Oxidative and Nitrosative Stress and Immune-Inflammatory Pathways in Patients with Myalgic Encephalomyelitis (ME)/Chronic Fatigue Syndrome (CFS)

Gerwyn Morris[1] and Michael Maes[2,3,*]

[1]*Tir Na Nog, Pembrey, Llanelli, UK;* [2]*Department of Psychiatry, Chulalongkorn University, Bangkok, Thailand;* [3]*Department of Psychiatry, Deakin University, Geelong, Australia*

Abstract: Myalgic Encephalomyelitis (ME) / Chronic Fatigue Syndrome (CFS) has been classified as a disease of the central nervous system by the WHO since 1969. Many patients carrying this diagnosis do demonstrate an almost bewildering array of biological abnormalities particularly the presence of oxidative and nitrosative stress (O&NS) and a chronically activated innate immune system. The proposal made herein is that once generated chronically activated O&NS and immune-inflammatory pathways conspire to generate a multitude of self-sustaining and self-amplifying pathological processes which are associated with the onset of ME/CFS. Sources of continuous activation of O&NS and immune-inflammatory pathways in ME/CFS are chronic, intermittent and opportunistic infections, bacterial translocation, autoimmune responses, mitochondrial dysfunctions, activation of the Toll-Like Receptor Radical Cycle, and decreased antioxidant levels. Consequences of chronically activated O&NS and immune-inflammatory pathways in ME/CFS are brain disorders, including neuroinflammation and brain hypometabolism / hypoperfusion, toxic effects of nitric oxide and peroxynitrite, lipid peroxidation and oxidative damage to DNA, secondary autoimmune responses directed against disrupted lipid membrane components and proteins, mitochondrial dysfunctions with a disruption of energy metabolism (e.g. compromised ATP production) and dysfunctional intracellular signaling pathways. The interplay between all of these factors leads to self-amplifying feed forward loops causing a chronic state of activated O&NS, immune-inflammatory and autoimmune pathways which may sustain the disease.

Keywords: Autoimmune, chronic fatigue syndrome, cytokines, inflammation, myalgic encephalomyelitis, nitrosative stress, oxidative.

1. INTRODUCTION

Myalgic Encephalomyelitis / chronic fatigue syndrome (ME/CFS) has been classified as a neurological disease by the WHO since 1969. In clinical practice ME/CFS people are classified as having ME/CFS merely because they present with pathological levels of fatigue accompanied by variable other symptoms whose origin cannot be determined by recourse to rudimentary biomedical measurements or whose origin is clearly psychiatric. However, there are patients who present with pathological levels of fatigue and muscle fatigueability accompanied by a wide range of neuro-cognitive and neuroimmune symptoms and biomedical abnormalities which become exacerbated following even minor increases in cognitive or physical activity [1,2]. The fatigue and intolerance to increased activity are associated with immune abnormalities consistent with activation of immuno-inflammatory and oxidative and nitrosative stress (O&NS) pathways [1].

This paper examines the development of a chronic illness initiated and maintained by self sustaining feed forward mechanisms based on initially prolonged elevations of systemic O&NS and immune-inflammatory pathways.

2. THE IMMUNE AND O&NS PATHOPHYSIOLOGY OF ME/CFS

2.1. Oxidative and Nitrosative Stress (O&NS)

Many studies involving peripheral blood measurements have demonstrated significant abnormalities related to increased O&NS in many patients with ME/CFS. These abnormalities include elevated concentrations of malondialdehyde (MDA), isoprostane, 8-OH-deoxyguanosine, 2,3 diphosphoglyceric acid, thiobutyric acid, and protein carbonyls [3-18]. Inducible Nitric Oxide (NO) synthase (iNOS) levels are also significantly higher in patients with ME/CFS compared to healthy controls [19]. Unsurprisingly there is also evidence of excessive production of NO [3,20]. Levels of oxidative stress following exercise are prolonged and excessive compared to raised oxidative stress following exercise in healthy people [21-23]. This may be one of the mechanisms underpinning the flu-like malaise experienced by patients for prolonged periods following aerobic exercise [1,20]. Exercise induces striking changes in the excitability of muscle membranes in people with this illness [21]. Oxidative stress in skeletal muscles contributes to elevated fatigability in muscles [24]. Several authors have reported that O&NS measures demonstrate a

significant and positive correlation with symptom severity [3-6,8,13,15,25]. Miwa and Fujita [18] reported that the entry of patients into a state of remission correlated with a significant amelioration of oxidative stress.

Secondary IgM and IgG mediated autoimmune reactions occur in many patients with ME/CFS [3], testifying to the severity of oxidative stress which can occur in people suffering from this illness. These autoimmune responses are directed against neoepitopes, which are the result of severe damage by O&NS pathways to endogenous epitopes [26], including disrupted lipid membrane components (e.g. palmitic, myristic and oleic acid), residue molecules of lipid peroxidation (e.g. azelaic acid and malondialdehyde), anchorage molecules, such as S-farnesyl-L-cysteine, and nitrosylated amino-acids, such as nitro-tyrosine, nitro-phenylalanine, nitro-tryptophan, nitro arginine and nitro-cysteine [3,20,26]. These molecules have been damaged undergoing conformational change because of high levels of O&NS and have thus become immunogenic. It is worthy of note that the concentration of these damaged molecules displays a significant and positive correlation with the severity of the symptoms experienced by patients [3].

2.2. Activation of Immuno-Inflammatory Pathways in ME/CFS

Several groups have reported elevated pro-inflammatory cytokines, e.g. tumor necrosis factor (TNF)α and interleukin (IL)-1β, in people diagnosed with ME/CFS [20,27,28]. Recent reports have challenged the previously dominant viewpoint that patients with ME/CFS display a chronically activated T helper (Th)2 biased immune system [1]. Pro-inflammatory and anti-inflammatory cytokines have been shown to co-occur in the same patients [29]. Nevertheless, in some ME/CFS patients pro-inflammatory cytokines are predominant [20,27,28,30,31]. Temporal changes in the cytokine population and concentrations have also been reported. Maes *et al*. [19] detected significantly increased levels of cyclo-oxygenase-2 (COX-2) and nuclear factor (NF)-κB in patients compared to healthy controls. Interestingly, they reported significant and positive correlations between the levels of COX-2 and NF-κB and the severity of selective symptoms.

2.3. Mitochondrial Dysfunction in ME/CFS

The weight of evidence implicates mitochondrial dysfunction, impaired oxidative phosphorylation and abnormally high lactate levels in the pathophysiology of ME/CFS [13,32-36]. Vermeulen *et al*. [32] reported that their patients displayed a decrease in ATP production and an increase in lactate concentration compared to healthy controls and that these relative abnormalities became even greater on repeat exercise tests. This echoed an earlier finding revealed by the use of NMR spectroscopy. Thus, Arnold *et al*. [37] and Kennedy *et al*. [13] reported elevated markers of oxidative stress in patients with ME/CFS and that the magnitude of the increase correlated positively and significantly with the degree of symptom worsening which followed increased energy expenditure. Behan [35] reported the presence of structural abnormalities in the mitochondria located in the skeletal muscle of ME/CFS patients. Lane *et al*. [38] also reported a significant increase in intracellular

lactate and a lowered rate of ATP resynthesis stemming from impaired oxidative phosphorylation in the aftermath of exercise compared to controls. A number of other authors display a grossly abnormal increase in lactate levels following even trivial exercise and an excessively slow recovery from this state of affairs [32,36,39,40]. Muscle fatigue may actually originate as a result of an accumulation of ROS in muscle cells and a subsequent depletion of available ATP [41].

Mitochondrial DNA damage resulting from elevated O&NS may also result in the genesis of progeny cells which are mutated and dysfunctional [42-44]. This may go some way to explaining the lack of clinically significant or practically important benefit of exercise therapy in these patients [45] and indeed highlight its potential to do serious harm [5,46,47]. Patients with ME/CFS become exhausted much earlier than healthy controls engaged in the same level of exercise. This relatively earlier onset of exhaustion correlates with grossly reduced intracellular levels of ATP combined with an increased utilization of glycolysis compared to healthy people indicating a defect of oxidative phosphorylation [48]. Significantly raised levels of cerebro-ventricular lactate have been detected in patients diagnosed with ME/CFS indicating mitochondrial dysfunction and oxidative stress related abnormalities in the brain of people suffering from this illness [25,49,50].

2.4. Brain Dysfunctions in ME/CFS

Evidence supports the existence of neuroinflammatory processes in individuals with ME/CFS. The presence of activated microglia using [11c] PK 1195 positron emission tomography (PET) and the presence of dysfunctional astrocytes using magnetic resonance imaging (MRI) were reported by Nakatomi *et al*. [51] and Barnden *et al*. [52]. Puri *et al*. [53] carried out the first systematic MR spectroscopy (MRS) study of metabolite levels in the brains of people with ME/CFS. One of the major advantages of MRS as an investigative tool is that its use can detect the presence of pathology in brain tissue which is undetectable using conventional MRI [54]. Puri *et al*. [53] reported that the choline / creatine ratio was significantly elevated in patients compared to controls. These findings were essentially replicated using proton MRI by Chaudhuri and others [55] who also reported a significantly increased choline peak in patients compared to controls. These findings echo an earlier finding by Tomoda *et al*. [56]. The choline peak includes contributions from a range of cell membrane phospholipids containing choline. This molecule is normally found in an insoluble state hence an increased choline peak represents abnormal levels of choline motility as a result of changes in myelin status, inflammation and general hypercellularity [57]. Elevated choline is held to be a reliable surrogate marker for the presence of gliosis [58] and is found in the brain tissue of people with Parkinson's [59] and Alzheimer's [60] disease and Multiple Sclerosis [61]. In the latter disease the presence of elevated choline peaks in normal appearing brain tissue is predictive of future lesion development [62,63]. The presence of significantly elevated ventricular lactate levels in patients with ME/CFS was reported by a team of researchers in three separate studies using ^{1}H nuclear

magnetic resonance (NMR) spectroscopy [25,49,50]. Lactate levels were reported to be increased by over 380% compared to levels in healthy volunteers. When considering the abnormalities underpinning these findings these workers considered elevated oxidative stress, mitochondrial dysfunction as well as cerebral hypoperfusion, which have all been reported in patients with ME/CFS in numerous studies [2]. They ultimately concluded that elevated O&NS was the most likely explanation for their findings [25].

