

# The NO/ONOO(-) cycle as the central cause of heart failure: Prevention by raising Nrf2 and probable cycle role in many other diseases.

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Stockholm 2016-03-09

I have published in my book and in 32 published papers, evidence and arguments that 23 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.

22 of these are shown on the next slide:

1. Myalgic encephalomyelitis/Chronic fatigue syndrome (ME/CFS)
2. Multiple chemical sensitivity (MCS)
3. Fibromyalgia
4. Post-traumatic stress disorder (PTSD)
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 (SCI)
21. Pulmonary arterial hypertension (PAH)
22. Glaucoma

The cases I have made for (or against) most of these 23, are fairly superficial, although I have published more detailed cases for ME/CFS, MCS, post-radiation syndrome, and tinnitus.

I have also published still more detailed cases for both pulmonary hypertension and heart failure.

It is the last of these that is the main focus of this talk.

- Before getting into my talk, it is important to acknowledge that a similar model was proposed by Guy C. Brown and his colleagues at Cambridge University:
- Brown & Bal-Price, *Molecular Neurobiology* 2003; 27:325-355;
- Brown & Neher, *Molecular Neurobiology* 2010;41:242-247.
- Brown's proposed mechanism and mine differ in several ways but their similarities are more important than their differences.

Over the past three years, I have published, very detailed cases for a NO/ONOO- cycle etiology on two additional diseases, both important cardiovascular diseases (both of these are public access papers):

Pall ML. 2013 Pulmonary hypertension is a probable NO/ONOO- cycle disease: A review. ISRN Hypertension 2013: Article ID 742418, 27 pages.

Pall ML. 2013 The NO/ONOO- Cycle as the Central Cause of Heart Failure. Int J Mol Sci. 2013 Nov 13;14(11):22274-330. doi: 10.3390/ijms141122274.

The heart failure paper, is a major focus of this talk. It is a 57 page paper, that was requested and was published in a special issue of the journal on oxidative stress in cardiovascular disease.

Heart failure is the #1 cause of hospitalization in the U.S. and is the #1, 2 or 3 cause of death depending on how this is calculated. The heart failure paper is important for several additional reasons, in my no doubt biased view.

1. There is a stunning amount of information about heart failure which can be used to test in great detail whether it is a NO/ONOO- cycle disease or not.
2. By detailed testing the cycle mechanism in a disease unrelated to the origin of the cycle, one can provide a detailed test of the general mechanism based on completely independent data. In this way, the NO/ONOO- cycle is greatly strengthened not only as the cause of heart failure but also a generic model of chronic inflammatory disease.
3. That detailed consideration has also revealed some new insights into the cycle.

The etiologic theory I will be discussing focuses on **nitric oxide** and its oxidant product **peroxynitrite**, a potent oxidant.



