NO/ONOO- cycle as the generic model for chronic inflammatory, oxidative/nitrosative stress diseases: Use of high dose vitamin and natural compound therapies

Martin L. Pall
Professor Emeritus of Biochemistry and Basic Medical Sciences
Washington State University
thetenthparadigm.org
martin_pall@wsu.edu
I have published in my book and in 27 published papers, evidence and arguments that 20 different chronic inflammatory diseases are probably caused by a biochemical vicious cycle mechanism known as the NO/ONOO- cycle. Each of these also are also known to have oxidative/nitrosative stress and most have been shown to have elevation of other cycle elements: mitochondrial dysfunction and elevated NMDA activity and many of them have been shown to also have elevated NF-kappa B activity and tetrahydrobiopterin (BH4) depletion as well.

The cycle is predicted to be a primarily local mechanism, such that depending on where it is located in the body, it can produce different symptoms and signs and different diagnoses. In this way, a single primary mechanism can produce a variety of diseases.
The etiologic theory I will be discussing focuses on nitric oxide and its oxidant product peroxynitrite, a potent oxidant.

\[
\cdot\text{NO} + \cdot\text{OO}^- \rightarrow \text{ONOOO}^-
\]

Nitric superoxide peroxynitrite oxide
<table>
<thead>
<tr>
<th>Illness</th>
<th>Stressors Implicated in Initiation of Illness</th>
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<tbody>
<tr>
<td>Chronic fatigue syndrome</td>
<td><strong>Viral infection, bacterial infection, organophosphorus pesticide exposure</strong>, carbon monoxide exposure, ciguatoxin poisoning, physical trauma, severe psychological stress, toxoplasmosis (protozoan) infection, ionizing radiation exposure</td>
</tr>
<tr>
<td>Multiple chemical sensitivity</td>
<td><strong>Volatile organic solvent exposure, organophosphorus/carbamate pesticide exposure</strong>, organochlorine pesticide exposure, pyrethroid exposure; hydrogen sulfide; carbon monoxide; mercury</td>
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<tr>
<td>Fibromyalgia</td>
<td><strong>Physical trauma (particularly head and neck trauma), viral infection, bacterial infection, severe psychological stress, pre-existing autoimmune disease</strong></td>
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<tr>
<td>Post-traumatic stress disorder</td>
<td><strong>Severe psychological stress</strong>, physical (head) trauma</td>
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Chemical Action in MCS

Organophosphorus/carbamate pesticides
- acetylcholinesterase
- acetylcholine
- muscarinic activity
- organochlorine pesticides
- acetylcholine
- GABAA receptors
- nitric oxide

Organic solvents
- H2S
- TRPV1, TRPA1
- other TRP receptors
- GABA receptors
- glutamate activity
- sodium transport
- nitric oxide
- glutamate activity

Pyrethroid pesticides
- Hg
- MeHg
- glutamate transport
- sodium channels
Five Principles
1. Cases can be initiated by short-term stressors that increase NO 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, NO, superoxide and ONOO-, 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 in 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 there are, but rather that the primarily local mechanisms can generate great variation in diagnosis and in the symptoms and signs, from one individual to another.
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.
There are 34 distinct, mechanisms that currently make up the NO/ONOO- cycle models as it was shown in the preceding figures. I have a few copies of this list for those who may be particularly interested in looking at it. All of these 34 mechanisms are well documented, well established biochemistry and physiology. Of those 31 have reported substantial pathophysiological roles.

Thus the only thing truly novel about the NO/ONOO- cycle, is that when these mechanisms are put into juxtaposition with each other, as they have been in the preceding figures, they serve collectively to integrate and explain a vast array of data about a large number of human diseases.

Therapy should focus on down-regulating parts of the NO/ONOO- cycle, rather than on symptomatic relief.
1. Extremely rapid, diffusion limited reaction between nitric oxide (NO\textsuperscript{-}) with superoxide (OO\textsuperscript{-}), forming peroxynitrite (ONOO\textsuperscript{-}).