A large survey concluded that the most common abnormalities generating excess lactate in the cerebro-spinal fluid (CSF) were associated with mitochondrial dysfunction involving the electron transport chain [64]. The bidirectional relationship between elevated oxidative stress, mitochondrial dysfunction and neuroinflammation is well documented [65].

Studies examining the proteome in the CSF of patients with ME/CFS have reported signatures of an activated compliment cascade [66,67]. The latter authors also reported proteomic signatures indicative of protein misfolding, apoptosis and activated microglia. Gene studies have revealed evidence of chronic immune activation, mitochondrial dysfunction and abnormal neural function [68,69]. Regland and others [70] reported elevated levels of homocysteine in the CSF of people diagnosed with ME/CFS which correlated significantly with disease severity. Elevated levels of homocysteine are known to be a cause of blood brain barrier disruption [71,72] and neurotoxicity [73,74]. In addition to disruption of the blood brain barrier, homocysteine is likely to also contribute to neurotoxicity by activating N-Methyl-D-aspartate (NDMA) receptors in the brain [75].

3. SOURCES OF ACTIVATION OF SYSTEMIC INFLAMMATORY AND O&NS PATHWAYS IN ME/CFS

3.1. Infections

The vast majority of ME/CFS patients endure recurrent, persistent or subacute bacterial and viral infections [76-78]. The existence of these infections associates positively with the number and severity of symptoms, including the many neurological symptoms [79]. We previously described the processes involved in generating a chronic inflammatory state involving elevated levels of pro-inflammatory cytokines and O&NS. Briefly, acute, prolonged or persistent infections lead to chronic activation of the immune system and subsequent elevated levels of pro-inflammatory cytokines and O&NS leading in turn to O&NS damage to self epitopes and recruitment of autoreactive T cells. The inflammatory state may persist following pathogen clearance and is sustained and indeed amplified by the conspiratorial action of a number of autoimmune processes involving molecular mimicry, epitope spreading and bystander activation together with the corrosive actions of activated macrophages and dendritic cells. Prolonged and elevated O&NS leads to chronic immune activation *via* the activation of NF-κB. The increased levels of cytokines generated by NF-κB activation leads in turn to the increased transcription of iNOS and ultimately elevated NO and peroxynitrite leading to a self sustaining inflammatory environment as described elsewhere [1].

3.2. Bacterial Translocation

A number of studies have reported the existence of bacterial translocation from the lumen of the gut into the systemic circulation in people with ME/CFS [80-82]. In ME/CFS the severity of bacterial translocation is significantly associated with increased levels of pro-inflammatory cytokines and neopterin, suggesting that bacterial translocation drives at least in part the activation of immuno-inflammatory pathways in ME/CFS [82]. Other studies have reported the presence of bacterial overgrowth in the gut of people diagnosed with ME/CFS [83-85]. Rao *et al*. [86] reported a disturbed gut interface in patients with ME/CFS either mediated by bacterial translocation or microbes within the gut. Increased bacterial overgrowth, particularly in the small intestine, may cause bacterial translocation [87,88]. Bacterial translocation is likely the cause of chronic immune activation in HIV positive people *via* the activation of Toll-Like Receptor (TLR)-4 receptors [89-91]. Circulating levels of bacterial lipopolysaccharides (LPS) disrupt the integrity of the blood brain barrier [92] and lead to the activation of microglia [93]. Microglia are responsible for activating and propagating the immune response in the central nervous system (CNS) following pathogen invasion [94,95]. Microglia (and astrocytes) are able to perceive pathogen signals *via* their pattern recognition receptors. Binding of pathogens promotes recruitment and antigen specific activation of infiltrating leucocytes [95]. Activation of microglia and subsequent activation of astrocytes leads to profound neuropathology [96,97].

3.3. Activation of the Toll-like Receptor - Radical Cycle

TLRs are indispensable moderators of the innate and adaptive immune system and are highly expressed on professional antigen presentation cells such as macrophages [98]. TLRs activate immune responses when engaged by microbial pathogen associated molecular patterns (PAMPS), such as bacterial endotoxins, viral nucleic acids, and endogenous danger associated molecular patterns (DAMPS) liberated by stressed, damaged host cells or oxidized phopsholipids [99]. Activation of the TLR-4 complex results in the upregulated transcription of NF-κB and the production of pro-inflammatory cytokines [99]. Elevated NF-κB also increases the transcription of iNOS, leading to the activation of NO, and genes encoding for COX-2 [19]. Increased bacterial translocation and raised concentrations of LPS in the periphery invokes a chronic pro-inflammatory state within the CNS *via* the engagement of TLR-4 receptors on microglia even in the absence of elevated cytokine levels [100-102]. TLR-4 expression is elevated in people with ME/CFS and may have a mediatory role in this illness [103,104].

3.4. Secondary Autoimmune Responses

Prolonged elevation of O&NS species damages lipids, proteins, DNA and other structures to such an extent that they lose immunogenic tolerance and autoantibodies are formed against them. Details of immune responses generated against these O&NS modified neoepitopes can be found in Morris and Maes [1]. Phospholipids and polyunsaturated

fatty acids (PUFAs) are very vulnerable to chemical modifications induced by oxidation. These corrupted molecules located on lipoproteins and cellular membranes convert ubiquitous molecular structures into DAMPs, which engage with TLR-4 to instigate or amplify chronic immune activation and even cell death [99]. Proteins which have undergone redox mediated conformational change may also be perceived as DAMPs by the immune system [105,106]. Prolonged lipid peroxidation generates α,β-unsaturated ketones and aldehydes, which form covalent bonds with a wide variety of proteins in the intracellular environment leading to the genesis of a wide variety of modified proteins, which function as DAMPs. These DAMPs are ligands of multiple proteins and their existence in large numbers lead to the chronic activation or upregulation of the adaptive and innate immune system [107,108].

Prolonged oxidative stress and elevated levels of reactive oxygen species produced as a result of chronic immune activation, inflammation or other stress signals can activate inflammasomes [109]. Inflammasomes are ultimately composed of multiple proteins located in the cytosol, which act as stress or pathogen sensors and are highly expressed in macrophages and other phagocytes such as dendritic cells. Once DAMPS or stress signals are detected the protein subunits oligomerise into a functional mature inflammasome unit leading to procaspase-1 cleavage generating active caspase-1, which in turn activates and secretes IL-1β and IL-18 [110,111]. This process produces a powerful inflammatory response *via* the release of pro-inflammatory cytokines, ATP or other pro-inflammatory mediators [112].

3.5. Lowered Omega-3 Polyunsaturated Fatty Acids (PUFAs)

PUFAs, such as arachidonic acid (AA) in the ω6 series, and docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) in the ω3 series have major roles in immune regulation and inflammation [113]. AA acts as a precursor molecule for the cyclooxygenase and lipoxygenase mediated synthesis of the eicosanoid family of inflammatory mediators such as prostaglandins and thromboxanes [113]. AA-generated eicosanoids are generally pro-inflammatory, whereas ω3 PUFAs promote anti-inflammatory activities leading to a decrease in the production of inflammatory eicosanoids, pro-inflammatory cytokines and reactive oxygen species [114]. Maes *et al*. [113] reported increased levels of ω6 PUFAs in patients with ME/CFS, while the ω6/ω3 ratios were significantly increased in patients than healthy controls and correlated negatively and significantly with the severity of the illness while the absolute levels of ω6 PUFAs displayed a significant and positive correlation with the severity of symptoms [113]. Interestingly these authors reported a significant positive correlation between the ω6/ω3 ratio and the level of impairment in the expression of CD69 following the mitogen stimulation of a wide range of T cells. This observation indicates a defect in early T cell activation [113]. Behan and others [115] published the results of a controlled trial of ω3 supplementation in 63 patients with ME/CFS over a three month period. These authors reported that ω3 PUFA levels in red cell membranes of patients were abnormal at baseline and had been corrected by supplementation in patients who reported significant

clinical improvement compared to patients in the placebo group. Puri *et al*. [116] reported clinical improvement in symptoms in all patients trialed on a ω3 supplementation over a 12 week period compared to control. This finding supported the results of a case study by the same group [116], which noted structural changes in the brain of a patient with ME/CFS detected by NMR spectroscopy following a course of EPA supplementation. Thus, lowered levels of ω3 PUFAs relative to increased ω6 PUFA levels could increase the inflammatory potential and thus activation of immune-inflammatory pathways in ME/CFS.