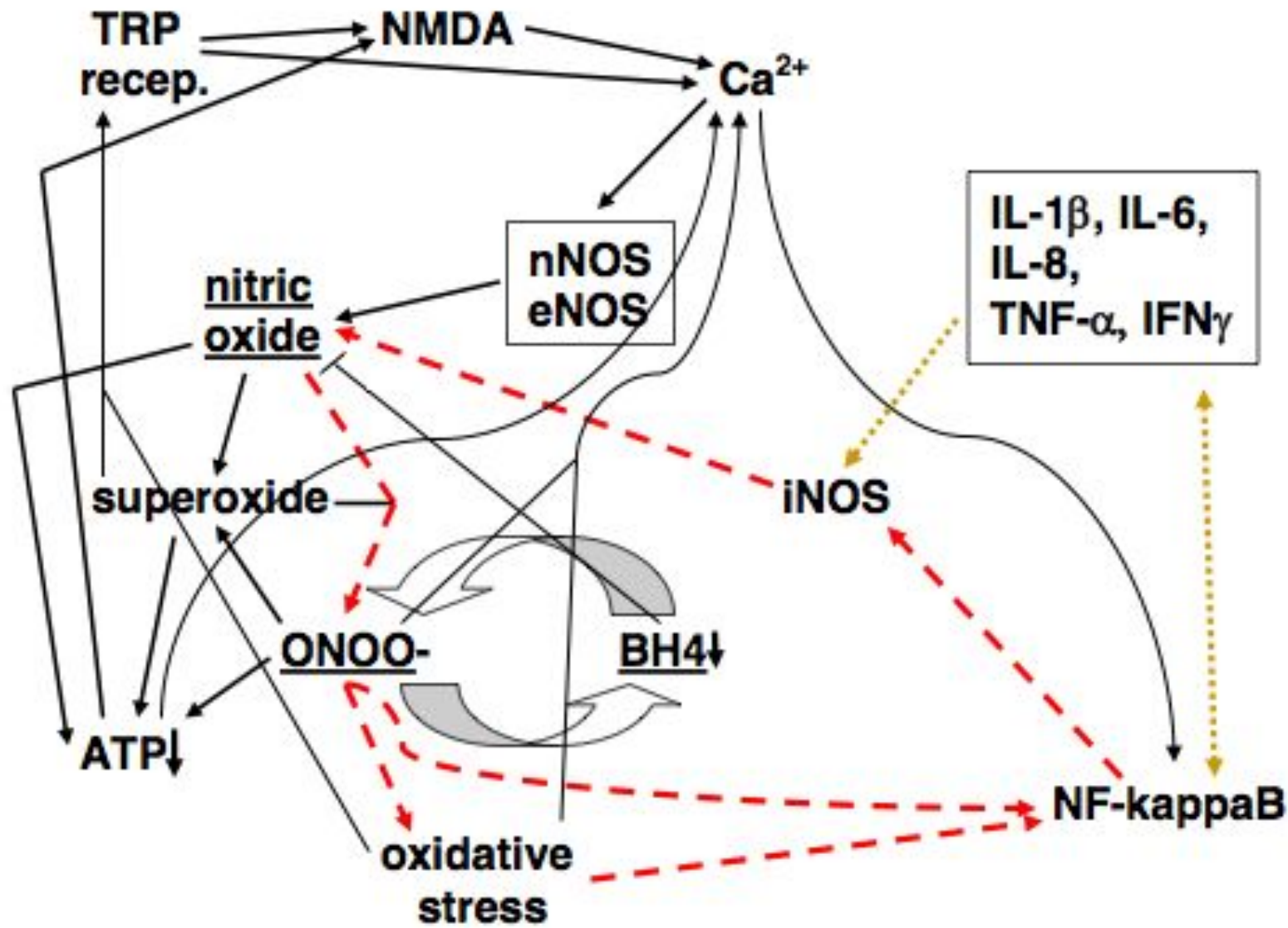
Nitric  
oxide

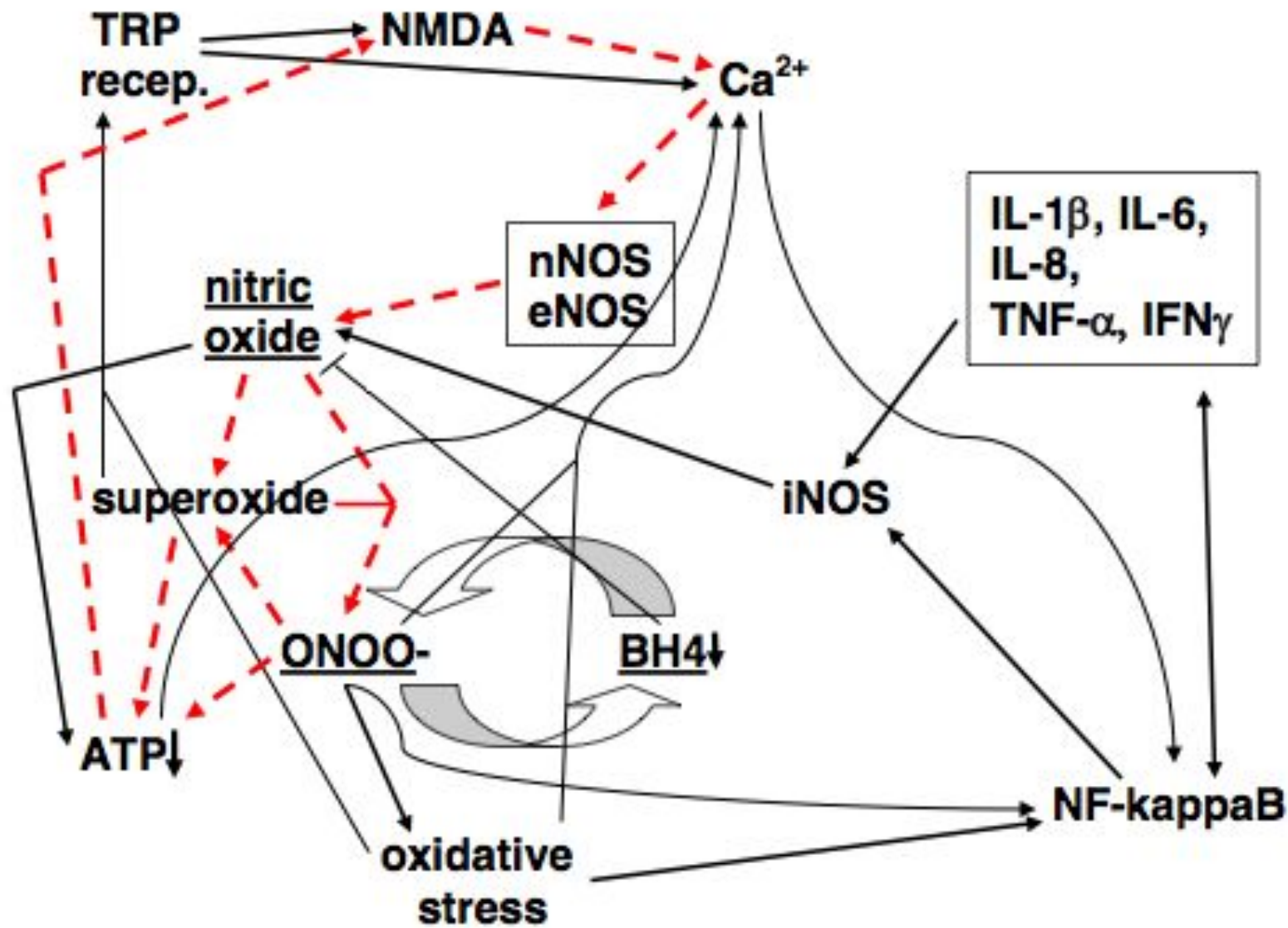
superoxide

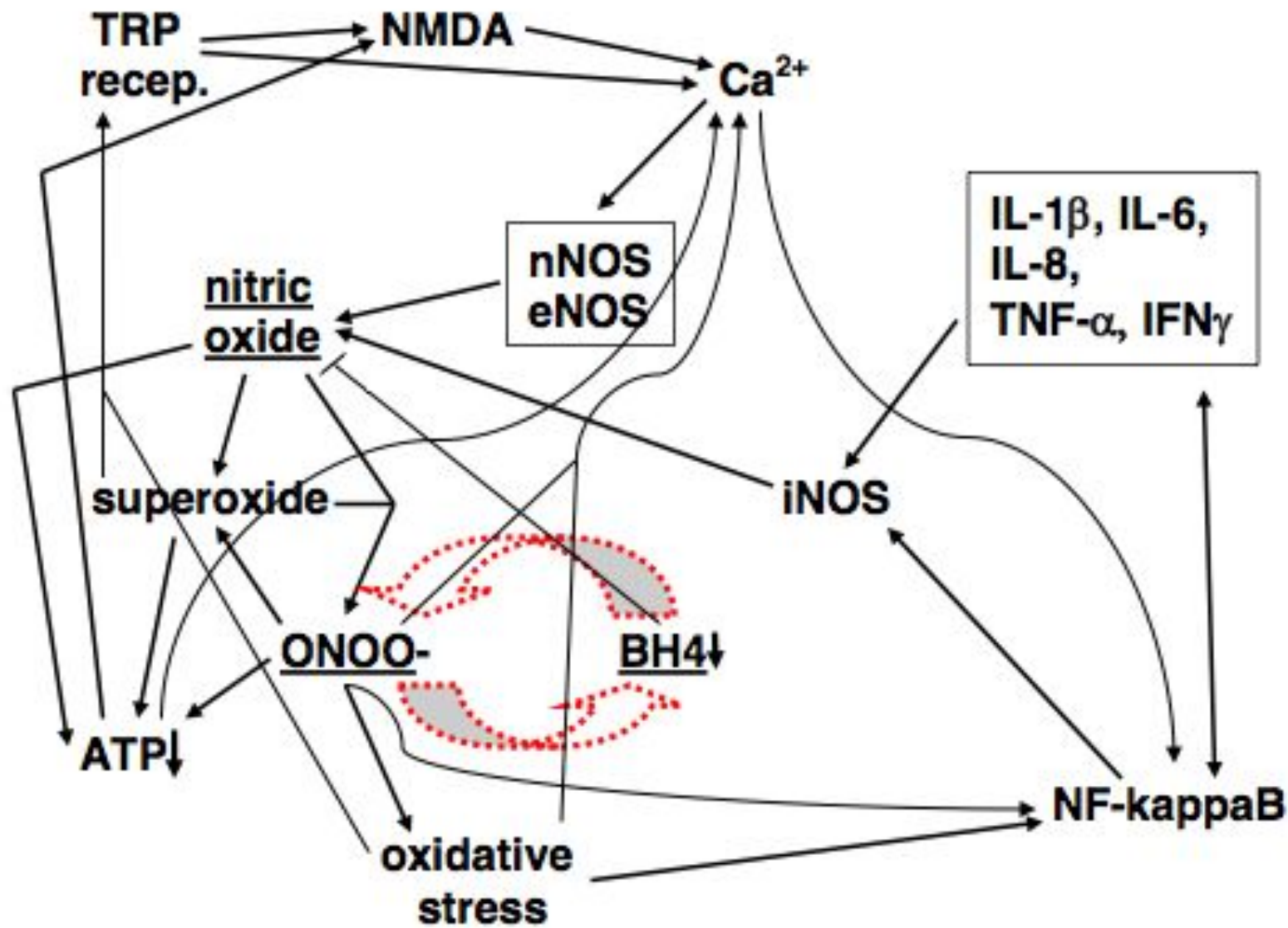
peroxynitrite

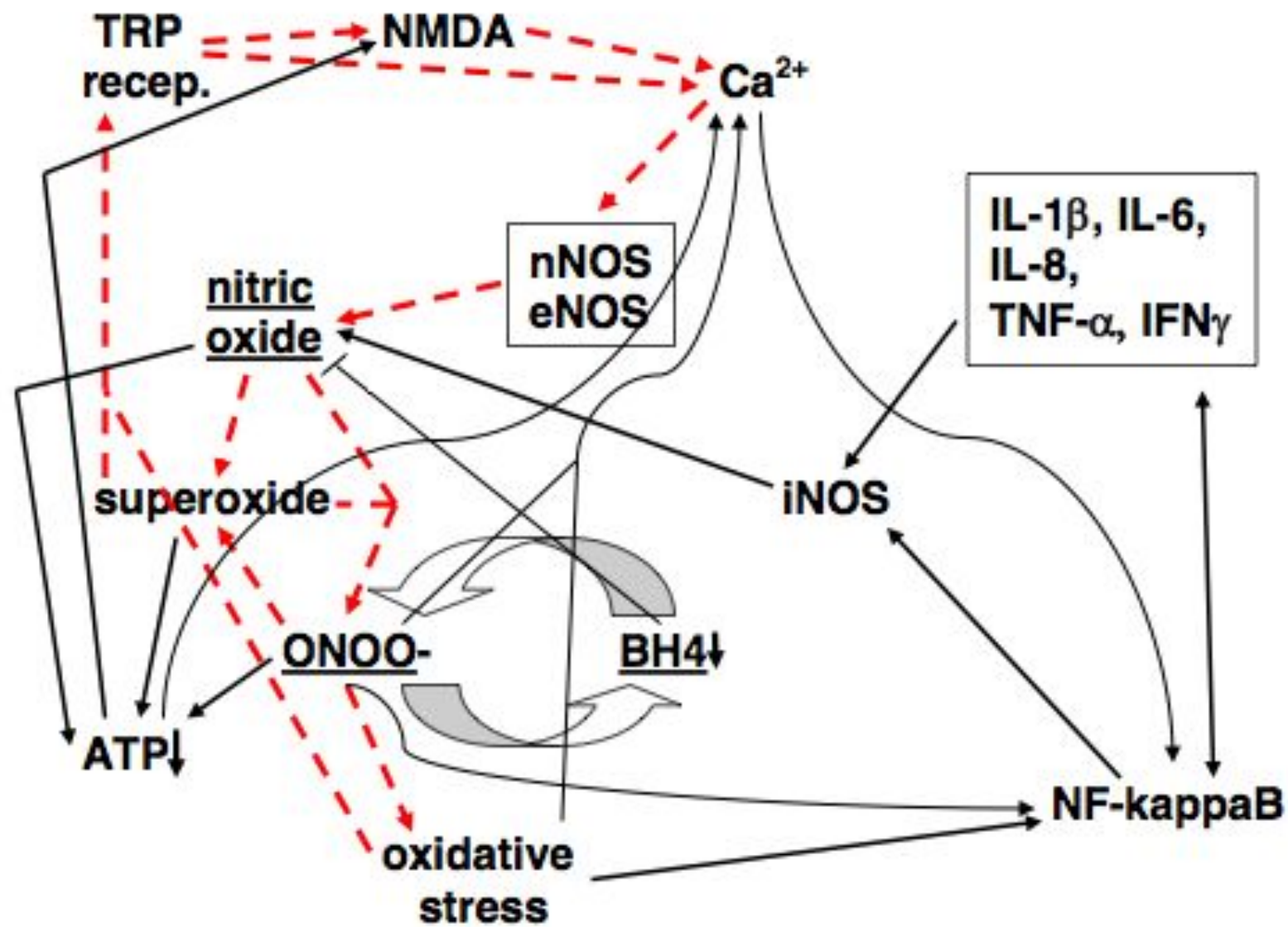


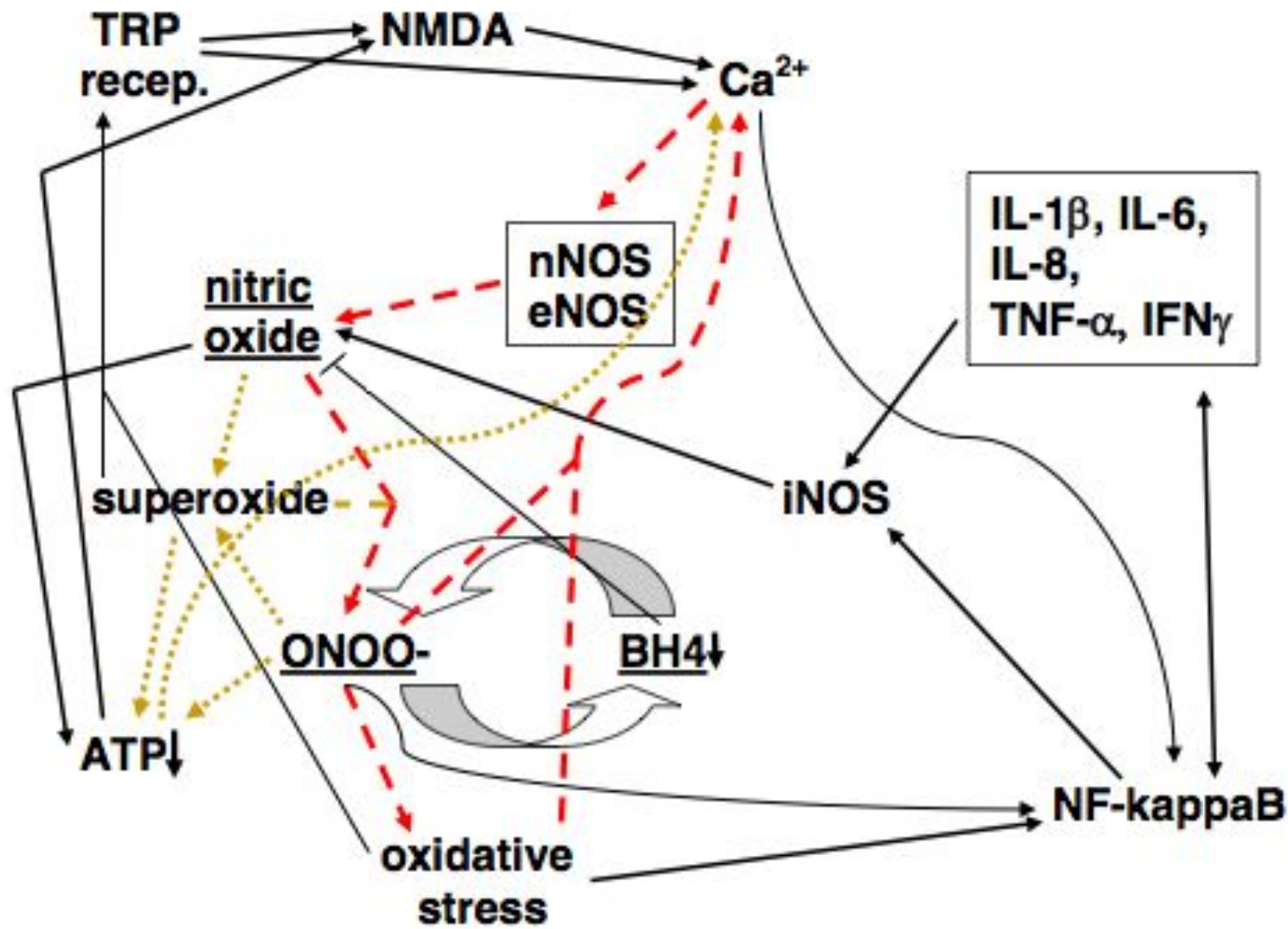












## Five Principles


1. Cases can be initiated by short-term stressors that increase cycle elements.
2. The chronic phase of illness is produced by the NO/ONOO<sup>-</sup> 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<sup>-</sup>, 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<sup>-</sup> cycle diseases should be treated by down-regulating the NO/ONOO<sup>-</sup> cycle biochemistry, rather than by symptomatic relief. In other words, we should treat the cause, rather than the symptoms. In some cases such causation is tested by genetic means rather than through therapy studies.