2. Peroxynitrite, a potent oxidant, can act mainly through its breakdown products to increase the activity of the transcription factor NF-kappaB.

3. Peroxynitrite breaks down both before and after reaction with carbon dioxide into the following free radicals, hydroxyl (HO\textsuperscript{-}), carbonate (CO3\textsuperscript{-}) and NO2 radical (NO2\textsuperscript{-}), each of which are responsible for a number of consequences produced by peroxynitrite.

4. Peroxynitrite being a potent oxidant produces oxidative stress, an imbalance between oxidants and antioxidants.

5. Oxidative stress also produces increases in NF-kappaB activity.

6. NF-kappaB produces increased transcription of the inducible nitric oxide synthase (iNOS), a gene whose transcription is known to be stimulated by NF-kappaB elevation.

7. NF-kappaB also stimulates the transcription of several inflammatory cytokines, including IL-1b, IL-6, IL-8, TNF-\alpha, and IFN\gamma.

8. Each of the five cytokines listed in 7 above, act directly and/or indirectly to stimulate the transcription of the iNOS gene.

9. When iNOS is induced, it produces large amounts of NO.

10. Peroxynitrite inactivates the calcium-ATPase, leading to increased levels of intracellular calcium.

11. Other oxidants also react with and inactivate the calcium-ATPase as well.

12. Lowered energy metabolism (decreased energy charge/ATP) also lowers calcium-ATPase activity, leading to increased levels of intracellular calcium.
14. Intracellular calcium stimulates the nNOS and eNOS forms of nitric oxide synthase, both of which are calcium dependent enzymes.
15. Increased nNOS and eNOS activity both produce increased NO synthesis.
16. Peroxynitrite oxidizes tetrahydrobiopterin (BH4), depleting BH4 levels.
17. BH4 depletion produces partial uncoupling of the three NO synthases, such that some of these enzymes produce superoxide in place of NO. Because of the very rapid reaction of these two compounds to produce peroxynitrite, this partial uncoupling is expected to produce an increase in peroxynitrite production.
18. Nicking of nuclear DNA by hydroxyl and carbonate radicals, can produce a massive stimulation of poly ADP-ribosylation of chromosomal proteins, leading, in turn to a massive depletion of NAD/NADH pools, because NAD is the substrate for such poly ADP-ribosylation. NADH depletion lowers, in turn, ATP production in the mitochondrion.
19. Other changes causing ATP depletion come from a cascade of events occurring within the mitochondrion. The cascade starts with NO, possibly produced by mitochondrial NO synthase (mtNOS which is thought to be largely a form of nNOS), with NO binding to cytochrome oxidase, competitively inhibiting the ability of molecular oxygen to bind. This inhibits the ability of cytochrome oxidase to serve as the terminal oxidase of the mitochondrial electron transport chain.
20. The action of NO in 18 above, produces increase superoxide production by the electron transport chain.
21. Peroxynitrite, produced from the combination 18 and 19 above, also acts to produce increased superoxide from the electron transport chain.
22. Peroxynitrite, superoxide and their products lead to lipid peroxidation of the cardiolipin in the inner membrane of the mitochondrion. Cardiolipin is highly susceptible to such peroxidation, because most of the fatty acids that make up its structure in mammals are polyunsaturated fatty acids, which are much more susceptible to peroxidation than are other fatty acids.

23. Cardiolipin peroxidation leads to lowered activity of some of the enzymes in the electron transport chain, leading to further lowering of ATP synthesis.

24. Cardiolipin peroxidation also leads to increased superoxide generation from the electron transport chain in the mitochondrion.

25. Peroxynitrite produces inactivation of the mitochondrial superoxide dismutase (Mn-SOD), leading in turn to increased superoxide levels in the mitochondrion.

26. Peroxynitrite, superoxide and nitric oxide all inactivate or inhibit the aconitase enzyme, lowering citric acid cycle activity and subsequent ATP synthesis.

