A Study of Sauna Therapy in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome Patients Shows Sauna Action via Raised Tetrahydrobiopterin and Confirms Three Predictions of the NO/ONOO- Cycle

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Abstract:

A series of myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) patients have been studied to test two previous predictions: 1. Sauna therapy acts, at least in part, by raising the availability of tetrahydrobiopterin (BH4) and 2. ME/CFS is caused by a biochemical vicious cycle mechanism known as the NO/ONOO- cycle. Sauna therapy is shown here to increase BH4 synthesis, acting by raising the rate limiting enzyme in BH4 synthesis, GPCH. This confirms, then, the first prediction. ME/CFS patients have very high levels of peroxynitrite as measured by the peroxynitrite marker 3-nitrotyrosine, averaging 5.43 times the levels of healthy controls with no overlap with those controls and also have low levels of BH4, confirming predictions of the NO/ONOO- cycle mechanism. Sauna treatment, presumably acting via increased BH4 availability, lowers peroxynitrite levels, again in agreement with prediction. Sauna treatment also lowers the very high levels of neopterin found in these patients, possibly by stimulating the signaling of the IL-1β cytokine. Four important conclusions can be drawn here. Sauna therapy does not act solely via detoxification, as has been widely assumed but acts, at least in part, by raising BH4 levels. Secondly, three important predictions of the NO/ONOO- cycle mechanism are confirmed for the first time in ME/CFS patients, providing support for this mechanism as the etiologic mechanism of this disease. Thirdly, three biochemical parameters are partially normalized by sauna treatment, BH4 levels, peroxynitrite levels and neopterin levels, suggesting that ME/CFS is fundamentally biochemical in nature. The very high, nonoverlapping levels of 3-nitrotyrosine (marker for peroxynitrite) in ME/CFS patients as compared with healthy controls, suggests that this may be a very useful objective test for severity in ME/CFS patients.

Introduction

This paper is focused on two interconnected series of predictions. The first is that sauna therapy works, at least in part, by raising the levels of tetrahydrobiopterin (BH4).[1] The second is that in studying the effect of sauna therapy on the biochemistry of human patients we can test not only the this first prediction but also a series of other biochemical changes that may be supportive of a biochemical vicious cycle mechanism thought to cause many chronic inflammatory diseases, called the NO/ONOO- cycle.[2-7] Let's consider these briefly. Sauna therapy has often been assumed to act entirely by a process of detoxification, acting to lower the levels of stored toxicants in the body.[8-18] While there is some published evidence that sauna therapy does lead to increased excretion of toxicants[14,18] and therefore some detoxification over a period of weeks, there is no evidence, to our knowledge that this is the sole or even primary mechanism leading to improvement in symptoms in response to sauna therapy.

In contrast to this view, it has been proposed that sauna therapy may act, to a substantial extent by raising the levels of BH4.[1] The evidence supporting this view (reviewed earlier[1]) is that:

1. Sauna therapy is reported to be helpful in the treatment of several diseases proposed to involve, among other things, BH4 depletion, namely multiple chemical sensitivity, fibromyalgia and myalgic encephalomyelitis/chronic fatigue

syndrome (ME/CFS) and also several diseases where BH4 depletion has been shown to play an important causal role, namely hypertension, vascular endothelial dysfunction and heart failure.

2. Sauna therapy can act via two distinct mechanisms to raise the levels of BH4, acting in both cases by raising the levels of the enzyme GTP cyclohydolase I (GPCH), the first and rate limiting enzyme in the *de novo* synthesis pathway for BH4. These two mechanisms are the stabilization of GPCH by the heat shock protein Hsp90 and the induction of GPCH produced by increased peripheral blood flow.
3. Both of the mechanisms listed in 2 above have been shown to lower uncoupling of eNOS nitric oxide synthase and the only known mechanism for such lowered uncoupling is to raise BH4 availability.

These two views of the action of sauna therapy, detoxification vs raising BH4 levels, are not mutually exclusive and are not presented as such here – both may have roles.

In this study of a series of ME/CFS patients, we show that:

1. Sauna therapy acts to raise the biosynthesis of BH4 by raising the levels of GPCH but that much of this BH4 can be subsequently oxidized to BH2 and biopterin, showing that there is still a lot of oxidative stress going on in these patients following sauna therapy.
2. Two NO/ONOO- cycle elements that have not previously been measured in ME/CFS patients are both elevated in these patients, notably peroxynitrite (measured through the marker 3-nitrotyrosine (3-NT)) and the depletion of BH4.
3. Sauna therapy, acting in part via raising of BH4 levels, can act to partially normalize the biochemistry in ME/CFS patient, raising BH4 levels, lowering 3-NT levels and also greatly lowering the levels of neopterin.
4. This partial normalization of the biochemistry following sauna therapy suggests that the etiology of ME/CFS is biochemical in nature and specifically provides confirmation for certain parts of what is called the NO/ONOO- cycle.