3.6. Lowered Antioxidant Levels

Oxidative stress results from an imbalance of O&NS species and antioxidants and can result from impaired cellular anti-oxidant defenses. Thus diminished activity of superoxide dismutase [117], zinc [118] and glutathione homeostasis [119,120] may contribute to increased O&NS. Prolonged O&NS is one of the major causes of mitochondrial dysfunction in neurological diseases [121]. Impaired antioxidant defenses and impaired glutathione homeostasis are found in Parkinson's [122], Alzheimer's [123] and Huntington's disease [124] and Amyotrophic Lateral Sclerosis [125]. We have reviewed elsewhere that ME/CFS is accompanied by lowered levels of key antioxidants, such as coenzyme Q10, zinc and glutathione [2].

4. CONSEQUENCES OF ACTIVATED INFLAMMA-TORY AND O&NS PATHWAYS

4.1. Neuroinflammation and Brain Disorders

Immunopathology in the periphery can result in central neuropathology *via* a number of different mechanisms. The simplest involves the disruption of endothelial tight junctions in the blood brain barrier and in the gut by elevated O&NS while more complex mechanisms all involve cytokine signaling between the peripheral immune system and the brain. We will deal with the simplest of these mechanisms first. Elevated levels of O&NS and TNFα increase the permeability of endothelial tight junctions in the blood brain barrier [126,127] and the gut [128-131]. These O&NS and inflammatory pathways may not only facilitate neuroinflammation by causing disruption of the blood brain barrier and allowing encephalitogenic T cells to enter into the CNS [132], but also do so by increasing the permeability of tight junctions in the gut *via* activation of NF-κB [133] and allowing translocation of bacteria and LPS into the bloodstream. This increased bacterial translocation occurs in many people with ME/CFS [134,82]. The entry LPS into the systemic circulation or mesenteric lymph nodes provokes the activation of a range of immune-inflammatory responses [4,19,135].

Activation of peripheral innate immune cells as a result of pathogen invasion leads to the production of a range of pro-inflammatory cytokines such as IL-1β, IL-6, and TNFα. This pro-inflammatory signal is transmitted across the blood brain barrier leading to the production of identical cytokines by glial cells in the brain. Glial cells (astrocytes and microglia) stimulated by the presence of cytokines also synthesise and release other pro-inflammatory molecules including prostaglandins [136,137] and NO [138]. The blood

brain barrier is responsible for conferring an immuno-privileged status on the brain and a number of mechanisms exist which enable a peripheral cytokine signal to overcome this formidable obstacle [139]. There are essentially three distinct pathways by which a pro-inflammatory cytokine message can transverse the blood brain barrier. Firstly, cytokines in the blood can simply diffuse into the brain through reasonably porous regions of the blood brain barrier which lie in close proximity to the circumventricular organs [140,141] and thus interact directly with astrocytes and microglia in the area of the brain called the glial limitans. A second major pathway involves interplay between cytokines and brain endothelial cells [142]. These specific endothelial cells possess receptors which are capable of transporting blood IL-1β into the brain or alternatively respond to engagement of receptors by manufacturing IL-1β *de novo* for release on the abluminal side [143]. The third route entails direct neural innervation by pro-inflammatory cytokines *via* the vagus nerve [144,145] and sympathetic nervous system circuits [146].

The first two routes of signal propagation considered above are dependent on the presence of elevated pro-inflammatory cytokines in the circulation. However, it has been demonstrated that inflammatory responses take place without the presence of detectable levels of cytokines in the blood [147]. This apparent paradox can be resolved by the fact that the afferent sensory fibres of the vagus nerve are able to detect minimally elevated levels of cytokines and relay the information indicating the presence of peripheral immune activation to the brain *via* ascending fibres [147,148]. Indeed, localized elevated levels of cytokines appear to be sufficient to activate afferent sensory fibres without the need for increased cytokine levels in the circulation. Interestingly, animal studies have revealed that a vagotomy may relieve fever because the brain no longer receives information indicating the presence of peripheral inflammation [148-150]. This explains why peripheral inflammation is often accompanied by microglial activation in the brain. Microglia have destructive capability by releasing a broad range of neurotoxins which includes pro-inflammatory cytokines [151-153], NO [154,155] and reactive oxygen species [156,155]. Upon activation, microglia rapidly release ATP and astrocytes act to amplify this release. Microglia are known to be regulators of neurotransmission in their own right as upstream partners of astrocytes. This is particularly relevant because activation of microglia and changes in patterns of neurotransmission are two early occurrences in the development of many brain diseases [156]. Microglia may also activate in response to the presence of elevated levels of LPS in the systemic circulation even in the absence of elevated pro-inflammatory cytokines [100]. The weight of evidence demonstrates that microglia are activated before astrocytes, and release ATP and other inflammatory messengers such as IL-1β, TNFα or prostaglandin (PG)E$_2$, which consequently trigger activation of astrocytes [157,156]. Activated astrocytes in turn upregulate their synthesis and secretion of TNFα and/or PGE$_2$ [158,159]. These multiple complex biderectional interactions between microglia, astrocytes and neurons *via* O&NS, cytokines and other inflammatory and anti-inflammatory molecules underpin neuroinflammation [160]. For a more detailed description of the processes and players involved in the generation of neuroinflammation the reader is referred to [161]. At this juncture it would seem worthy of note that microglia may remain chronically activated long after the initial infection or other activating stimulus is past [162]. Once microglia have been activated by pathogen invasion for example they can remain activated when the initiating pathogen has been cleared by the immune system.

Activation of microglia is not the only trigger of reactive astrogliosis. Other triggering factors involve the presence of cytokines, PGE2 and O&NS. Mediators of innate immunity such as TLRs and LPS as well as certain neurotransmitters such as ATP, noradrenaline and glutamate also act as major activators of astrocytes [163,164]. In response to such indices of pathology, astrocytes abandon their role as maintainers of CNS homeostasis and acquire the status of immune cells and act to exacerbate the inflammatory milieu by releasing inflammatory mediators, such as IL-1β, TNFα, interferon-γ and PGE2 [165-168]. This intimate and conspirational relationship between the neuroglia may form the basis of another feedforward mechanism amplifying and sustaining chronic neuroinflammation, neuroprogression or overt neurodegeneration. Indeed once initiated, neuro-inflammation becomes a self-sustaining, self-amplifying process [160,169,170]. Moreover, the change in astrocyte phenotype means that the master control of CNS homeostasis is compromised and thus the function of the entire brain is placed in peril [171]. Neuroinflammatory processes underpin the pathophysiology in neurological illnesses [172] being a source of pathology in people with Parkinson's disease [173,174], Alzheimer's Disease [175,176], Amyotrophic Lateral Sclerosis [177,172], Huntington's disease [160,178] and Multiple Sclerosis [179,180].

4.2. Toxic Effects of Increased NO Production

There are several lines of evidence demonstrating the existence of elevated NO in people with ME/CFS which were discussed in an earlier section. The formation of NO is catalyzed by an ubiquitous family of different NO synthase isoforms. Three members of the family are calcium ion / calmodium dependent and generate NO with a fleetingly short half life. One of these, i.e. iNOS, is expressed as a result of inflammation or immune activation [181]. This enzyme generates NO which may persist for hours or sometimes days [182]. A number of other stimuli can provoke the genesis of iNOS such as the activation of the tyrosine kinases, mitogen-activated protein kinases (MAPKs) and Janus Kinases. Tyrosine phosphatases on the other hand inhibit the transcription of iNOS. Hyper-methylation is probably another mechanism which inhibits the transcription of this enzyme [183].

NO is a multifunctional molecule capable of acting as a vasorelaxant, a neurotransmitter and an effector molecule in the immune response. It is a thermodynamically unstable entity and is prone to react with other molecules, particularly proteins leading to their oxidation, nitrosylation or nitration [181]. This NO-induced chemical modification can alter the structure and function of proteins and cause widespread disruption on signaling cascades [181]. NO can function directly as an intracellular molecule *via* the activation of

guanylate cyclase. NO can also interact with MAPKs and proteins associated with the mitochondrial respiratory chain. It is also involved in post translational modification and cellular degradation of proteins [181].

At physiological concentrations, NO is a major player in neuromodulation. Endogenous NO regulates cholinergic transmission in the basal forebrain [184] and the rate of release of gamma-aminobutyric acid (GABA) [185]. NO also plays an important role in managing the local levels of synaptic plasticity [181], and long term potentiation which is considered to be the neural mode of learning [186,187]. The concentration of NO also has a profound effect on glutamate release. Physiological levels inhibit the release of this neurotransmitter but at higher concentrations NO actually promotes the release of glutamate [188]. Indeed at higher concentrations NO ceases to be an essential and beneficial neuromodulator but instead becomes a promoter of neuropathology [181]. For example NO and NDMA receptors are jointly responsible for the regulation of noradrenaline release [189]. However NO together with peroxinitrite may deactivate the release of noradrenaline [190,191] and be an activator of NDMA mediated neuropathology [192].