27. Oxidative stress leads to oxidation of cysteine residues in the enzyme xanthine reductase, converting it into xanthine oxidase which produces superoxide as a product, thus increasing superoxide generation.

28. Increased activity of the enzyme NADPH oxidase, which produces superoxide as a product, is an important part of the inflammatory cascade, and contributes, therefore, to the cascade by producing increased superoxide.

29. Activity of the NMDA receptors, allow calcium influx into the cell, raising intracellular calcium levels.

30. Activity of transfer receptor potential (TRP) receptors also allows calcium influx into the cell, again raising intracellular calcium levels, presumably leading to increased nitric oxide production.
31. The main physiological agonist of the NMDA receptors is glutamate whose extracellular concentration is lowered after release, by energy dependent transport. It follows that ATP depletion produces increased NMDA stimulation by lowering glutamate transport.

32. The activity of the NMDA receptors is also greatly increased by ATP depletion within the cells containing the NMDA receptors. The mechanism here is that the ATP depletion lowers the electrical potential across the plasma membrane, which produces, in turn, increased susceptibility of the NMDA receptors to stimulation.

33. Three of the TRP group of receptors have been shown to be stimulated by increased superoxide and/or oxidative stress or their downstream consequences, these being the TRPV1, TRPA1 and TRPM2 receptors, with the increased TRPV1 and TRPA1 activity being produced in part through the oxidation of cysteine residue side chains. Several TRP receptors are also activated by nitric oxide mediated nitrosylation.

34. TRPV1, TRPA1 and probably several other TRP group receptors, receptor stimulation has each been repeatedly shown to lead to increased NMDA activity, with neurons containing these TRP family of receptors acting in part by releasing glutamate, the major physiological NMDA agonist.
<table>
<thead>
<tr>
<th></th>
<th>Chronic fatigue syndrome/myalgic encephalomyelitis</th>
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<tr>
<td>2</td>
<td>Multiple chemical sensitivity</td>
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<tr>
<td>3</td>
<td>Fibromyalgia</td>
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<td>4</td>
<td>Post-traumatic stress disorder</td>
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<tr>
<td>5</td>
<td>Tinnitus</td>
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<tr>
<td>6</td>
<td>Post-Radiation Syndrome</td>
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<tr>
<td>7</td>
<td>Multiple Sclerosis (MS)</td>
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<tr>
<td>8</td>
<td>Autism</td>
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<td>9</td>
<td>Overtraining Syndrome</td>
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<tr>
<td>10</td>
<td>Silicone Implant Associated Syndrome</td>
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<td>11</td>
<td>Sudeck’s Atrophy</td>
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<tr>
<td>12</td>
<td>Post-Herpetic Neuralgia (Pain)</td>
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<tr>
<td>13</td>
<td>Chronic Whiplash Associated Disorder</td>
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<td>14</td>
<td>Amyotrophic Lateral Sclerosis (ALS)</td>
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<tr>
<td>15</td>
<td>Parkinson’s Disease</td>
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<td>16</td>
<td>Alzheimer’s Disease</td>
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<td>17</td>
<td>Asthma</td>
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<tr>
<td>18</td>
<td>Irritable Bowel Syndrome</td>
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<tr>
<td>19</td>
<td>Epilepsy</td>
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<tr>
<td>20</td>
<td>Spinal cord injury</td>
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We don’t have time to review the evidence that each of these are probable NO/ONOO- cycle diseases. However the evidence for each of them is the fit to the five principles underlying the NO/ONOO-cycle.
Let’s talk about therapy. Conventional allopathic medicine has been unable to cure any of these 20 proposed NO/ONOO- cycle diseases. In most cases, we don’t have even any effective treatments, or more rarely we have treatments that are effective in some cases but often have severe side effects. My opinion is that the most promising agents available to treat these diseases are nutritional and also some other naturopathic approaches such as sauna therapy. I also think that it is likely that most approaches to treatment will involve large numbers of such agents, Although there may come a time where this will no longer be true. What should be clear is that the NO/ONOO-cycle is very robust because of all the positive feedback loops that reinforce each other to keep it going. Any effective therapy must deal with this robust nature.