Materials and Methods:

17 patients (12 female and 5 male) who each met the Fukuda[19] criteria for myalgic encephalomyelitis/chronic fatigue syndrome were studied before and after 4 sauna treatments, given at two day intervals, with fasting, venous blood samples and urine samples taken before the first and immediately after the last sauna treatment. Sauna treatments were for 50 minutes each, 122 to 125 degrees F in a far infrared sauna. Of these 17 patients, 15 had been previously been diagnosed as having Lyme disease, but had been treated with antibiotics and had been apparently Borrelia-free for the preceding six months at the time of study. They still suffered from ME/CFS, as indicated above.

Blood for biochemical analysis was obtained from fasting venous samples. For analysis of plasma levels of biopterin, dihydrobiopterin (BH2) and tetrahydrobiopterin (BH4), we used reversed phase HPLC with electrochemical detection and fluorescence detection. Detailed methods were previously described by Cai et. al.[20]

For the 3-NT assay, a cold vacutainer tube (7 ml) containing EDTA, PMSF and a proprietary stabilizer, obtained from Health Diagnostics Research Institute, South Amboy, NJ .was used to draw blood and after mixing was placed on ice immediately. After centrifugation the plasma was used for analysis. Samples were derivatized by the method of Crowley et al [21] and then assayed for 3-NT as described by Schwedhelm et al.[22]

GTP cyclohydrolase I (GPCH), in lymphocytes isolated from blood, was assayed by the method of Werner et al.[23]

Each of these assays, as well as assays for neopterin are available from Health Diagnostics Research Institute, South Amboy, NJ for clinical measurements and for experimental studies.

Results and Conclusions:

The levels of various biochemical parameters were measured before and after sauna therapy in ME/CFS patients, as shown in Table 1, including that of biopterin, (Biop in the urine, columns 2&3), dihydrobiopterin (BH2, columns 4&5), tetrahydrobiopterin (BH4, columns 6&7), the enzyme GTP cyclohydrolase I in lymphocytes (GPCH, columns 8&9), 3-nitrotyrosine (3-NT, columns 10&11), and neopterin in the urine (Neo, columns 12&13). Let's examine columns 2-9 first. It can be seen that Biop, BH2, BH4 and GPCH all show highly statistically significant increases following sauna therapy [all $p<0.0001$, using a paired t-test for statistical analysis (Table 1)]. To analyze these further, it is important to consider the specific treatment protocol used. As was noted above in the preceding section, it involved four sauna treatments at two day intervals with blood and urine samples taken before the first sauna treatment and immediately after the last sauna treatment. However, since the last sauna treatment had just occurred immediately before the final samples were taken, it is unlikely that it had time to have much impact on these final parameters. It follows that there would have been 2 to 6 days between the previous three treatments and the taking of the final samples. This 2 to 6 day period means that if there is a lot of oxidative stress that is still occurring in these patients, there will be much time to oxidize the end product of the pathway (BH4) to its oxidation products, BH2 and biopterin. It seems clear that this is what happened in this study such that there are even larger % increases in BH2 levels and in biopterin levels than in BH4 levels after sauna treatment. Because BH4 is the end product of the biosynthetic pathway, it must be the case that the increased BH2 and biopterin reflect an increase in BH4 biosynthesis. This interpretation is also supported by the increase in GPCH following sauna treatment, the first and rate-limiting enzymatic step in the de novo pathway for BH4 synthesis ($P<0.0001$).

It should be useful to compare the values of BH4 and 3-NT before sauna therapy in these ME/CFS patients with those of healthy controls. Here we use values of 111 healthy controls, of widely variable ages, ranging from 18 to 73. The BH4 levels of healthy

controls were 1.82 +/- 0.51 as compared values of the ME/CFS patients levels of 1.29 +/- 0.192, ($p<0.0001$, non-paired t-test), (data presented as mean +/- standard deviation). The values from 3-NT for healthy controls were 4.29 +/- 1.72 as compared with 23.3 +/- for the ME/CFS patients (5.43 times higher). The levels of 3-NT for the 111 healthy controls were completely non-overlapping, with the highest value for the 111 healthy controls being 6.88 as compared with the lowest value for the ME/CFS patients being 15.6 or over 2 ¼ times as high. This is quite a striking difference. Because the probability of each ME/CFS 3-NT value being above the total range of healthy controls by chance being less than $1/100^{th}$, the probability of all 17 of these being above the health control range simply by chance can be estimated as $p<10^{-34}$.