4.3. Toxic Effects of Increased Peroxynitrite

Increased production of NO and superoxide elevates the generation of peroxynitrite anion, a very toxic product [193-195]. No enzyme is needed in the formation of peroxinitrite from superoxide and NO because no enzyme could possibly catalyze any reaction that occurs so rapidly. NO is produced at relatively high levels *in vivo* and reacts more readily with superoxide than superoxide dismutase and thus NO can overcome the antioxidant effect of this molecule. Peroxynitrite is significantly more reactive than O_2 and NO despite not being a free radical [196-198]. The half-life of peroxynitrite is extremely short (of the order of 10-20 ms), but that is easily sufficient to diffuse across biological membranes over sufficient distance [199]. Peroxynitrite only reacts with a restricted number of functional groups, such as thiols and iron / sulfur centers [198]. The development and refinement of proteomic analyses confirms the highly selective nature of nitration and that this process is confined to a restricted range of tyrosine functional groups on a very small number of proteins [200-203]. Peroxynitrite can modify heme functional groups, e.g. hemoglobin [204], myoglobin [205] or cytochrome c [206], and inactivate enzymes such as iNOS by inducing oxidative modification of the heme group within its active site [207]. This reaction may be one of the prime mechanisms in the negative feedback mechanism designed to limit peroxinitrite levels in an inflammatory environment. Peroxynitrite reacts especially rapidly with iron-sulfur clusters, leading to the inactivation of enzymes normally involved in the regulation of crucial metabolic processes, such as mitochondrial aconitase [208] and phoshogluconate dehydratase [209], eNOS [210] and alcohol dehydrogenase [211]. Peroxynitrite reacts with various amino acids in a polypeptide chain inducing conformational changes affecting gross structure and therefore normal functioning. The most common reaction occurs with cysteine, so that thiol oxidation by peroxynitrite

is a major cause of conformational change in protein molecules [212].

It is worthy of note that cysteine modification does not always result in the inactivation of an enzyme but can sometimes result in enzyme activation as evidenced for matrix metalloproteinases, now understood to be the mechanism underpinning peroxynitrite-related effects on heart disease [213-215] and stroke [216]. Peroxynitrite additionally inhibits superoxide dismutase [217-219], glutaredoxin [220] etc. Peroxynitrite directly oxidizes reduced glutathione and other thiols [221-223]. Therefore the sensitivity of cells to peroxynitrite is highly dependent on glutathione levels in the cells. Glutathione depletion exacerbates peroxynitrite induced pathology [224,222]. A number of authors have suggested that the relationship between diminished levels of glutathione and enhanced peroxynitrite toxicity is a major contributing factor underpinning the development of neurological illnesses such as Parkinson's disease and Amyotrophic Lateral Sclerosis. The depletion of vital cellular antioxidants by peroxinitrite could induce a feedback loop of increased oxidants within cells and increased oxidative damage [222].

4.4. Protein Nitration

The majority of research teams have reported that nitration of tyrosine in proteins is associated with a loss of function. The inactivation of mitochondrial Mn-SOD is achieved by the nitration of a unitary tyrosine residue (Tyr-34) [218]. Nitrotyrosination suppresses tyrosine phosphorylation and hence disrupts many signaling pathways [225]. Moreover, nitrotyrosination has been observed in neurological illnesses connected to elevated levels of oxidative stress, such as Alzheimer's [226,227] and Parkinson's disease [228] and Amyotrophic Lateral Sclerosis [229]. Peroxynitrite also inactivates prostacyclin synthase by the nitration of a single tyrosine residue [Tyr-430] [230]. Tyrosine nitration modulates tyrosine kinase-dependent signaling and is responsible for the generation of neoepitopes on proteins, which are immunogenic. Studies have demonstrated cellular and humoral immune responses to a wide range of nitrotyrosine-carrying proteins [231,232] and nitrated proteins have a role in the pathophysiology of several autoimmune diseases, including Systemic Lupus Erythematosus [233,234].

4.5. Lipid Peroxidation and Modification of DNA

Major cytotoxic effects of peroxynitrite are lipid peroxidation in membranes [235], peroxidation of myelin lipids in the CNS [236-238] and damage to DNA by inducing oxidative changes in nucleoside bases [239,240] and guanine [241]. The nitration of guanine yields 8-nitro-guanine ultimately generating basic sites susceptible to cleavage [239-241]. Peroxynitrite attacks may extract hydrogen atoms from deoxyribose molecules in the sugar phosphate backbone resulting in sugar ring opening once again leading to the genesis of DNA strand breaks [239].

4.6. O&NS Affect Energy Production

The peroxynitrite-induced oxidation of crucial cysteine groups inactivates several enzymes that play a role in

energetic processes, including creatine kinase, glyceraldehyde-3-phosphate dehydrogenase, NADH dehydrogenase (complex 1) succinate dehydrogenase (complex 2), cytochrome c reductase (complex 3) and ATP synthase (complex 5) from the mitochondrial electron transport respiratory chain [242-249]. Oxidation of cysteine may inactivate tyrosine phosphatases [250,251]. Cytochrome nitration of cytochrome C severely impairs its redox activity and hence this is another target of peroxynitrite toxicity. Notably, nitration of cytochrome c upregulates its peroxidatic activity, which increases the production of hydrogen peroxide causing more oxidative damage to mitochondria [252,253]. The inhibition of aconitase by disruption of its ferrous-sulphur active site is yet another mechanism by which peroxinitrite impairs energy production [208,254]. Nicotinamide nucleotide transhydrogenase, an enzyme which enables the reduction of NAD, is another crucial mitochondrial enzyme inactivated by peroxynitrite mediated oxidation and nitration [255]. The depletion of NADPH which follows such inactivation reduces the ability of mitochondria to regenerate reduced glutathione, which plays a major contribution in the augmentation of oxidative stress already existing within the organelle [256,257]. Peroxynitrite-mediated oxidation of cysteine-bound thiols is a probable cause of permeability transition pore opening observed in isolated mitochondria in an environment containing a moderately high concentration of this entity [257].

Finally from the perspective of the adverse effects of peroxinitrite on cellular energetics we consider the effect of peroxinitrite induced activation of poly [ADP-ribose] polymerase-1 (PARP-1) [258,181]. Chronic activation of PARP-1 diminishes cellular reserves of NAD+, which is an indispensable cofactor of the tricarboxylic acid cycle, glycolytic pathway, and the electron transport chain [258-261]. Therefore depletion of NAD+ results in marked decrease in cellular ATP stores causing cellular dysfunction or even programmed death *via* the necrotic pathway [262,263]. Reactive oxygen species not only exert oxidative effects, but also engage in signaling by influencing the activity of a number of transcription factors, such as MAPK, that are associated with inflammation [264-266].

4.7. Intracellular Signaling Networks

The foundation of signal transduction within cells is based on rapidly reversible protein phosphorylation, regulated by phosphorylating kinase enzymes described as serine / threonine kinases or tyrosine kinases and dephosphorylating phosphatase enzymes [267]. The weight of evidence now strongly indicates that peroxynitrite modulates phosphotyrosine signaling by modulating the activities of phosphotyrosine phosphatases and phosphotyrosine kinases. Thus, peroxynitrite even at miniscule doses irrevocably inhibits phosphotyrosine phosphatases [250,268]. Peroxynitrite impairs tyrosine phosphorylation which may cause deregulation of vital cellular activities [269,270]. In T cells, nitration processes by peroxynitrite may block tyrosine phosphorylation which normally occurs in response to cell activation. This results in a diminished proliferative activity in activated T cells, hence impairing T cell responses [271]. The second mechanism resulting in phosphotyrosine upregulation is dependent on direct activation of phosphotyrosine kinases. Peroxynitrite

promotes rather than suppresses tyrosine phosphorylation in several cell types [272-274] and strongly activates MAPKs, which are all transactivated as a result of double phosphorylation at a particular tripeptide enabled *via* a protein kinase cascade [275]. p38 MAPK and c-Jun NH2-terminal kinase (JNK) are collectively labeled as stress-activated protein kinases (SAPKs) [276]. The MAPKs have many targets, whose downstream activation modulates many critical cellular functions [277]. JNK activation by elevated levels of peroxynitrite has been repeatedly reported [278,279]. Peroxynitrite can also activate extracellular signal-regulated kinases (ERK) [280,281] with the consequence of increased neutrophil migration and superoxide synthesis in inflammatory conditions [282,274]. The Src family of kinases, which play a key role in mitogenesis and immune cell activation are also known to be activated by peroxinitrite [283-285] either by oxidation of cysteine residues or inhibition of tyrosine 527 binding to sulphydryl groups [285,284].

Exposure to elevated O&NS leads to the oxidation, nitration and covalent modification of amino acid residues within the p53 molecule inducing conformational change resulting in an altered tertiary structure [286-288]. This change in structure impairs or eliminates the ability of the p53 protein to bind to DNA and thus carry out its normal functions [289,286]. Another result of prolonged excessively high levels of O&NS is the rapid activation of signal transducer and activator of transcription 3 (STAT-3) [290-292] and NF-kappa B [293]. Activated STAT3 also constitutively activates NF-kappa β directly [294]. STAT3 and NF-6β engage in mutual reciprocal regulation *via* feedback loops [295]. Moreover, a functional antagonism exists between NF-kappa β and p53. Initiation of NF-kappa β transcription of NF-kappa β within a cell leads to the termination of p53 transcription [296]. Loss of p53 allows the change to anaerobic glycolysis as an ATP source [297,298] resulting in diminished oxygen uptake and aerobic respiration in mitochondria. [299] leading to greatly diminished exercise capacity [300]. Loss of p53 not only leads to a reduced rate of mitochondrial respiration but also leads to greatly increased generation of free radicals in mitochondria [301].

Activation of NF-kappa β in response to lowered p53 activity instigates aerobic glycolysis also called the Warburg effect. This leads to a marked increase in the metabolism of glucose in an attempt to compensate for a fall in ATP production [302,303] and fosters glycolytic ATP generation [303,304]. A mechanistic explanation of the relapsing remitting pattern of ME/CFS based on interplay between p53 and NF-kappa β has been proposed [305]. p53 plays an indispensable role in coordinating metabolic activity within the cell to match energy requirements [306-308] and loss of its function would likely impair a person's ability to raise energy production in response to the increased demands provoked by exercise.