There are 34 distinct, mechanisms that currently make up the NO/ONOO- cycle models as it was shown in the preceding figures. These are all copied on subsequent slides and are all documented in my pulmonary hypertension, NO/ONOO- cycle review. All 34 of these are documented in the pulmonary hypertension paper.

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.



1. Extremely rapid, diffusion limited reaction between nitric oxide ( $\text{NO}\cdot$ ) with superoxide ( $\text{OO}\cdot^-$ ), forming peroxynitrite ( $\text{ONOO}^-$ ).
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 ( $\text{HO}\cdot$ ), carbonate ( $\text{CO}_3\cdot^-$ ) and  $\text{NO}_2$  radical ( $\text{NO}_2\cdot$ ), 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-1 $\beta$ , 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, acting in some cases via the double headed arrow linking it to NF-kappaB.
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. Large increases in intracellular calcium raise intramitochondrial calcium, which if large, lead to increased superoxide generation in the mitochondria and in some cases to apoptotic cell death.
13. Lowered energy metabolism (decreased energy charge/ATP) also lowers calcium-ATPase activity, leading to increased levels of intracellular calcium.

- 
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.

OK, let's consider how all this may or may not explain the etiology of heart failure. The first principle predicts that stressors initiating cases of heart failure must be able to raise cycle elements. Is this true of heart failure?