In summary, then, these data confirm three important predictions of the NO/ONOO- cycle mechanism is, as proposed, it is the central etiologic mechanism for ME/CFS: BH4 levels are predicted to be low in ME/CFS patients, peroxynitrite levels are predicted to be high and that a treatment that raises BH4 levels, notably sauna treatment, lowers peroxynitrite levels. However, it seems clear that peroxynitrite levels are still highly elevated after sauna treatment, and this is presumably a key part of the mechanism causing increased BH4 synthesis to show up mainly as the oxidation products, BH2 and biopterin. They also support the theory that this cycle is the central etiologic mechanism of ME/CFS.

There are two additional comparisons that need to be discussed from these data. Total biopterin in plasma is composed primarily of BH4 and BH2, with relatively little of the fully oxidized product,biopterin being present because biopterin is fairly rapidly excreted into the urine. That is why the biopterin measurements reported here are all urine measurements. It follows from this that total biopterin, as shown in Table 2 can be compared with BH4 plus BH2 levels shown in Table 1. Such a comparison shows that all of the BH4 plus BH2 values for the 17 patients before sauna therapy are within the normal reference range, showing that BH4 biosynthesis is normal and that the low BH4 levels are caused by BH4 oxidation (presumably by peroxynitrite). This is confirmed by the observation that 6 out of 17 patients have BH4/BH2 ratios below the reference range. Surprisingly, however, 2 out of 17 patients have BH4 /BH2 ratios above the reference range. When one studies the BH4 + BH2 values after sauna treatment, there is a great increase, such that 12 of 17 patients are above the reference range and only patients 8, 10, 13, 15 and 17 are within the reference range and these are all in the upper half of that range (compare Table 1 and 2). Thus there is a striking increase in BH4 synthesis following sauna therapy leading to a striking increase in total biopterin.

The most surprising result seen in Table 1 is that the elevated neopterin levels in ME/CFS patients are greatly reduced following sauna therapy. An understanding of the mechanism of neopterin generation is needed here in order to interpret these results. Under inflammatory conditions, several inflammatory cytokines induce both the inducible nitric oxide synthase (iNOS, see Fig. 1) and also GPCH, with the GPCH induction providing the increased BH4 needed to prevent widespread uncoupling of the iNOS enzyme.[23-25] Such widespread uncoupling would lead to the production of large amounts of superoxide by that enzyme[7]. However in primates there is little induction of

the second enzyme in the pathway, pyruvoyl tetrahydropterin synthase (PTPS) [23-25]. This leads to accumulation of the second intermediate in the pathway, dihydroneopterin triphosphate which is degraded to neopterin which is then excreted in the urine [23-25]. It is surprising, therefore, that sauna therapy which we show leads to increased levels of GPCH also leads to lowered neopterin rather than raised neopterin. There is only one logical explanation for this finding. This is that sauna therapy must act to produce a large increase in the second enzyme in the pathway, PTPS, as well, leading to increased metabolism of the dihydroneopterin triphosphate in the BH4 biosynthetic pathway and therefore decreased generation of neopterin. So the question is whether there is a mechanism by which sauna therapy can lead to increased PTPS? The answer is that there is.

The cytokine IL-1β has been shown to induce PTPS but not GPCH [26], unlike other cytokines and the action of IL-1β has been shown to be greatly stimulated by the heat shock protein Hsp90 [27-29] which is induced by the heat of sauna therapy. The mechanism of stimulation of IL-1β action is though the stimulation of the action of its receptor [30]. It should be noted that we have no independent evidence that this is the mechanism of sauna therapy in raising PTPS activity, but it would be surprising if there were a second such mechanism by which sauna treatment raises PTPS activity. In any case, the raising of PTPS activity provides a second mechanism by which sauna therapy raises BH4 synthesis, since lowering the degradative loss of neopterin triphosphate in the BH4 biosynthetic pathway, will, of course, lead to increased biosynthesis of BH4.