5. CONCLUSIONS AND FUTURE DIRECTIONS

The proposal made herein is that once generated chronically increased levels of O&NS and immune-inflammatory substances in ME/CFS conspire to generate a multitude of self-sustaining and self-amplifying pathological

processes which lead to the development of a chronic illness. The pathology stemming from chronically elevated levels of NO and peroxinitrite cannot readily be overstated. At high levels NO becomes a neurotoxin and a facilitator of glutamate-generated exitotoxicity and other NDMA receptor related pathology. Its role in modifying the activity of vital cellular enzymes and signaling pathways coupled with its ability to inhibit the performance of mitochondrial electron transport chain and compromise the production of ATP merely serves to magnify its importance as a source of pathology.

Peroxinitrite is a major regulator of cellular processes and elevated levels lead to chronically elevated inflammatory pathways and inactivation of vital regulatory enzymes responsible for the maintenance of other vital cell systems. The toxic effects of peroxinitrite on energy production are multiple, ranging from inhibition of enzymes within the mitochondrial respiratory chain, depletion or inactivation of vital players in the tricarboxylic acid cycle, disruption of the mitochondrial membrane potential and inactivation of p53. Loss of function of the latter molecule compromises the cells ability to respond to increasing energy demands and, *via* a range of processes, leads to loss of immune homeostasis. Compromised electron transfer chain function leads to increased production of O&NS species which in turn further compromise energy production both directly and indirectly and is a stark example of pathology which becomes self-sustaining and self-amplifying once initiated. It is held that the genesis of these self-sustaining pathological systems stems from prolonged and or intense pathogen invasion. Prolonged and or persistent infections, resulting in the development of autoimmune processes and bacterial translocation lead to chronic activation of the immune system and subsequent elevated levels of pro-inflammatory cytokines and O&NS leading in turn to O&NS damage to self epitopes and likely activation of the inflammasome which further acts to maintain chronic immune activation and increase the magnitude of inflammation.

The flame of chronic immune activation and elevated O&NS can be fanned by TLR engagement in the periphery and the CNS, lowered antioxidant defenses and a deficiency in ω3PUFA levels. The processes underpinning neuro-inflammation involving conspirational interactions between astrocytes, microglia, O&NS, pro-inflammatory cytokines and other inflammatory mediators are also self-sustaining once initiated. The chronic activation of inflammatory pathways also makes a major contribution to disease persistence. It is also worthy of note that a pathogen invasion of sufficient intensity can leave microglia, and hence astrocytes, chronically active even once the initial invasion has been cleared. The interplay between all of these factors leads to the formation of self-sustaining, self-amplifying auto inflammatory feed forward loops leading to a state of elevated oxidative stress and chronic immune activation which sustains the disease.

Investigation into pathogens as causative agents need to be mindful of the experiences in Multiple Sclerosis where initial skepticism has now evaporated and the consensus view is that at least two pathogens are responsible for initiating and maintaining the disease in a significant proportion of patients. This consensus has developed following the realization that the blood compartment may reveal no association between viruses and disease causation while examination of other body compartments for the presence of virus reveal strong associations. Hence further research into virus causation in ME/CFS should focus on lymphoid and muscle tissue in the periphery and sampling CSF. The exploration of relationships between polymorphisms and the presence of actively replicating viruses in people with ME/CFS appears to be urgently required. There would also seem to be a great need for the investigation of intracellular signaling networks, such as NF-kappa β, MAPK, JAK-STAT, p53, the role of apoptosis and mitochondrial signaling cascades by means of high throughput methods analyzed by systems biology. Pharmaceuticals and nutraceuticals aimed at reducing O&NS levels are needed in order to treat individuals with ME/CFS. Endotherapia would seem to be a worthy candidate as its actions in ameliorating oxidative stress has provided objectively measureable improvements in patients with MS. Minocycline would be a candidate drug for reducing neuroinflammatory processes because of its nature as an inhibitor of microglia. Dietary supplementation with ω3 PUFAs, zinc and other antioxidants has already demonstrated promise and supplementation with IV glutathione or its precursors such as N-acetyl cysteine should be considered.

AUTHORS' CONTRIBUTIONS

COMPETING INTERESTS

ACKNOWLEDGEMENTS

ABBREVIATIONS

ME/CFS	=	Myalgic Encephalomyelitis / chronic fatigue syndrome
O&NS	=	oxidative and nitrosative stress
MDA	=	malondialdehyde
iNOS	=	inducible Nitric Oxide (NO) synthase
TNF	=	tumor necrosis factor
IL-1	=	interleukin-1
Th	=	T helper
COX-2	=	cyclo-oxygenase-2
NF-kappa B	=	nuclear factor-κB
PET	=	positron emission tomography
MRI	=	magnetic resonance imaging

MRS	=	magnetic resonance spectroscopy
NMR	=	nuclear magnetic resonance
CSF	=	cerebro-spinal fluid
TLR	=	Toll-Like Receptor
LPS	=	lipopolysaccharides
CNS	=	central nervous system
PAMPs	=	pathogen associated molecular patterns
DAMPs	=	danger associated molecular patterns
PUFA	=	polyunsaturated fatty acid
AA	=	arachidonic acid
EPA	=	eicosapentaenoic acid
PG	=	prostaglandin
MAPK	=	mitogen-activated protein kinases
GABA	=	gamma-aminobutyric acid
PARP-1	=	poly [ADP-ribose] polymerase-1
JNK	=	c-Jun NH2-terminal kinase
SAPKs	=	stress-activated protein kinases
ERK	=	extracellular signal-regulated kinases
STAT3	=	signal transducer and activator of transcription 3

REFERENCES

[1]   Morris, G.; Maes, M. A neuro-immune model of Myalgic Encephalomyelitis/Chronic fatigue syndrome. *Metab. Brain Dis.*, [Epub ahead of print] **2012**. PMID: 22718491.

[2]   Morris, G.; Anderson, G.; Galecki, P.; Berk, M.; Maes, M. A narrative review on the similarities and dissimilarities between myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) and sickness behavior. *BMC Med.*, **2013**, *11*, 64. http://dx.doi.org/10.1186/1741-7015-11-64

[3]   Maes, M.; Mihaylova, I.; De Ruyter, M. Lower serum zinc in Chronic Fatigue Syndrome (CFS): relationships to immune dysfunctions and relevance for the oxidative stress status in CFS. *J. Affect. Disord.,* **2006**, *90*(2-3), 141-147. http://dx.doi.org/10.1016/j.jad.2005.11.002

[4]   Maes, M., Mihaylova, I., Kubera, M., Uytterhoeven, M., Vrydags, N., Bosmans, E. Coenzyme Q10 deficiency in Myalgic ncephalomyelitis/ chronic fatigue syndrome (ME/CFS) is related to fatigue, autonomic and neurocognitive symptoms and is another risk factor explaining the early mortality in ME/CFS due to cardiovascular disorder. *Neuro. Endocrinol. Lett.,* **2009**, *30*(4), 470-476. PMID: 20010505.

[5]   Maes, M., Twisk, F.N. Chronic fatigue syndrome: Harvey and Wessely's (bio)psychosocial model versus a bio(psychosocial) model based on inflammatory and oxidative and nitrosative stress pathways. *BMC Med.,* **2010**, *8*, 35. http://dx.doi.org/10.1186/1741-7015-8-35

[6]   Maes, M.; Twisk, F.N.M.; Kubera, M.; Ringel, K. Evidence for inflammation and activation of cell-mediated immunity in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS): Increased interleukin-1, tumor necrosis factor-a, PMN-elastase, lysozyme and neopterin. *J. Affect. Disord.,* **2011**, *136*(3), 933-939. http://dx.doi.org/10.1016/j.jad.2011.09.004

[7]   Manuel y Keenoy, B.; Moorkens, G.; Vertommen, J.; De Leeuw, I. Antioxidant status and lipoprotein peroxidation in chronic fatigue syndrome. *Life Sci.,* **2001**, *68*(17),2037-2049. http://dx.doi.org/10.1016/S0024-3205(01)01001-3

[8]   Vecchiet, J.; Cipollone, F.; Falasca, K.; Mezzetti, A.; Pizzigallo, E.; Bucciarelli, T.; De Laurentis, S.; Affaitati, G.; De Cesare, D.; Giamberardino, M.A. Relationship between musculoskeletal symptoms and blood markers of oxidative stress in patients with chronic fatigue syndrome. *Neurosci. lett.,* **2003**, *335*(3), 151-154. http://dx.doi.org/10.1016/S0304-3940(02)01058-3

[9]   Smirnova, I.V.; Pall, M.L.; Elevated levels of protein carbonyls in sera of chronic fatigue syndrome patients. *Mol. Cell. Biochem.,* **2003**, *248*(1-2), 93-95. DOI: 10.1023/A:1024176016962

[10]   Miwa, K.; Fujita, M. Increased oxidative stress suggested by low serum vitamin E concentrations in patients with chronic fatigue syndrome. *Int. J. Cardiol.,* **2009**, *136*(2), 238-239. http://dx.doi.org/10.1016/j.ijcard.2008.04.051

[11]   Jason, L.; Sorenson, M.; Sebally, K.; Alkazemi, D.; Lerch, A.; Porter, N.; Kubow, S. Increased HDAC in association with decreased plasma cortisol in older adults with chronic fatigue syndrome. *Brain Behav. Immun.* **2011**, *25*(8), 1544-5417. http://dx.doi.org/10.1016/j.bbi.2011.04.007