While there may be a time where conventional allopathic medicine is much more effective than it is now, it cannot be more effective without first recognizing the complexity of the problem that it faces with these diseases. Let’s consider how nutritional and other natural agents may be useful.
Agents Predicted to Down-Regulate the NO/ONOO- Cycle for which We Have Clinical Trial Data of Efficacy

| IV high-dose ascorbate; CFS/ME, MCS | Probably works in 3 ways to lower the central couplet: Scavenges peroxynitrite; Reduces BH3 back to BH4; Produces H2O2, which induces GTP cyclohydrolase I, leading to increased de novo synthesis of BH4. |
| Flavonoids; CFS/ME and FM | Chain breaking antioxidants; may also scavenge peroxynitrite breakdown products and/or superoxide |
| Carnitine/acetyl carnitine; CFS/ME | Improves mitochondrial function |
| Hydroxocobalamin form of B-12; CFS/ME | A potent nitric oxide scavenger after reduction to the cobalt II form in vivo; lowers nitric oxide |
| High dose folate; CFS/ME and also other proposed NO/ONOO cycle diseases | Raises levels of 5-methyltetrahydrofolate, a very potent scavenger of peroxynitrite; This is different from the usual interpretation which argues that the primary role is in raising methylation. |
Agents Predicted to Down-Regulate the NO/ONOO– Cycle for which We Have Clinical Trial Data of Efficacy

NMDA antagonists, other agents that indirectly lower NMDA activity, magnesium; FM, CFS/ME

Implicates excessive NMDA activity

Fish oil (long chain omega-3); FM, CFS/ME

Has substantial antiinflammatory activity; may also act on neuronal tissue through roles of long chain omega-3 fatty acid in the plasma membrane and inner mitochondrial membrane

Coenzyme Q10; FM; clinical observations in CFS/ME

Improves mitochondrial function; may protect mitochondria from peroxynitrite mediated damage

NT-factor; CFS/ME

Allows regeneration of oxidized cardiolipin in mitochondrial inner membrane

Ecklonia cava extract; FM

Polyphenolic chain breaking antioxidant; scavenges peroxynitrite and superoxide

D-ribose; CFS/ME & FM

Helps restore purine nucleotides including ATP; subsequent degradation will raise uric acid levels
There is one other agent that is thought to act by lowering a NO/ONOO- cycle element: sauna therapy. Sauna therapy has been reported to be effective in clinical trials in MCS, CFS/ME and FM, as well as in several other diseases characterized by BH4 depletion. It is thought to act in two ways to raise the levels of GTP cyclohydrolase I, the rate-limiting enzyme in the de novo pathway for BH4 synthesis. There is unpublished data that sauna therapy does raise blood levels of BH4.
Note: A number of these agents should be used with care. The fact that they are mentioned here does not suggest that their use is risk-free.

Quite a number of agents influence the central couplet or precursors of peroxynitrite leading into the central couplet.

High dose ascorbate (vitamin C) may be particularly useful agent in this regard and while IV ascorbate can achieve much higher blood levels, high dose oral ascorbate may be useful as well. Why?

Ascorbate is a peroxynitrite scavenger, but is only a weak one at normal physiological levels. When tetrahydrobiopterin (BH4) is oxidized by peroxynitrite, BH3 is the initial product. BH3 can be reduced back to BH4 by ascorbate (must occur rapidly because BH3 is unstable and is further oxidized to BH2 and other oxidation products). Finally, high levels of ascorbate generates H2O2 (hydrogen peroxide) and H2O2 induces the enzyme GTP cyclohydrolase I, the first and rate limiting step in the de novo pathway for the synthesis of BH4.
Reduced glutathione has many antioxidant roles. One of them is that reduced glutathione reduces BH2 to BH4. We will come back later to the issue of how to maintain high levels of reduced glutathione.