**Table 2.** Initiating Stressors in Cases of Heart Failure and NO/ONOO- cycle Elements Raised by Them.

<b>Initiating stressor</b>	<b>Raised NO/ONOO-cycle elements</b>	<b>Citations</b>
Hypertension/pressure overload	Mitochondrial and general oxidative stress, peroxynitrite, superoxide, NF-κB, BH4 depletion	40,57,58,139-141
Mouse mitochondrial superoxide dismutase knockout	Superoxide, oxidative stress	36,37
Doxorubicin	Peroxynitrite, superoxide, oxidative stress, Ca <sup>2+</sup> (particularly in the mitochondrion), NF-κB, iNOS, cytokines TNF-α	11,34,35,62,208
Homocysteine elevation	NMDA activity, NO, peroxynitrite, Ca <sup>2+</sup> , probable BH4 depletion	187-189
Transplantation – severe ischemia-reperfusion	Superoxide elevation, oxidative stress, mitochondrial dysfunction, peroxynitrite	16
Endothelin-1 (ET-1)	Superoxide, iNOS, oxidative stress, Ca <sup>2+</sup>	38,74,209
Ovariectomy	BH4 depletion and oxidation; superoxide	143
Cardiomyocyte-specific NF-κB elevation (transgenic)	NF-κB, cytokine elevation	59
Transgenic calcineurin elevation	Ca <sup>2+</sup> , mitochondrial dysfunction, superoxide	210
Post-viral, autoimmune?	iNOS induction, NO peroxynitrite, inflammatory cytokines,	211,212

Duchenne muscular dystrophy	Ca <sup>2+</sup> , NO, iNOS induction, mitochondrial dysfunction, oxidative stress and elevated levels of several TRPC channels, superoxide	213-215
Endotoxin exposure; sepsis	iNOS, NF-κB, cytokines, superoxide, oxidative stress, Ca <sup>2+</sup> , mitochondrial dysfunction, NO, peroxynitrite	24,75-78, 218-223
Cardiac-specific transgenic iNOS overexpression	iNOS, NO, oxidative stress, mitochondrial dysfunction	44,79,80
Tachypacing	iNOS, BH4 depletion, superoxide, peroxynitrite, Ca <sup>2+</sup> , oxidative stress, mitochondrial dysfunction	144,224-226
Myocardial infarction	Ca <sup>2+</sup> , oxidative stress, mitochondrial dysfunction, iNOS, peroxynitrite, NF-κB	66,208,227
Hypothyroid [216,217]	Oxidative stress, mitochondrial dysfunction, cytokines	228,229
Hyperthyroid [216,217]	Oxidative stress, mitochondrial dysfunction	39,230-233
Chagas disease	Ca <sup>2+</sup> , mitochondrial dysfunction, NO, cytokines, iNOS, oxidative stress, superoxide	234-239
Cytokines (IL-1β, IFNγ & TNF-α)	Cytokines, iNOS, NO, superoxide, peroxynitrite	19

**The fourth principle states that the NO/ONOO(-) cycle is primarily local.**

The primarily local nature of heart failure shows up in the many observable changes to the myocardium, including local “remodeling” changes, local biochemical changes, local inflammatory changes and local changes in gene expression.

The local nature is also strongly suggested by the fact that transgenic mouse models are created by simply changing cardiac- limited genetic activity.

Finally, it is likely that differences between different types of heart failure, i.e. right vs left heart failure, heart failure with and without arrhythmia or with or without valve impact can be easily explained by having different tissue distribution in different cases of a primarily local mechanism.

The second, third and fifth principles are discussed through much of the paper. The second principle states that the elements of the cycle should be elevated. Each of the 12 elements of the cycle are elevated in heart failure, with one possible exception, that of intracellular calcium, where the levels stay high during the heart beat in heart failure except that the very highest levels of intracellular calcium in heart failure are lower than the very highest levels in the normal heart.

The fifth principle states that the elements of the cycle have roles in generating the disease. Here all 12 elements of the cycle have such roles, based on studies of specific pharmacological agents and based in transgenic animal studies. That is even true with intracellular calcium where three calcium receptors, calpains, CaMKII and calcineurin all have important roles in heart failure and in producing specific correlates of heart failure.



The third principle states that the correlates of the disease must be produced by elements of the cycle. There is a very large table in the paper that shows how in the incredible complexity of heart failure, each of the many correlates are produced by elements of the cycle.

**Table 3.** Heart Failure Correlates Produced by NO/ONOO- Cycle Elements

<b>Citation(s)</b>	<b>Cycle element(s)</b>	<b>HF correlate changes produced by cycle element</b>
11	Peroxynitrite and iNOS (both)	MMP activation, lipid peroxidation
13,14	Peroxynitrite, oxidative stress	Tyrosine nitration, oxidation, sulfonylation and consequent inactivation of SERCA2a; lowered rate of relaxation
15	peroxynitrite	Creatine kinase tyrosine nitration and inactivation; lowered energy storage and utilization in the myocardium
18	peroxynitrite	Cardiomyocyte action potential changes; slowed Ca <sup>2+</sup> cycling
20	peroxynitrite	Decreased response to isoproterenol; lessened ability of isoproterenol to increase Ca <sup>2+</sup> transients or shortening; increased Tyr284 nitration on protein phosphatase 2a; produces effect by decreasing Ser16 phosphorylation on phospholamban.
24	peroxynitrite	Produces overall increase in protein-bound 3-NT, oxidative stress, NF-κB elevation, TNF-α elevation
37	Mitochondrial superoxide	Mitochondrial energy metabolism dysfunction
40	Hydrogen peroxide derived from mitochondrial superoxide	Changes in the mitochondrial proteome associated with HF
41	Oxidative stress, probably peroxynitrite	Ventricular remodeling; cavity dilatation and dysfunction