[12]   Brkic, S.; Tomic, S.; Maric, D.; Novakov, Mikic, A.; Turkulov, V. Lipid peroxidation is elevated in female patients with chronic fatigue syndrome. *Med. Sci. Monit.,* **2010**, *16*(12), CR628-CR632. ISSN: 15076164

[13]   Kennedy, G.; Spence, V.A.; McLaren, M.; Hill, A.; Underwood, C.; Belch, J.J. Oxidative stress levels are raised in chronic fatigue syndrome and are associated with clinical symptoms. *Free Radic. Biol. Med.,* **2005**, *39*(5), 584-589. http://dx.doi.org/10.1016/j.freeradbiomed.2005.04.020

[14]   Manuel y Keenoy, B.; Moorkens, G.; Vertommen, J.; Noe, M.; Nève, J.; De Leeuw, I. Magnesium status and parameters of thestick oxidant-antioxidant balance in patients with chronic fatigue: effects of supplementation with magnesium. *J. Am. Coll. Nutr.,* **2000**, *19*(3), 374-382. http://dx.doi.org/10.1080/07315724.2000.10718934

[15]   Richards, R.S.; Roberts, T.K.; McGregor, N.R.; Dunstan, R.H.; Butt, H.L. Blood parameters indicative of oxidative stress are associated with symptom expression in chronic fatigue syndrome. *Redox Rep.,* **2000**, *5*(1), 35-41. PubMed ID: 10905542

[16]   Richards, R.S.; Wang, L., Jelinek, H. Erythrocyte oxidative damage in chronic fatigue syndrome. *Arch. Med. Res.,* **2007**, *38*(1), 94-8. http://dx.doi.org/10.1016/j.arcmed.2006.06.008

[17]   Kurup, R.K.; Kurup, P.A. Isoprenoid pathway dysfunction in chronic fatigue syndrome. *Acta. Neuropsychiatrica.,* **2003**, *15*(5), 266-273. http://dx.doi.org/10.1034/j.1601-5215.2003.00045.x

[18]   Miwa, K.; Fujita, M. Fluctuation of serum vitamin E (alpha-tocopherol) concentrations during exacerbation and remission phases in patients with chronic fatigue syndrome. *Heart Vessels.,* **2010**, *25*(4), 319-323. http://dx.doi.org/10.1007/s00380-009-1206-6

[19]   Maes, M.; Mihaylova, I.; Kubera, M.; Bosmans, E. 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.,* **2007**, *28*(4), 463-469. PMID: 17693978

[20]   Maes, M.; Twisk, F.N.; Johnson, C. Myalgic Encephalomyelitis (ME), Chronic Fatigue Syndrome (CFS), and Chronic Fatigue (CF) are distinguished accurately: Results of supervised learning techniques applied on clinical and inflammatory data. *Psychiatry Res.,* **2012**, *200*(2-3), 754-760. http://dx.doi.org/10.1016/j.psychres.2012.03.031

[21]   Jammes, Y.; Steinberg, J.G.; Mambrini, O.; Brégeon, F.; Delliaux, S. Chronic fatigue syndrome: assessment of increased oxidative stress and altered muscle excitability in response to incremental exercise. *J. Intern. Med.* **2005**, *257*(3), 299-310. http://dx.doi.org/10.1111/j.1365-2796.2005.01452.x

[22]   Jammes, Y.; Steinberg, J.G.; Delliaux, S.; Bregeon, F. Chronic fatigue syndrome combines increased exercise-induced oxidative stress and reduced cytokine and Hsp responses. *J. Intern. Med.* **2009**, *266*(2), 196-206. http://dx.doi.org/10.1111/j.1365-2796.2009.02079.x

[23]   Jammes, Y.; Steinberg, J.G.; Delliaux, S. Chronic fatigue syndrome: acute infection and history of physical activity affect resting levels and response to exercise of plasma oxidant/ antioxidant status and heat shock proteins. *J. Intern. Med.,* **2012**, *272*(1), 74-84. http://dx.doi.org/10.1111/j.1365-2796.2011.02488.x

[24]   Fulle, S.; Pietrangelo, T.; Mancinelli, R.; Saggini, R.; Fanò, G. Specific correlations between muscle oxidative stress and chronic fatigue syndrome: a working hypothesis. *J. Muscle Res. Cell.*

Motil., **2007**, *28*(6), 355-362. http://dx.doi.org/10.1007/s10974-008-9128-y

[25]   Shungu, D.C.; Weiduschat, N.; Murrough, J.W.; Mao, X.; Pillemer, S.; Dyke, J.P.; Medow, M.S.; Natelson, B.H.; Stewart, J.M.; Mathew, S.J. Increased ventricular lactate in chronic fatigue syndrome. III. Relationships to cortical glutathione and clinical symptoms implicate oxidative stress in disorder pathophysiology. *NMR Biomed.,* **2012**, *25*(9),1073-1087. http://dx.doi.org/10.1002/nbm.2772

[26]   Geffard, M.; Bodet, D.; Martinet, Y.; Dabadie, M.P. Detection of the specific IgM and IgA circulating in sera of multiple sclerosis patients: interest and perspectives. *Immunoanalyse & Biologie Specialisee.,* **2002**, 17(5), 302-310. http://dx.doi.org/10.1016/S0923-2532(02)01214-0

[27]   Maes, M.; Twisk, F.N.; Kubera, M.; Ringel, K. Evidence for inflammation and activation of cell-mediated immunity in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS): increased interleukin-1, tumor necrosis factor-α, PMN-elastase, lysozyme and neopterin. *J. Affect. Disord.,* **2012**, *136*(3), 933-939. http://dx.doi.org/10.1016/j.jad.2011.09.004

[28]   Maes, M.; Twisk, F.N.; Ringel, K. Inflammatory and cell-mediated immune biomarkers in myalgic encephalomyelitis/chronic fatigue syndrome and depression: inflammatory markers are higher in myalgic encephalomyelitis/chronic fatigue syndrome than in depression. *Psychother. Psychosom.,* **2012**, *81*(5), 286-95. http://dx.doi.org/10.1159/000336803

[29]   Lombardi, V.C.; Hagen, K.S.; Hunter, K.W.; Diamond, J.W.; Smith-Gagen, J.; Yang, W.; Mikovits, J.A. Xenotropic murine leukemia virus-related virus-associated chronic fatigue syndrome reveals a distinct inflammatory signature. *In Vivo.,* **2011**, *25*(3), 307-314. PubMed ID: 21576403

[30]   Maher, K.J.; Klimas, N.G.; Fletcher, M.A. Chronic fatigue syndrome is associated with diminished intracellular perforin. *Clin. Exp. Immunol.,* **2005**, *142*, 505-511. DOI: 10.1111/j.1365-2249.2005.02935.x

[31]   Broderick, G.; Fuite, J.; Kreitz, A.; Vernon, S.D.; Klimas, N.; Fletcher, M.A. A formal analysis of cytokine networks in Chronic Fatigue Syndrome. *Brain Behav. Immun.,* **2010**, *24*(7), 1209-1217. http://dx.doi.org/10.1016/j.bbi.2010.04.012

[32]   Vermeulen, R.C.W.; Kurk, R.M.; Visser, F.C.; Sluiter, W.; Scholte, H.R. Patients with chronic fatigue syndrome performed worse than controls in a controlled repeated exercise study despite a normal oxidative phosphorylation capacity. *J. Transl. Med.,* **2010**, *8*, 93. http://dx.doi.org/10.1186/1479-5876-8-93

[33]   Myhill, S.; Booth, N.E.; McLaren-Howard, J. Chronic fatigue syndrome and mitochondrial dysfunction. *Int. J. Clin. Exp. Med.,* **2009**, *2*(1), 1-16. ISSN: 19405901

[34]   Booth, N.E.; Myhill, S.; McLaren-Howard, J. Mitochondrial dysfunction and the pathophysiology of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS). *Int. J. Clin. Exp. Med.,* **2012**, *5*(3), 208-20. PMID: 22837795.

[35]   Behan, W.M.; More, I.A.; Behan, P.O. Mitochondrial Abnormalities in the Postviral Fatigue Syndrome. *Acta. Neuropathologica,* **1991**, *83*(1), 61-65. http://dx.doi.org/10.1007/BF00294431

[36]   Plioplys, A.V.; Plioplys, S. Electron-microscopic Investigation of Muscle Mitochondria in Chronic Fatigue Syndrome. *Neuro-psychobiology.* **1995**, *32*(4), 175-181. http://dx.doi.org/10.1159/000119233

[37]   Arnold, D.L.; Bore, P.J.; Radda, G.K.; Styles, P.; Taylor, D.J. Excessive intracellular acidosis of skeletal muscle on exercise in a patient with a post-viral exhaustion/fatigue syndrome. *Lancet.,* **1984**, *1*, 1367-1369. PMID: 6145831

[38]   Lane, R.J.; Barrett, M.C.; Taylor, D.J.; Kemp, G.J.; Lodi, R. Heterogeneity in Chronic Fatigue Syndrome: Evidence from Magnetic Resonance Spectroscopy of Muscle. *Neuromuscl. Disord.,* **1998**, *8*(3-4), 204-209. http://dx.doi.org/10.1016/S0960-8966(98)00021-2

[39]   Paul, L.; Wood, L.; Behan, W.M.; Maclaren, W.M. Demonstration of Delayed Recovery from Fatiguing Exercise in Chronic Fatigue Syndrome. *Eur. J. Neurol.,* **1999**, *6*(1), 63-69. http://dx.doi.org/10.1046/j.1468-1331.1999.610063.x