5-Methyltetrahydrofolate (5-MTHF) is usually the most common folate in the body. 5-MTHF is a potent peroxynitrite (ONOO-)-scavenger. Consequently, substantially raising its levels can lower ONOO- and thus lower the central couple and the cycle, in general. One can do this by using reduced folates, typically either 5-MTHF or folinic acid. Folic acid is less effective. The reaction between 5-MTHF and ONOO- works both ways. Diseases characterized by ONOO-elevation often produce deficiencies in reduced folates, presumably because oxidation products of 5-MTHF are lost to the folate pools. Because 5-MTHF is an important methyl donor, this can lead to some lowering of methylation cycle activity. It is my view that lowered methylation in relationship to various diseases is usually caused by elevated ONOO- leading to lowered 5-MTHF.

In any case, this appears to be another case, where relatively high levels of supplements may be useful in treatment, acting by lowering an important part of the cycle.
Another agent that has been used to treat several of the apparent NO/ONOO- cycle diseases is high dose hydroxocobalamin (a form of vitamin B-12). Typically such high levels are obtained by using either IM or subcutaneous injection, or using nasal spray or inhaled nebulized hydroxocobalamin. Hydroxocobalamin is a potent NO scavenger, because the initial cobalt III form of the compound is reduced \textit{in vivo} to the cobalt II form which scavenges NO.

As best I can determine, from peoples’ subjective responses, both methylcobalamin and cyanocobalamin forms of B-12 also may be useful but that methylcobalamin is less active and cyanocobalamin is still less active than is hydroxocobalamin. In an early placebo-controlled trial, 5 mg IM injections were used twice a week and as far as I can determine, this is still a good dosage for most people.

Note: oral B-12 absorption is limited by the availability of intrinsic factor, a glycoprotein needed for efficient absorption. Therefore standard oral hydroxocobalamin is of very limited value – much higher blood levels must be obtained to get a good clinical response.
Magnesium is perhaps the most commonly used nutritional agent to treat presumed NO/ONOO- cycle diseases. Magnesium ions have multiple functions in the body but perhaps the most relevant here is that it lowers that activity of the NMDA receptors. Because many of our diets are relatively low in magnesium, this may be particularly important.
Reduced glutathione (GSH) is often stated to THE most important antioxidant produced in the body. It is universally depleted under conditions of oxidative stress. While such depletion is NOT the cause of oxidative stress but rather is caused by oxidative stress, nevertheless recovery from oxidative stress requires at least partial recovery of GSH pools.

Such recovery requires restoration of functional glutathione peroxidase, the enzyme that reduces oxidized glutathione (GSSG) to GSH, but also restoration of total glutathione (GSH + GSSG) pools as well. Total pools are depleted by oxidative stress because whenever there is substantial, prolonged oxidative stress, the massive conversion of GSH to GSSG in the stress tissue, leads to efflux of the GSSG from the cell and subsequent extracellular degradation of the GSSG. Thus the total pools are depleted in tissues under oxidative stress and restoring pools requires either supplying glutathione to the tissue or precursors (notably cysteine) that allow the tissue to resynthesize GSH. This whole process is very complex.

Let’s talk about the issue of restoring functions glutathione peroxidase.
Pentose phosphate shunt [TPP(thiamine)]
Dependent process

NADP
GSH

Glutathione reductase

NADPH
[FAD(riboflavin)]-dependent process
½ GSSG

So the generation of NADPH is a TPP-dependent process and the action of NADPH in reducing glutathione is a FAD (riboflavin)-dependent process. Thiamine is reported to have “antioxidant” properties, which means it reacts with free radicals and other oxidants. It may well, therefore be depleted in tissues with high levels of ONOO-. I am unaware of any similar data on riboflavin and its co-enzymes but they do have complex oxidative chemistry and so may also be depleted by high levels of ONOO-. For these reasons, it may well be the high doses of these B-vitamins may be needed to help restore GSH pools in these oxidized tissues.
While there may be some question about the need to use high doses of thiamine and riboflavin or their coenzymes to help restore GSH pools in tissues impacted by the NO/ONOO- cycle, there is no similar uncertainty about another B-vitamin, niacin.