45	Mitochondrial oxidative stress	Oxidative changes in enzymes involved in mitochondrial ATP synthesis; energy metabolism dysfunction
46,50	Mitochondrial oxidative stress	Myocyte hypertrophy, apoptosis, interstitial fibrosis and MMP activation, producing maladaptive cardiac remodeling and failure; oxidative mtDNA damage and lowered mtDNA copy number.
47-49	Mitochondrial oxidative stress	Cardiolipin peroxidation
56	Oxidative stress	Lowered myocardial Akt signaling, increased connective tissue growth factor
102,103	Oxidative stress	Oxidation of heme iron in soluble guanylate cyclase, lowered cGMP synthesis
159,160	Oxidative stress	Protein oxidation of RyR2, causes Ca <sup>2+</sup> leakiness
57	NF-κB	Fibrosis, cardiomyocyte hypertrophy; MMP-2 activation; decreased fractional shortening
58	NF-κB	Fibrosis and associated increased collagen and fibronectin synthesis; increased connective tissue growth factor
59	NF-κB	Myocarditis, inflammatory dilated cardiomyopathy, muscle fiber atrophy; dilated ventricles and atria, strong systolic dysfunction and some diastolic dysfunction
50,61	NF-κB	Cardiac hypertrophy
53-65	NF-κB	Il-1β, TNF-α, IL-6 elevation
56	NF-κB	Systolic dysfunction, lowered chamber remodeling, cytokine expression, fibrosis and apoptosis
58	Cytokines, NO	Lowered contractility
59,70	Cytokine (TNF-α)	Cardiomyopathy
71	Cytokine signaling	Cardiomyocyte mortality, contractile dysfunction, ventricular arrhythmia.
19	Il-1β, TNF-α, IL-6	iNOS, NO, superoxide, peroxynitrite, lowered cardiac function

65	IL-6	Fetal gene expression, cardiomyocyte growth
76	iNOS	Cardiac contractile dysfunction
77	iNOS, NO	TNF- $\alpha$ elevation, oxidative stress, energy metabolism dysfunction
79,44	iNOS, NO	Cardiac hypertrophy, ventricular dilatation, interstitial fibrosis, reactivation of the fetal gene expression; reduced contractility, ejection fraction, and cardiac energetics; up-regulation of peroxiredoxins (a possible protective response)
80	iNOS	Mild inflammatory cell infiltrate, cardiac fibrosis, hypertrophy, dilatation; bradyarrhythmia
81	iNOS, NO	Lowered isoproterenol responsiveness
83	iNOS	Cardiac contractile dysfunction
123	Mitochondrial dysfunction (caused by mtDNA mutation)	Dilated cardiomyopathy
127	Mitochondrial dysfunction	Cardiac hypertrophy, remodeling
130	Mitochondrial Ca <sup>2+</sup> via CaMKII	Mitochondrial transition pore opening; myocyte apoptosis
131	Mitochondrial dysfunction	Lowered cardiomyocyte shortening; aberrant Ca <sup>2+</sup> cycling

132	Mitochondrial dysfunction	Systolic dysfunction; hypertrophy
137	Mitochondrial dysfunction	Lowered ejection fraction
139	BH4 depletion	NOS partial uncoupling, dephosphorylated phospholamban, diastolic dysfunction, impaired relaxation.
140	BH4 depletion (acting via eNOS partial uncoupling)	Fibrosis, myocyte hypertrophy, fetal gene expression, oxidative stress, peroxynitrite, MMP-2/9 activation
141	BH4 depletion	Hypertrophy, fibrosis, NO synthase uncoupling, oxidative stress
144,147	BH4 depletion, iNOS	Both have roles in producing atrial fibrillation and probably cardiomyopathy; NO synthase uncoupling
165-174 (see Table 1)	Ca <sup>2+</sup> stimulating calpain(s)	Calpain(s) partial proteolysis is thought to: degrade dystrophin, myofibrils, gelsolin, sarcolemma proteins; activate TGF-β, caspase-3, apoptosis inducing factor, NF-κB; aggregate talin, α-actinin and vinculin. These, in turn are thought to contribute to: fibrosis and remodeling, apoptosis, necrosis, hypertrophy, right ventricular dysfunction, atrial fibrillation.
175-179 (see Table 1)	Ca <sup>2+</sup> stimulating CaMKII	Phosphorylation of titin springs (contributes to diastolic stress, of Na(V)1.5 (changes action potential, stimulates arrhythmia), of mitochondrial Ca <sup>2+</sup> uniporter (lowers ΔPsi, may stimulate opening of mitochondrial transition pore and apoptosis)
180-184 (see Table 1)	Ca <sup>2+</sup> stimulating calcineurin	NFAT pathway, leading to maladaptive hypertrophy; systolic dysfunction, fetal gene expression, fibroblast growth and fibrosis

185	NMDA	Negative inotropic effects
186	NMDA	Sudden cardiomyopathic death
187-192	NMDA	MMP-9 elevation; decreased cell shortening, maximal contraction and relaxation rate, decay of Ca <sup>2+</sup> transient; raised levels of NO, cytosolic Ca <sup>2+</sup> , calpain activity; cardiac arrhythmia and sudden cardiac death; oxidative stress, mitochondrial dysfunction, NO, cytokines, apoptosis
197	TRPC3/TRPC6	Ventricular tachyarrhythmia
198	TRPC6	Cardiac hypertrophy, calcineurin/NFAT signaling, beta-myosin overexpression, pathologic remodeling
203	TRPC3/ TRPC6/ TRPC4	Pathologic cardiac hypertrophy, calcineurin/NFAT signaling
204	TRPC1	Maladaptive cardiac hypertrophy
205	TRPM4	Arrhythmia

Ten out of 36 cycle mechanisms act to produce mitochondrial dysfunction/lowered ATP:

12. Large increases in intracellular calcium raise intramitochondrial calcium, which if large, lead to increased super oxide generation in the mitochondria and in some cases to apoptotic cell death.

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 19 above, produces increase superoxide production by the electron transport chain.

21. Peroxynitrite, produced from the combination of several mechanisms, 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.
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26. Peroxynitrite, superoxide and nitric oxide all inactivate or inhibit the aconitase enzyme, lowering citric acid cycle activity and subsequent ATP synthesis.



The evidence you have seen shows that heart failure is a NO/ONOO(-) cycle disease:

- Stressors initiating cases of heart failure all act to raise cycle elements!
- Each element of the cycle is elevated and has roles in causing heart failure.
- Among those roles is that each of the complex correlates of heart failure is raised by cycle elements AND each cycle element has roles in causing many different correlates of heart failure!
- Heart failure clearly has a local mechanism – different tissues may be impacted in different cases of heart failure.

In the discussion section of my heart failure paper,  
I discussed two areas in the paper that show roles of two novel things that have not been incorporated into the NO/ONOO- cycle:

The reciprocal relationship between the NO signaling pathway and the peroxynitrite pathway – discussed shortly below.