[40]   Lane, R.J.M.; Barrett, M.C.; Woodrow, D.; Moss, J.; Fletcher, R.; Archard, L.C. Muscle Fibre Characteristics and Lactate Responses to Exercise in Chronic Fatigue Syndrome. *J. Neurol. Neurosurg.*

Psychiatry, **1998**, *64* (3), 362-367. http://dx.doi.org/10.1136/jnnp.64.3.362

[41]   Allen, D.G.; Lamb, G.D.; Westerbland, H. Skeletal Muscle Fatigue: Cellular Mechanisms. *Physiol. Rev.,* **2008**, *88*(1), 287-332. http://dx.doi.org/10.1152/physrev.00015.2007

[42]   Adam-Vizi, V.; Chinopoulos, C. Bioenergetics and the Formation of Mitochondrial Reactive Oxygen Species. *Trends Pharmacol. Sci.,* **2006**, *27*(12), 639-645. http://dx.doi.org/10.1016/j.tips.2006.10.005

[43]   Cadenas, E.; Davies, K.J.; Mitochondrial free radical generation, oxidative stress, and aging. *Free Radic. Biol. Med.,* **2000**, *29*(3-4), 222-230. http://dx.doi.org/10.1016/S0891-5849(00)00317-8

[44]   Smith, R.; Murphy, M. Animal and Human Studies with the Mitochondrial-targeted Antioxidant MitoQ. *Annals. N.Y. Acad. Sci.* **2010**, *1201*, 96-103. http://dx.doi.org/10.1111/j.1749-6632.2010.05627.x

[45]   White, P.D.; Goldsmith, K.A.; Johnson, A.L.; Potts, L.; Walwyn, R.; DeCesare, J.C.; Baber, H.L.; Burgess, M.; Clark, L.V.; Cox, D.L.; Bavinton, J.; Angus, B.J.; Murphy, G.; Murphy, M.; O'Dowd, H.; Wilks, D.; McCrone, P.; Chalder, T.; Sharpe, M. PACE trial management group. 2011. Comparison of adaptive pacing therapy, cognitive behaviour therapy, graded exercise therapy, and specialist medical care for chronic fatigue syndrome (PACE): a randomised trial. *Lancet.* **2011**, 377(9768), 823-836. http://dx.doi.org/10.1016/S0140-6736(11)60096-2

[46]   Twisk, F.N.; Maes, M. A review on cognitive behavorial therapy (CBT) and graded exercise therapy (GET) in myalgic encephalomyelitis (ME) / chronic fatigue syndrome (CFS): CBT/GET is not only ineffective and not evidence-based, but also potentially harmful for many patients with ME/CFS. *Neuro. Endocrinol. Lett.,* **2009**, 30(3), 284-299.

[47]   Kindlon, T. Reporting of Harms Associated with Graded Exercise Therapy and Cognitive Behavioural Therapy in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome. *Bull. IACFS/ME.,* **2011**, *19*(2), 59-111.

[48]   Wong, R.; Lopaschuk, G.; Zhu, G.; Walker, D.; Catellier, D.; Burton, D.; Teo, K.; Collins-Nakai, R.; Montague, T. Skeletal muscle metabolism in the chronic fatigue syndrome. *In vivo* assessment by 31P nuclear magnetic resonance spectroscopy. *Chest.,* **1992**, *102*(6), 1716-1722. PubMed ID: 1446478

[49]   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. 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.,* **2009**, *22*(3), 251-258. http://dx.doi.org/10.1002/nbm.1315

[50]   Murrough, J.W.; Mao, X.; Collins, K.A.; Kelly, C.; Andrade, G.; Nestadt, P.; Levine, S.M.; Mathew, S.J.; Shungu, D.C. Increased ventricular lactate in chronic fatigue syndrome measured by 1H MRS imaging at 3.0 T. II: comparison with major depressive disorder. *NMR Biomed.,* **2010**, *23*(6), 643-650. http://dx.doi.org/10.1002/nbm.1512

[51]   Nakatomi, Y.; Mizuno, K.; Ishii, A.; Wada, Y.; Tanaka, M.; Tazawa, S.; Onoe, K.; Fukuda, S.; Kawabe, J.; Takahashi, K.; Kataoka, Y.; Shiomi, S.; Yamaguti, K.; Inaba, M.; KuraFranktsune, H.; Watanabe, Y. Microglial activation in patients with Chronic Fatigue Syndrome: a Positron Emission Tomography study with [11C]PK11195. *Neurosci. Res.,* **2011**. 71, issue (September 2011), p. e200. DOI: 10.1016/j.neures.2011.07.862

[52]   Barnden, L.R.; Crouch, B.; Kwiatek, R.; Burnet, R.; Mernone, A.; Chryssidis, S.; Scroop, G.; Del Fante, P. A brain MRI study of chronic fatigue syndrome: evidence of brainstem dysfunction and altered homeostasis. *NMR Biomed.,* **2011**, *24*(10), 1302-1312. http://dx.doi.org/10.1002/nbm.1692

[53]   Puri, B.K.; Counsell S.J.; Zaman, R.; Main, J.; Collins, A.G.; Hajnal, J.V.; Davey, N.J. Relative increase in choline in the occipital cortex in chronic fatigue syndrome. *Acta Psychiatr. Scand.,* **2002**, *106*(3), 224-226. http://dx.doi.org/10.1034/j.1600-0447.2002.01300.x

[54]   Ge, Y. Seeing is believing: *in vivo* evolution of multiple sclerosis pathology with magnetic resonance. *Top. Magn. Reson. Imaging.,* **2006**, *17*(4), 295-306. http://dx.doi.org/10.1097/RMR.0b013e3180417d14

[55]   Chaudhuri, A.; Condon, B.; Gow, J.; Brennan, D.; Hadley, D. Proton magnetic resonance spectroscopy of basal ganglia in chronic

fatigue syndrome. *Brain Imaging.* **2003**, *14*(2), 225-228. http://dx.doi.org/10.1097/00001756-200302100-00013

[56]   Tomoda, A.; Miike, T.; Yamada, E.; Honda, H.; Moroi, T.; Ogawa, M.; Ohtani, Y.; Morishita, S. Chronic fatigue syndrome in childhood. *Brain Dev.,* **2000**, *22*(1), 60-64. http://dx.doi.org/10.1016/S0387-7604(99)00111-4

[57]   Narayana, P.A. Magnetic resonance spectroscopy in the monitoring of multiple sclerosis. *J. Neuroimaging,* **2005**, *15*(4 Suppl),46S-57S. http://dx.doi.org/10.1177/1051228405284200

[58]   Wehrl, H.F.; Schwab, J.; Hasenbach, K.; Reischl, G.; Tabatabai, G.; Quintanilla-Martinez, L.; Jiru, F.; Chughtai, K.; Kiss, A.; Cay, F.; Bukala, D.; Heeren, R.M.; Pichler, B.J.; Sauter, A.W. Multimodal elucidation of choline metabolism in a murine glioma model using magnetic resonance spectroscopy and 11C-choline positron emission tomography. *Cancer Res.,* **2013**, 73(5), 1470-1480. http://dx.doi.org/10.1158/0008-5472.CAN-12-2532

[59]   Clarke, C.E.; Lowry, M. Basal ganglia metabolite concentrations in idiopathic Parkinson's disease and multiple system atrophy measured by proton magnetic resonance spectroscopy. *Eur. J. Neurol.,* **2000**, *7*(6), 661-665. http://dx.doi.org/10.1046/j.1468-1331.2000.00111.x

[60]   Satlin, A.; Bodick, N.; Offen, W.W.; Renshaw, P.F. Brain proton magnetic resonance spectroscopy (1H-MRS) in Alzheimer's disease: changes after treatment with xanomeline, an M1 selective cholinergic agonist. *Am. J. Psychiatry,* **1997**, *154*(10), 1459-1461. 9326834

[61]   Arnold, D.L.; Matthews, P.M.; Francis, G.S.; O'Connor, J.; Antel, J.P. Proton magnetic resonance spectroscopic imaging for metabolic characterization of demyelinating plaques. *Ann. Neurol.,* **1992**, *31*(3), 235-241. http://dx.doi.org/10.1002/ana.410310302

[62]   Tartaglia, M.C.; Narayanan, S.; De Stefano, N.; Arnaoutelis, R.; Antel, S.B.; Francis, S.J.; Santos, A.C.; Lapierre, Y.; Arnold, D.L. Choline is increased in pre-lesional normal appearing white matter in multiple sclerosis. *J. Neurol.,* **2002**, *249*(10), 1382-1390. http://dx.doi.org/10.1007/s00415-002-0846-6

[63]   Narayana, P.A.; Doyle, T.J.; Lai, D.; Wolinsky, J.S. Serial proton magnetic resonance spectroscopic imaging, contrast-enhanced magnetic resonance imaging, and quantitative lesion volumetry in multiple sclerosis. *Ann. Neurol.,* **1998**, *43*(1), 56-71. http://dx.doi. org/10.1002/ana.410430112

[64]   Chow, S.L.; Rooney, Z.J.; Cleary, M.A.; Clayton, P.T.; Leonard, J.V. The significance of elevated CSF lactate. *Arch. Dis. Child.,* **2005**, *90*(11), 1188-1189. http://dx.doi.org/10.1136/adc.2005.075317

[65]   Cui, H.; Kong, Y.; Zhang, H. Oxidative Stress, Mitochondrial Dysfunction, and Aging**.** *J. Sign. Transd.* **2012**, *2012*, 646354. PMID: 21977319

[66]   Schutzer, S.E.; Angel, T.E.; Liu, T.; Schepmoes, A.A.; Clauss, T.R.. Adkins, J.N.; Camp, D.G.; Holland, B.K.; Bergquist, J.; Coyle, P.K.; Smith, R.D.; Fallon, B.A.; Natelson, B.H. Distinct Cerebrospinal Fluid Proteomes Differentiate Post-Treatment Lyme Disease from Chronic Fatigue Syndrome. *PLoS ONE.,* **2011**, *6*(2), e17287. http://dx.doi.org/10.1371/journal.pone.0017287

[67]   Baraniuk, J.N.; Casado, B.; Maibach, H.; Clauw, D.J.; Pannell, L.K.; Hess, S.S. A Chronic Fatigue Syndrome - related proteome in human cerebrospinal fluid. *BMC Neurol.,* **2005**, *5*, 22. DOI: 10.1186/1471-2377-5-22

[68]   Fang, H.; Xie, Q.; Boneva, R.; Fostel, J.; Perkins, R.; Tong, W. Gene expression profile exploration of a large dataset on chronhttp://dx.doi.org/10.2217/14622416.7.3.429ic fatigue syndrome. *Pharmacogenomics.,* **2006**, *7*(3), 429-440.