It is known that for tissues having high levels of ONOO-, one gets nicking of DNA in the nucleus of cells leading to massive poly-ADP-ribosylation of chromosomal proteins. This process uses NAD as substrate and often leads to massive depletion of NAD/NADH pools. Because of the essential role of NADH in mitochondria, this leads to a drastic lowering of mitochondrial function, leading to ATP depletion. High doses of niacin in the form of nicotinamide and nicotinic acid will help restore such mitochondrial function and nicotinamide also inhibits the poly ADP-ribose polymerase, the enzyme that catalyzes this poly-ADP-ribosylation. Very high levels of niacin are thought to be needed for both of these action and such high levels are, therefore, needed to help restore mitochondrial metabolism in NO/ONOO- cycle diseases.
In summary, then, there are strong cases that very high doses of vitamins including ascorbate, the hydroxocobalamin form of B-12, reduced folate and both the nicotinic acid and nicotinamide forms of niacin are useful in lowering important aspects of the NO/ONOO-cycle. There are also weaker but still substantial cases that can be made for high dose therapy using riboflavin (or riboflavin 5’-phosphate) and thiamine, as well.

There is also a case for using relatively high doses of vitamin B-6 (in the form of pyridoxine or pyridoxal phosphate, although we don’t have time to consider this B vitamin. All of these considerations argue against the European policy of only allowing low doses of these vitamins to be sold.
There are a large number of nutritional agents that appear to be useful when used in substantial amounts to treat apparent NO/ONOO- cycle diseases.

1. Some of these are agents that act to help restore mitochondrial function;
2. some are antiinflammatory agents;
3. and some are antioxidants, particularly phenolic antioxidants including flavonoids, ellagic acid, curcumin and other polyphenolics.
4. There are large number of agents, both natural and synthetic that lower NF-kappa B activity and these may be helpful. I will not discuss these types of agents further.

Let’s consider some of these.
Three agents, L-carnitine/acetyl carnitine, Coenzyme Q10 and phospholipids probably mainly act to help improve mitochondrial function. Carnitine acts to allow fatty acids to enter the inner mitochondrial space. Thus both carnitine and phospholipids can probably help to restore cardiolipin in the inner membrane and it is not unusual for these to be used in clinical practice. Garth Nicolson has developed a protocol for CFS/ME that is mainly focused on phospholipids. Coenzyme Q10 has important functions in the electron transport chain and it also acts as an intramitochondrial chain breaking antioxidant. One other thing that may be important is that thyroid hormone, particularly the T3 most active form, can act to lower the mitochondrial cascade that is thought to be part of the NO/ONOO- cycle. Jacob Teitelbaum uses thyroid hormone in patients with either low or low normal levels, in order to raise hormone levels into the middle of the normal range. This may be a useful approach, as well.
Long chain fatty acids in fish oil have important antiinflammatory properties and fish oil supplements have been used to treat at least a dozen different apparent NO/ONOO- cycle diseases.
The issue of how best to use antioxidants to treat apparent NO/ONOO- cycle diseases is, in many ways, very complex. I think that the most important aspects of this topic go way beyond the issue of increasing availability of chain-breaking antioxidants. No doubt some of my comments will be viewed as controversial.

A number of phenolic antioxidants including silymarin, green tea extract, curcumin and resveratrol have been shown to be able to induce increased levels of superoxide dismutase without first increasing oxidative stress. This induction may be useful in lowering levels of superoxide and thus most of the cycle.

