energy metabolism and ATP utilization	Magnesium is the agent that is most widely studied and found to be useful in the treatment of the multisystem illnesses
Fish oil (long chain omega-3 fatty acids)	Lowers iNOS induction; lowers production of inflammatory eicosonoids; important for brain function	Highly susceptible to lipid peroxidation and may, therefore be depleted; four studies reported improvements in clinical trials, 3 with CFS and one with FM
Flavonoids	Chain breaking antioxidants; some scavenge peroxynitrite, some scavenge superoxide; some reported to induce SOD; All three types are found in FlaviNox; some flavonoids may also act to help restore BH4 levels; lower NF-kappa B activity	Ginkgo extract tested in CFS; anthocyanidin flavonoids in FM; other flavonoids tested in CFS animal model
NMDA antagonists	Lower NMDA activity	Four different antagonists reported to be effective in the treatment of fibromyalgia; anecdotal reports of effectiveness for MCS
Agents that indirectly lower excitotoxicity including NMDA activity		Only clinical trials done with pregabalin for fibromyalgia, but other members of this class often used clinically
Acetyl L-carnitine/ carnitine	Helps transport fatty acids into mitochondria; may be important here not only directly for energy metabolism but also to restore the oxidized fatty acid residues that may be produced in the cardiolipin of the inner membrane	May also help lower reductive stress; two trials in CFS
Ecklonia cava extract	Polyphenolic chain breaking antioxidant; reported to help scavenge both peroxynitrite and superoxide; based on its reported properties, it may also help restore BH4 levels	Appears to stay in the body much longer than do the flavonoids, a useful property; reported to be helpful in a clinical trial study of fibromyalgia
Reductive stress relieving agents	These include S-adenosyl methionine (SAM or SAMe), trimethylglycine (betaine), carnitine and choline	SAM reported to be effective in multiple clinical trials with FM and CFS patients; betaine widely used clinically
Hydroxocobalamin form of vitamin B-12	Potent nitric oxide scavenger, lowers nitric oxide levels	Limited intestinal transport; often taken by IM injection or as a nasal spray or inhalant; clinical

		trial with CFS-like illnesses; widely used for treatment of CFS, FM and MCS
Folic acid	Relatively high doses will lower the partial uncoupling of the nitric oxide synthases by helping to restore tetrahydrobiopterin (BH4)	Reacts with oxidants and therefore may be depleted due to the NO/ONOO- cycle
Algal supplements	Probably act as antioxidants	
Hyperbaric oxygen	May act to help restore cytochrome oxidase activity by competing with nitric oxide	My impression is that this approach needs to be used with substantial care – too high or prolonged dosage can cause damage
Trimethyl glycine (betaine), S-adenosyl methionine (SAM), choline, carnitine	Lower reductive stress; also helps with the generation of S-adenosyl methionine (SAM)	While lowering reductive stress may be the main concern, SAM generation may also be of concern; the enzyme methionine synthase is inhibited by nitric oxide and inactivated under conditions of oxidative stress, thus leading to lowered SAM and lowered methylation
Coenzyme Q10 (ubiquinone)	Important in mitochondrial function; important antioxidant, especially in mitochondrion; reported to scavenge peroxynitrite	Optimal dosage may vary considerably among different individuals; suggest taking early in day
D-ribose, RNA or inosine	Two important functions: Provides adenosine for restoring adenine nucleotide pools after energy metabolism dysfunction; when catabolized, the purine bases generate uric acid, a peroxynitrite scavenger	Each of these may act somewhat similarly; however only D-ribose has been tested in a clinical trial and reported to be effective; each of these agents has distinct drawbacks

Modified from the author's web site, used with permission. References provided in Chapter 15, ref.1, except as follows [96-102]. In a number of cases, only one of a class of agents has been tested via clinical trial with CFS and/or FM.