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1. Pall ML Do sauna therapy and exercise act by raising the availability of tetrahydrobiopterin? Medical Hypoth 2009;73:610–613.
2. Pall ML. Pulmonary hypertension is a probable NO/ONOO- cycle disease: A review. ISRN Hypertension 2013: Article ID 742418, 27 pages.
3. Pall M. L. 2007 "Explaining 'Unexplained Illness': Disease Paradigm for Chronic Fatigue Syndrome, Multiple Chemical Sensitivity, Fibromyalgia, Post-Traumatic Stress Disorder, Gulf War Syndrome and Others", 16 Chapter book, Harrington Park (Haworth) Press.
4. Pall M. L. 2010 The NO/ONOO- Vicious Cycle Mechanism as the Cause of Chronic Fatigue Syndrome/Myalgic Encephalomyelitis, In Chronic Fatigue Syndrome: Symptoms, Causes and Prevention, Edita Svoboda and Kristof Zelenjcik, eds., Nova Publishers, pp 27-56.
5. Pall ML. Elevated, sustained peroxynitrite levels as the cause of chronic fatigue syndrome. Med Hypotheses 2000;54:115-125.

6. Pall M. L. 2009 Multiple chemical sensitivity: Toxicological questions and mechanisms. In General and Applied Toxicology, 3rd Edition, John Wiley & Sons, pp. 2303-2352.
7. Pall ML. Nitric oxide synthase partial uncoupling as a key switching mechanism for the NO/ONOO- cycle. Med Hypotheses 2007;69:821-825.
8. Gibson PR, Elms AN-M, Ruding LA. Perceived treatment efficacy for conventional and alternative therapies reported by persons with multiple chemical sensitivity. Environ Health Perspect 2003;111:1498–504.
9. Rea WJ. Chemical sensitivity. Boca Raton, FL: Lewis Publishers; 1992.
10. Crinnion W. Components of practical clinical detox programs – sauna as atherapeutic tool. Altern Ther Health Med 2007;13:S154–6.
11. Baird DN, Rea WJ. The temperomandibular joint implant controversy. Part II: its clinical implications. J Nutr Environ Med 1999;9:209–222.
12. Rogers SA. Detoxify or die. Syracuse, New York: Prestige Publishers; 2002.
13. Krop J. Chemical sensitivity after intoxication at work with solvents: response to sauna therapy. J Altern Complement Med 1998;4:77–86.
14. Genuis SJ, Birkhloz D, Rodushkin I, Beesoon S. 2011 Blood, usine, sweat (BUS) study: monitoring and eliminating bioaccumulated toxic elements. Arch Environ Contamination Toxicol 61:344-357.
15. Cecchini M, LoPresti V. Drug residues store in the body following cessation of use: impacts on neuroendocrine balance and behavior – use of Hubbard sauna regimen to remove toxins and restore health. Medical Hypoth 2007;68:868-879.
16. Cecchini MA, Root DE, Rachunow JR, Gelb PM. Chemical exposures at the World Trade Center: use of Hubbard detoxification regimen to improve health status of New York City rescue workers exposed to toxicants. Townsend Letter 2006;273:58-65.
17. Ross GH, Sternquist MC. Methamphetamine exposure and chronic illness in police officers: significant improvement with sauna-based detoxification therapy. Toxicol Ind Health 2012; 28:758-768.
18. Parpaleĭ IA, Prokof'eva LG, Obertas VG. [The use of the sauna for disease prevention in the workers of enterprises with chemical and physical occupational hazards].[Article in Russian] Vrach Delo. 1991 May;(5):93-95.
19. Fukuda et al. The chronic fatigue syndrome: a comprehensive approach to its definition and study. International Chronic Fatigue Syndrome Study Group. Ann Intern Med 1994;121:953-959.
20. Cai S, Alp, NJ, McDonald D., et al. GTP cyclohydrolase I gene transfer augments intracellular tetrahydrobiopterin in human endothelial cells: effects on nitric oxide synthase activity, protein levels and dimerisation. Cardiovascular Research 2002;55; 838-849.
21. Crowley JR, Yarasheski K, Leeuwenburgh C, Turk J, and Heinecke JW Isotope dilution mass spectrometric quantification of 3-nitrotyrosine in proteins and tissues is facilitated by reduction to 3-aminotyrosine. Anal Biochem 1998;259, 127-135.
22. Schwedhelm E, Tsikas D, Gurzke FM Frolich J. Gas chromatographic-tandem mass spectrometric quantification of free 3-nitrotyrosine in human plasma at the basal state. Anal Biochem 1999;276:195-203.