The role of three calcium receptors as having important roles in heart failure: calpains, CaMKII, calcineurin.

This is not surprising, our models are rarely if ever complete – the real biology is often much more complex.

There is one other consideration of the properties of heart failure which also supports the conclusion that it is a NO/ONOO- cycle disease. In any proposed NO/ONOO- cycle disease, if there other factors which act causally, these factors must be acting as NO/ONOO- cycle elements - this logically follows from the cycle mechanism.

In both heart failure and pulmonary hypertension, both endothelin-1 (ET-1) and RhoA/rho kinase signaling act as causal factors. These were both shown in my pulmonary hypertension paper to act as cycle elements. That is they are both elevated by a number of cycle elements and they act, in turn, to raise cycle elements.

*So here we have specific predictions - that each of these causal factors must acts as cycle elements, and they do!*

Heart failure is the #1 cause of hospitalization in the U.S. and is the #1, 2 or 3 cause of death depending on how this is calculated. The heart failure paper is important for this reason and for several additional reasons.

1. There is a stunning amount of information about heart failure which can be used to test in great detail whether it is a NO/ONOO- cycle disease or not. It has been and provides very strong support for such an etiology.
2. By detailed testing the cycle mechanism in a disease unrelated to the origin of the cycle, one can provide a detailed test of the general mechanism based on completely independent data. In this way, the NO/ONOO- cycle is greatly strengthened not only as the cause of heart failure but also a generic model of chronic inflammatory disease.
3. That detailed consideration of heart failure has revealed some new insights into the cycle.

1. Myalgic encephalomyelitis/Chronic fatigue syndrome (ME/CFS)
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  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
- Asthma
  - Irritable Bowel Syndrome
  - Epilepsy
  - Spinal cord injury (SCI)
  - Pulmonary arterial hypertension (PAH)
  - Glaucoma

One of the things that some of you may have noted, is that I have stated that nitric oxide (NO) is elevated in heart failure and that this elevation has multiple causal roles in the disease. The evidence for this is very clear.

And yet it has been stated over and over again, that not only heart failure but other types of cardiovascular disease are caused by “insufficient bioavailability of nitric oxide.” You should be asking here, these are obviously conflicting views, how can they be resolved?

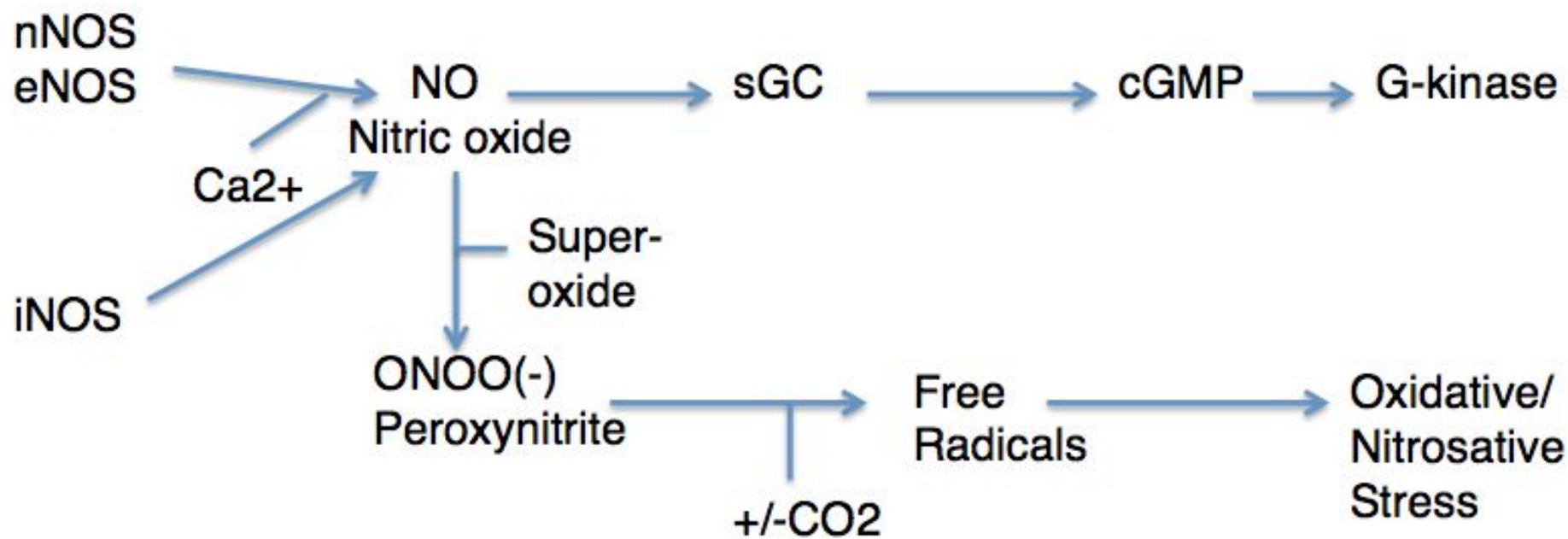
What I argue in the heart failure paper, is that this other point of view is basically a misinterpretation of the observations that NO signaling via cGMP and cGMP-dependent protein kinase helps prevent heart failure & other forms of cardiovascular disease.

But the reason for such low signaling is not a deficiency in NO!

Most responses physiological responses to  $\text{Ca}^{2+}$  and NO act as follows:

NO increasing levels of cGMP, leading in turn to stimulation of the cGMP-dependent protein kinase (protein kinase G).

In contrast, most pathophysiological effects of NO are mediated through its role as a precursor of peroxynitrite ( $\text{ONOO}^-$ ), leading to free radical generation and oxidative stress.





## So how does the peroxynitrite pathway lower NO signaling?

- Oxidants oxidize the heme group on sGC, making it inactive.
- Free radicals produce a thiyl group on sGC, leading to nitrosylation and inactivation.
- Peroxynitrite can cause nitration of a specific tyrosine in Protein Kinase G, leading to inactivation.
- Oxidative stress increases expression of PDE5, leading to increased degradation of cGMP.