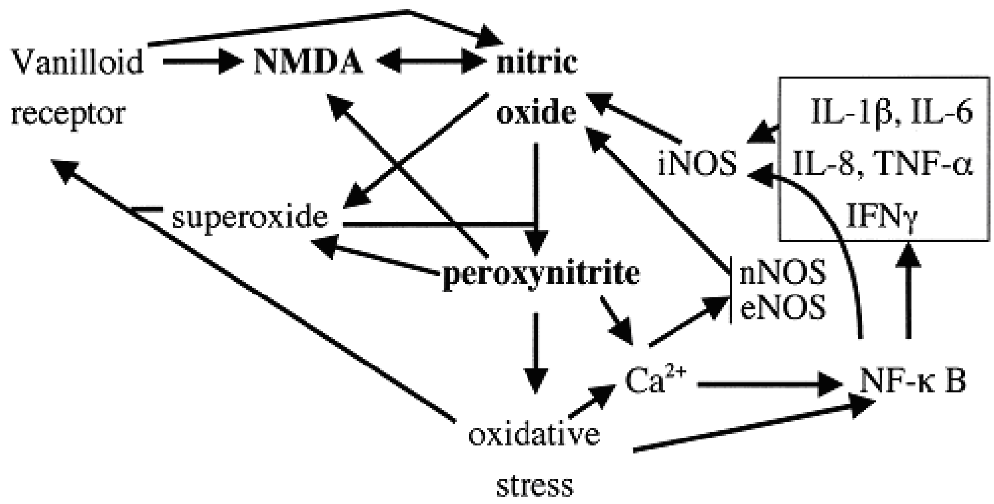
Table 4. Additional agents, not explicitly discussed in Table 3, that are included in the Allergy Research Group nutritional support protocol.

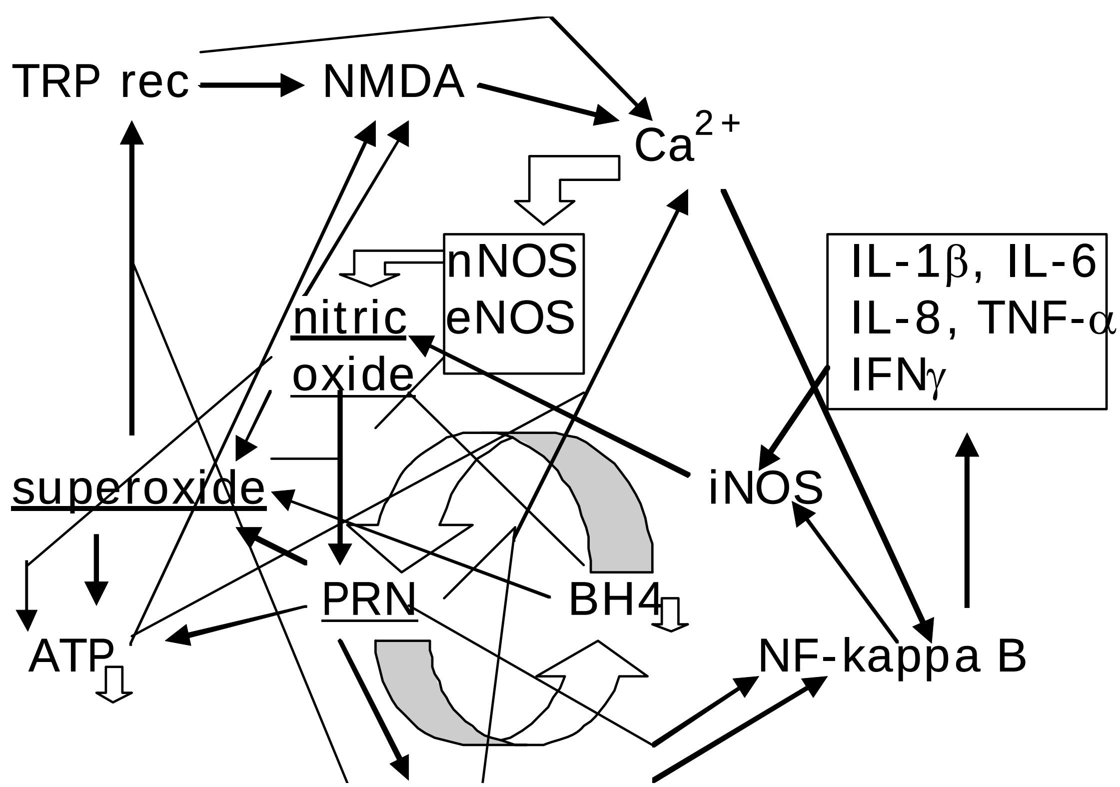
Vitamin B6, including pyridoxal phosphate	multiple functions, most relevant may be to stimulate glutamate decarboxylase activity, limit excitotoxicity
Niacin, including nicotinic acid and nicotinamide	Helps restore NAD/NADH pools after poly-ADP ribosylation leads to pool depletion; important for energy metabolism
Thiamine	Is depleted by oxidants; essential for two steps in pentose phosphate shunt and is needed, therefore to help provide NADPH for glutathione reductase
Riboflavin including 5'-phosphate	Depletion can limit glutathione reductase activity
Carotenoids including natural -carotene, lycopene, lutein	Helps scavenge peroxynitrite, especially in biological membranes
Natural vitamin E, including -tocopherol and the tocotrienols	-tocopherol thought to have special role in scavenging NO2 radical (from peroxynitrite); tocotrienols may have special role in protecting from excitotoxicity and/or mitochondrial oxidation
Taurine	thought to lower excitoxicity by stimulating gabaergic activity
Zinc, manganese, copper	Modest doses used; may increase superoxide dismutase activity
-lipoic acid	Multiple antioxidant roles on reduction to dihydrolipoic acid; helps restore reduced glutathione pools
N-acetyl cysteine	Helps restore reduced glutathione pools; modest doses used to prevent or lower possible excitotoxicity
Selenium as seleno-L-methionine	Important antioxidant; a variety of organic selenium compounds are peroxynitrite scavengers; selenium levels often low in multisystem illnesses