A number of phenolic antioxidants including ellagic acid, grapeseed, Ginkgo biloba, citrus flavonoid and green tea extract scavenge ONOO-. Many of the deeply colored pro-anthocyanidin flavonoids scavenge superoxide, as well.

Resveratrol which was mentioned above, also increases availability of BH4 by inducing the rate-limiting enzyme, GTP cyclohydrolase I.
Natural carotenoids which contain cis-double bonds act as peroxynitrite scavengers, as well. Regrettably, most the clinical trials on carotenoids have used synthetic beta-carotene, which contains almost no cis-double bonds.
Natural carotenoids which contain cis-double bonds act as peroxynitrite scavengers, as well. Regrettably, most the clinical trials on carotenoids have used synthetic beta-carotene, which contains almost no cis-double bonds.
Most of the clinical trials on high dose “vitamin E” have used synthetic \( \alpha \)-tocopherol as mixture of 8 forms only one of which is natural. Natural vitamin E contains various amounts of 8 natural forms, 4 tocopherols and 4 tocotrienols. When high doses of \( \alpha \)-tocopherol are used in treatment, it induces an enzyme, CYP4F2, that degrades all forms of vitamin E. This leads to a deficiency in three of the tocopherols and all four tocotrienols.

**Properties of forms of vitamin E not shared by \( \alpha \)-tocopherol:**
The most important tocopherol that becomes depleted is \( \gamma \)-tocopherol, which scavenges NO2 radical, a breakdown product of ONOO-, lowers NF-kappa B activity and also has important antiinflammatory properties. The tocotrienols have been reported to lower NF-kappa B activity, to lower the consequences of excessive NMDA activity, to lower inflammatory cytokine production and to apparently increase BH4 availability. One study reported that tocotrienols had a special role in lowering oxidative stress in mitochondria.

For all of these reasons, I suggest that “vitamin E” treatment of NO/ONOO-cycle disease should be performed using combinations of natural vitamin E with relatively modest amounts of \( \alpha \)-tocopherol and higher amounts of \( \gamma \)-tocopherol and the tocotrienols.
In summary, modestly high to very high doses of multiple natural compounds are likely to be useful in the therapy of various probable NO/ONOO- cycle diseases including the following: Ascorbate, riboflavin (or still better it’s 5’ phosphate), thiamine, nicotinamide and nicotinic acid, hydroxocobalamin, reduced folate, magnesium, reduced glutathione and/or its precursors, \( \alpha \)-lipoic acid, a large number of flavonoids and other phenolic antioxidants, natural carotenoids, phospholipids, coenzyme Q10, agents that lower NF-kappa B, carnitine/acetyl-carnitine, \( \gamma \)-tocopherol and the tocotrienols.
Five Principles

1. Cases can be initiated by short-term stressors that increase NO 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, NO, 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 in 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 there are, but rather that the primarily local mechanisms can generate great variation in diagnosis and in the symptoms and signs.
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.
Major Disease Paradigms:

1. Infectious diseases
2. Genetic diseases
3. Nutritional deficiency diseases
4. Hormone dysfunction diseases
5. Allergies
6. Autoimmune diseases
7. Somatic mutation/selection (cancer)
8. Ischemic cardiovascular diseases
9. Amyloid (including prion) diseases
10. NO/ONOO- cycle diseases
1. Chronic fatigue syndrome/myalgic encephalomyelitis
2. Multiple chemical sensitivity
3. Fibromyalgia
4. Post-traumatic stress disorder
5. Tinnitus
6. Post-Radiation Syndrome
7. Multiple Sclerosis (MS)
8. Autism
9. Overtraining Syndrome
10. Silicone Implant Associated Syndrome
11. Sudeck’s Atrophy
12. Post-Herpetic Neuralgia (Pain)
13. Chronic Whiplash Associated Disorder
14. Amyotrophic Lateral Sclerosis (ALS)
15. Parkinson’s Disease
16. Alzheimer’s Disease
17. Asthma
18. Irritable Bowel Syndrome
19. Epilepsy
20. Spinal cord injury