## So how does NO signaling act to lower the peroxynitrite pathway??

- It works primarily by raising Nrf2!

## **Conclusion:**

**Each of these two pathways of action of NO inhibits the other, with the peroxynitrite/ oxidative stress pathway acting to lower consequent cGMP signaling.**

**That lowered signaling has been misinterpreted as being due to lowered NO bioavailability, but it is actually due to the action of peroxynitrite/oxidative stress.**

**Agents that increase cGMP levels or protein kinase G activity, and there are a number of them including viagra type agents, can be useful, therefore in the treatment of cardiovascular disease generally and may also be useful in the treatment of other NO/ONOO- cycle diseases.**

➤ Nrf2 is also raised by electroacupuncture (according to 3 2015 studies), by BRCA1 and by APOE

➤ Nrf2 may be nature's way of avoiding and/or treating NO/ONOO(-) cycle diseases.

Nrf2 lowers the three most central parts of the cycle: Oxidative/nitrosative stress; inflammation; and mitochondrial dysfunction.

There is some evidence in our Nrf2 paper that it also lowers certain other aspects of the cycle.

Healthful diets  
incl. Traditional  
Mediterranean  
& Okinawan

Probable  
Paleolithic  
Diet

Many  
Modern  
Diets

Many phenolic  
antioxidants  
Isothiocyanates  
Allium sulfur  
compounds  
DHA, EPA  
(Omega-3s)  
Carotenoids  
Terpenoids  
 $\gamma, \delta$ -tocopherols,  
tocotrienols

Other health promoting  
factors:

- Low level oxid.  
stress (hormesis)
- Exercise
- Calorie restriction

→ **Nrf2** ↑

Transcriptional  
stimulation  
~500 genes

Antioxidant proteins  
Mitochondrial biogenesis/  
energy metabolism  
Detoxification, carbon-cont.  
toxicants, toxic metals  
Autophagy  
Lowered inflammation

Possible Increased  
Lifespan & Healthspan

Chronic diseases of oxid.  
stress, inflammation,  
mitochondrial dysfunction  
including NO/ONOO(-)  
cycle diseases



Pall and Levine 2015 Nrf2, a master regulator of detoxification and also antioxidant, anti-inflammatory and other cytoprotective mechanisms, is raised by health promoting factors. Acta Physiologica Sinica February 25, 2015, 67(1): 1–18

## **Why do I think that Nrf2 is such an important regulatory system in our bodies. It is for two main reasons:**

1. Nrf2 occurs in all types of metazoa and has therefore been around for perhaps a billion years of evolution. Equally important is that when one looks at the stunning coordination of the complex protective biochemical mechanisms, particularly the antioxidant mechanisms and the detoxification mechanisms, it is clear that Nrf2 is a highly evolved system. The only way that can happen is for it to be a major target of natural selection, telling us that it is very important for the survival and reproduction of the species.
2. When one sees that it lowers oxidative stress, inflammation and improves mitochondrial function and that these each have important roles in many chronic diseases, these argue for a system of great importance

Because inflammation, oxidative stress and mitochondrial dysfunction are so important to the NO/ONOO(-) cycle suggests that Nrf2 may be nature's way of avoiding and/or treating NO/ONOO(-) cycle diseases.

That is also suggested by a few studies suggesting that raising Nrf2 also ameliorates the effects of three other cycle elements: NMDA activity, excessive  $[Ca^{2+}]_i$ , and BH4 depletion.

These considerations suggest an additional important connection. The list of 23 diseases that I suggest may be NO/ONOO(-) cycle diseases may be too conservative. There are many other diseases which are prevented and/or treated by raising Nrf2 in animals and/or humans and consequently each of these may be good candidates to be NO/ONOO(-) diseases.

Before leaving the topic of cycle diseases, I wanted to discuss some important new (at least to me) observations regarding Alzheimer's and Parkinson's diseases, which were proposed to the NO/ONOO(-) cycle diseases in Chapter 14 of my book. The 12 elements of the cycle are all elevated in these two diseases and various stressors that are reported to act as risk factors for them can raise cycle elements.

One of the important predictions of the NO/ONOO(-) cycle is that any causal factor for a cycle-caused disease must act as a NO/ONOO(-) cycle element.

It is important, therefore, to ask whether the amyloid-beta protein (A-beta), which acts as causal factor for Alzheimer's through the formation of small aggregates and also alpha-synuclein aggregates which also acts as a causal factor for Parkinson's disease, both function as NO/ONOO(-) cycle elements?

Both do – that is they are raised by cycle elements and they both raise cycle elements.

A-beta has been shown to be raised by both NF-kappaB and by  $[Ca^{2+}]_i$  (intracellular calcium). Furthermore, small aggregates of A-beta insert themselves into the plasma membranes of cells and act as calcium channels, allowing calcium ions to flow into the cell, thus increasing  $[Ca^{2+}]_i$ .

The formation of alpha-synuclein aggregates from the protein monomers has been shown to be greatly increased by oxidation and nitration (both caused by peroxynitrite products). And alpha-synuclein aggregates have been shown to greatly lower Nrf2 activity, in this way raising essentially the entire NO/ONOO(-) cycle.

Both of these observations, then provide strong support for the view that these two neurodegenerative diseases are NO/ONOO(-) cycle diseases.



## Summary:

Heart failure has been tested in many specific ways and shown to be an NO/ONOO(-) cycle disease.

This argues that the NO/ONOO(-) cycle is a major paradigm of human disease and provides further strengthens the case that 22 other diseases may also be NO/ONOO(-) diseases.

There are specific observations, regarding the roles of amyloid-beta small aggregates in Alzheimer's disease and of alpha-synuclein aggregates in Parkinson's disease that further strengthens the case that these are both NO/ONOO(-) cycle diseases.

Nrf2 is an amazing regulatory protein whose activity is raised by many health-promoting factors and which acts to prevent or treat many different chronic diseases.

Nrf2 lowers oxidative stress and inflammation and also improves mitochondrial function, thus lowering much of the NO/ONOO(-) cycle.

Nrf2 apparently acts in extending both lifespan and healthspan.

Nrf2 may be nature's way of preventing and/or treating NO/ONOO(-) cycle diseases.