Modified from the author's web site with permission.

Figure 1. Vicious (NO/ONOO-) cycle diagram. Each arrow represents one or more mechanisms by which the variable at the foot of the arrow can stimulate the level of the variable at the head of the arrow. It can be seen that these arrows form a series of loops that can potentially continue to stimulate each other. An example of this would be that nitric oxide can increase peroxynitrite which can stimulate oxidative stress which can stimulate NF- B which can increase the production of iNOS which can, in turn increase nitric oxide. This loop alone constitutes a potential vicious cycle and there are a number of other loops, diagrammed in the figure that can collectively make up a much larger vicious cycle. The challenge, according to this view, in these illnesses is to lower this whole pattern of elevations to get back into a normal range. You will note that the cycle not only includes the compounds nitric oxide, superoxide and peroxynitrite but a series of other elements, including the transcription factor NF- B, oxidative stress, inflammatory cytokines (in box, upper right), the three different forms of the enzymes that make nitric oxide (the nitric oxide synthases iNOS, nNOS and eNOS), and two neurological receptors the vanilloid (TRPV1) receptor and the NMDA receptor. The figure and legend are taken from the author's web site with permission.

Figure 2. A more complete NO/ONOO- cycle diagram. Central to the figure are the reciprocal interactions between peroxynitrite, abbreviated as PRN and tetrahydrobiopterin (BH4) depletion. Also indicated is the ATP depletion produced by peroxynitrite, superoxide and nitric oxide. And in the upper left corner, TRP represents the three TRP receptors, TRPV1, TRPA1 and TRPM2, each of which is stimulated via distinct mechanisms by oxidative stress. Each arrow in the figure represents one or more mechanisms by which one element of the cycle stimulates another element of the cycle. Figure and legend is taken from the author's web site with permission.


Vanilloid
receptor
NMDA
nitric
oxide
superoxide
peroxynitrite
iNOS
IL-1β, IL-6
IL-8, TNF-α
IFNγ
nNOS
eNOS
Ca2+
NF-κ B
oxidative
stress


TRP rec
NMDA
Ca2+
nNOS
eNOS
nitric
oxide
IL-1β, IL-6
IL-8, TNF-α
IFNγ
superoxide
iNOS
PRN
BH4
ATP
NF-kappa B