23. Werner ER, Werner-Felmayer G, Fuchs D, et al. Tetrahydrobiopterin biosynthetic activities in human macrophages, fibroblasts, THP-1, and T 24 cells. GTP-cyclohydrolase I is stimulated by interferon-gamma, and 6-pyruvoyl tetrahydropterin synthase and sepiapterin reductase are constitutively present. J Biol Chem 1990; 265:3189-3192.
24. Werner ER, Werner-Felmayer G, Wachter H. Tetrahydrobiopterin and cytokines. Proc Soc Exp Biol Med 1993;203:1-12.
25. Wachter H, Fuchs D, Hausen A, et al. 1992 Neopterin Biochemistry—Methods—Clinical Application. Berlin: Walter De Gruyter.
26. Franscini N, Blau N, Walter RB, Schaffner A, Schoeden G. 2003 Critical role of interleukin-1beta for transciptional regulation of endothelial 6-pyruvoyltetrahydropterin synthase. Anterioscler Thromb Vasc Biol 23:e50-e53.
27. Shi L, Zhang , Fang S, et al. 2009 Heat shock protein 90 (Hsp90) regulates the stability of transforming growth factor beta-activated kinase 1 (TAK1) in interleukin-1beta-induced cell signalling. Mol Immunol 46:541-550.
28. Rice JW, Veal JM, Fadden RP, et al. 2008 Small molecule inhibitors of Hsp90 potently affect inflammatory disease pathways and exhibit activity in models of rheumatoid arthritis. Arthritis Rheum 58:3765-3775.
29. Lukart SD, Panoskaltis-Mortari A, Hinkel T, et al. 2009 Mactinin, a fragment of cytoskeletal alpha-actinin, is a novel inducer of heat shock protein (Hsp-90) mediated monocyte activation. BMC Cell Biol 2009 Aug 28;10:60.
30. De Nardo D, Masendycz P, Ho S, et al. 2005 A central role for the Hip90-Cdc37 molecular chaperone module in Interleukin-1 receptor-associated-kinase-dependent signalling by toll-like receptors. J Biol Chem 280:9813-9822.

Table 1. Biochemical Parameters Before and After Sauna Treatments

Patient #	Biop	Biop aft.	BH2	BH2 aft	BH4	BH4 aft	GPCH	GPCH aft	3-NT	3-NT aft	Neo	Neo-aft
1	0.48	2.66	1.06	4.3	1.34	1.45	4.37	4.81	21.6	17.3	6.32	1.28
2	0.53	4.67	1.11	3.84	1.09	1.22	2.76	3.12	37.5	31.8	4.39	1.01
3	0.46	4.21	1.26	3.47	1.2	1.39	3.01	3.64	16.9	13.8	3.76	0.98
4	0.51	5.8	1.32	2.91	1.13	1.36	2.92	3.45	21.5	19.2	5.62	1.69
5	0.84	1.67	1.27	4.62	1.2	1.31	2.6	2.82	17.7	13.1	5.81	1.41
6	1.06	2.12	1.04	3.02	1.11	1.3	3.26	3.82	19.3	17.8	7.07	1.84
7	0.93	5.91	1.13	3.9	1.2	1.56	2.77	3.63	15.6	12	6.84	2.11
8	0.84	2.16	1.06	2.07	1.12	1.03	1.92	1.91	32.5	25.7	6.63	0.93
9	0.47	0.88	1.13	3.57	1.13	1.32	2.08	2.4	27.5	22.5	5.93	1.04
10	0.56	3.53	0.93	2.06	1.3	1.55	2.17	2.6	19.3	17.7	5.7	1.21
11	0.82	0.44	0.86	2.81	1.42	1.55	1.84	1.99	18.5	17.2	5.66	1.24
12	0.67	1.38	0.94	2.06	1.87	1.8	1.7	1.82	21.3	15.5	5.76	1.2
13	0.73	1.58	1.12	1.93	1.25	1.57	2.62	3.23	32.7	27.4	7.22	2.31
14	0.94	4.12	1.34	2.17	1.36	1.74	2.59	3.28	27.8	26.7	4.27	0.77
15	1.07	1.44	0.97	1.63	1.49	1.86	1.97	2.4	16.7	12.8	5.84	1.57
16	0.73	0.82	1.11	1.92	1.31	1.82	3.62	4.14	23.7	19.4	5.68	4.5
17	0.81	0.87	1.29	1.38	1.4	1.49	3.37	3.48	27.2	21.6	5.98	5.02
Mean	0.732	2.603	1.114	2.803	1.28	1.489	2.68	3.090	23.3	19.5	5.79	1.771
paired t-test		p< .0001		p< .0001		p< .0001		p< .0001		p< .0001		p< .0001

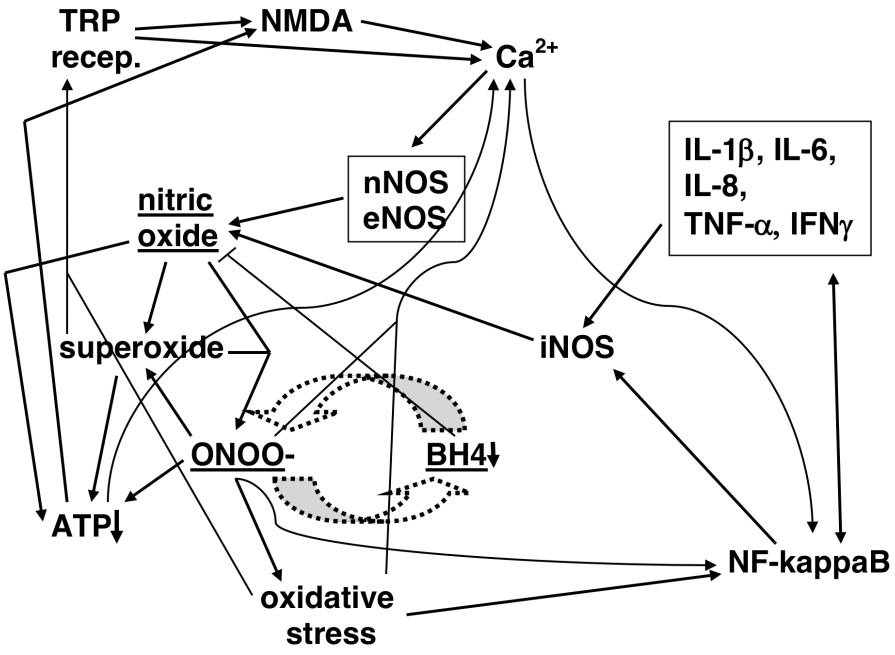
Biop = biopterin in urine, μg/mg creatinine; BH2 = dihydrobiopterin in plasma, ng/ml; BH4=tetrahydrobiopterin in plasma, ng/ml; GPCH = GTP cyclohydrolase I in lymphocytes, ; 3-NT=3-nitrotyrosine in plasma, ng/ml; Neo=neopterin in urine, μg/mg creatinine.

Table 2. Normal Reference Range for Biochemical Parameters

Total biopterin in plasma (ng/ml)	1.84-3.70
Dihydrobiopterin (BH2) in plasma (ng/ml)	1.16-2.49
Tetrahydrobiopterin (BH4) in plasma (ng/ml)	1.24-2.93
BH4/BH2 ratio	1.05-1.31
Biopterin (urine, μg/mg creatinine)	0.05-1.54
Neopterin (urine, ng/ml)	0.08-2.70
3-nitrotyrosine (3-NT) in plasma (ng/ml)	1.1-6.88

Fig.1 NO/ONOO- Diagram

Each arrow represents one or more mechanisms by which one element of the cycle increases another. The various elements include: nitric oxide (NO), central upper left, superoxide, central left, peroxynitrite (ONOO-), central lower left, oxidative stress (an imbalance between oxidants and antioxidants) bottom central, NF-kappaB, an important transcription factor that stimulates transcription of many genes (lower right corner), including the inflammatory cytokines (upper right box) and iNOS, central, slightly right, intracellular calcium (Ca2+) top, a cascade of events leading to mitochondrial dysfunction and lowered APTY pools (lower left corner), excessive activity of the NMDA receptors (top, left central), lowered levels of tetrahydrobiopterin (BH4, center slightly right) and increased of activity of several of the TRP family of receptors. The two arrows that are most relevant to this study are the dashed arrows linking peroxynitrite (ONOO-) with BH4 depletion. The right pointing arrow mechanism is extensively documented and involves peroxynitrite oxidation of BH4; the left pointing arrow must be viewed, however as somewhat speculative and it is important, therefore, that this study provides some evidence supporting this arrow.


TRP
recep.
NMDA
Ca2+
nNOS
eNOS
IL-1β, IL-6,
IL-8,
TNF-α, IFNγ
nitric
oxide
superoxide
iNOS
ONOO-
BH4
ATP
NF-kappaB
oxidative
stress