[69]   Kaushik, N.; Fear, D.; Richards, S.C.; McDermott, C.R.; Nuwaysir, E.F.; Kellam, P.; Harrison, T.J.; Wilkinson, R.J.; Tyrrell, D.A.; Holgate, S.T.; Kerr, J.R. Gene expression in peripheral blood mononuclear cells from patients with chronic fatigue syndrome. *J. Clin. Pathol.,* **2005**, *58*(8), 826-32. http://dx.doi.org/10.1136/jcp.2005.025718

[70]   Regland, B.; Andersson, M.; Abrahamsson, L.; Bagby, J.; Dyrehag, L.E.; Gottfries, C.G.; Increased concentrations of homocysteine in the cerebrospinal fluid in patients with fibromyalgia and chronic fatigue syndrome. *Scand. J. Rheumatol.,* **1997**, *26*(4), 301-307. http://dx.doi.org/10.3109/03009749709105320

[71]   Tyagi, N.; Kumar, M.; S.B.; Pushpakumar.; Lominadze, D.; Moshal, K,S.; Sen, U.; Vacek, T.P.; Tyagi, S.C. Homocysteine attenuates blood brain barrier function by inducing oxidative stress

and the junctional proteins. *FASEB J.,* **2008**, 22 (Meeting Abstract Supplement) 734.7

[72]   Tyagi, N.; Gillespie, W.; Vacek, J.C.; Sen, U.; Tyagi, S.C.; Lominadze, D. Activation of GABA-A receptor ameliorates homocysteine-induced MMP-9 activation by ERK pathway. *J. Cell. Physiol.,* **2009**, *220*(1), 257-266. http://dx.doi.org/10.1002/jcp.21757

[73]   Obeid, R.; Herrmann, W. Mechanisms of homocysteine neuro-toxicity in neurodegenerative diseases with special reference to dementia. *FEBS Lett.,* **2006**, *580*(13), 2994-3005. http://dx.doi.org/10.1016/j.febslet.2006.04.088

[74]   Chacón, I.J.; Molero, A.E.; Pino-Ramírez, G.; Luchsinger, J.A.; Lee, J.H.; Maestre; G.E. Risk of dementia associated with elevated plasma homocysteine in a latin american population. *Int. J. Alzheimers Dis.* **2009**, *23*, 2009. PMID: 20798752

[75]   Boldyrev, A. Molecular mechanisms of homocysteine toxicity and possible protection against hyperhomocysteinemia. *Recent Adv. Nutr. Prevent. Alzheimer's Dis.,* **2010**, 127-143. ISSN 978-81-7895-470-7.

[76]   Nicolson, G.L.; Nicolson, N.L.; Haier, J. Chronic Fatigue Syndrome patients subsequently diagnosed with Lyme Disease Borrelia burgdorferi: evidence for mycoplasma species co-infections. *JCFS.,* **2008**, *14*(4), 5-17. http://dx.doi.org/10.1080/10573320802091809

[77]   Seishima, M.; Mizutani, Y.; Shibuya, Y.; Arakawa, C. Chronic fatigue syndrome after human parvovirus B19 infection without persistent viremia. *Dermatology,* **2008**, *216*(4), 341-346. http://dx. doi.org/10.1159/000116723

[78]   Chia, J.K.; Chia, A.Y. Chronic fatigue syndrome is associated with chronic enterovirus infection of the stomach. *J. Clin. Pathol.,* **2008**, *61*(1), 43-48. http://dx.doi.org/10.1136/jcp.2007.050054

[79]   Nicolson, G.L.; Gan, R.; Haier, J. Multiple co-infections (Mycoplasma, Chlamydia, human herpesvirus-6) in blood of chronic fatigue syndrome patients: association with signs and symptoms. *APMIS,* **2003**, *111*, 557-566. http://dx.doi.org/10.1034/j.1600-0463.2003.1110504.x

[80]   Maes, M.; Mihaylova, I.; Leunis, J.C. Increased serum IgA and IgM against LPS of enterobacteria in chronic fatigue syndrome (CFS): indication for the involvement of gram-negative entero-bacteria in the etiology of CFS and for the presence of an increased gut-intestinal permeability. *J. Affect. Disord.,* **2007**, 99(1-3), 237-240. http://dx.doi.org/10.1016/j.jad.2006.08.021

[81]   Maes, M.; Kubera, M.; Leunis, J.C. The gut-brain barrier in major depression: intestinal mucosal dysfunction with an increased translocation of LPS from gram negative enterobacteria (leaky gut) plays a role in the inflammatory pathophysiology of depression*. Neuro-Endocrinol. Lett.,* **2008**, *29*(1), 117-124. PMID: 18283240

[82]   Maes, M.; Twisk, F.N.; Kubera, M.; Ringel, K.; Leunis, J.C.; Geffard, M. Increased IgA responses to the LPS of commensal bacteria is associated with inflammation and activation of cell-mediated immunity in chronic fatigue syndrome. *J. Affect Disord.,* **2012**, *136*(3), 909-917. http://dx.doi.org/10.1016/j.jad.2011.09.010

[83]   Sheedy, J.R.; Wettenhall, R.E.; Scanlon, D.; Gooley, P.R.; Lewis, D.P.; McGregor, N.; Stapleton, D.I.; Butt, H.L.; De Meirleir, K.L. Increased d-lactic Acid intestinal bacteria in patients with chronic fatigue syndrome. *In Vivo.,* **2009**, *23,*621-628. PubMed ID: 19567398

[84]   Evengard, B.; Nord, C.E.; Sullivan, A. Patients with chronic fatigue syndrome have higher numbers of anaerobic bacteria in the intestine compared to healthy subjects. *Eur. Soc. Clin. Microbiol. Infect. Dis.,* **2007**, *29,* S340-S340. http://dx.doi.org/10.1016/S0924-8579(07)71079-8

[85]   Butt, H.L.; Dunstan, R.H.; McGregor, N.R.; Roberts, T.K.; Zerbes, M.; Klineberg, I.J. Alteration of the Bacterial Microbial Flora in Chronic Fatigue/Pain Patients. In: Clinical and scientific basis of chronic fatigue syndrome: from myth towards management International scientific meeting. *Roberts, T.K.,* **1999**, *95*. https://getinfo. de/app/Alteration-of-the-Bacterial-Microbial-Flora-in/id/BLCP%3 ACN033068530. ISBN: 0646369059

[86]   Rao, A.V.; Bested, A.C.; Beaulne, T.M.; Katzman, M.A.; Iorio, C.; Berardi, J.M.; Logan, A.C. A randomized, double-blind, placebo-controlled pilot study of a probiotic in emotional symptoms of chronic fatigue syndrome. *Gut. Pathog.,* **2009**, *1*(1), 6. http://dx.doi.org/10.1186/1757-4749-1-6

[87]   Lin HC. 2004. Small intestinal bacterial overgrowth: a framework for understanding irritable bowel syndrome. *JAMA, 292*(7),852-8. DOI: 10.1001/jama.292.7.852

[88]   Deitch, E.A.; Sittig, K.; Li, M.; Berg, R.; Specian, R.D. Obstructive jaundice promotes bacterial translocation from the gut. *Am. J. Surg.* **1990**, *159*(1), 79-84. http://dx.doi.org/10.1016/S0002-9610(05)80610-5

[89]   Marchetti, G.; Tincati, C.; Silvestri, G. Microbial translocation in the pathogenesis of HIV infection and AIDS. *Clin. Microbiol. Rev.,* **2013**, 26(1), 2-18. http://dx.doi.org/10.1128/CMR.00050-12

[90]   Appay, V.; Sauce, D. Immune activation and inflammation in HIV-1 infection: causes and consequences. *J. Pathol.,* **2008**, *214*(2), 231-241. http://dx.doi.org/10.1002/path.2276

[91]   Brenchley, J.M.; Price, D.A.; Schacker, T.W.; Asher, T.E.; Silvestri, G.; Rao, S.; Kazzaz, Z.; Bornstein, E.; Lambotte, O.; Altmann, D.; Blazar, B.R.; Rodriguez, B.; Teixeira-Johnson, L.; Landay, A.; Martin, J.N.; Hecht, F.M.; Picker, L.J.; Lederman, M.M.; Deeks, S.G.; Douek, D.C. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat. Med.,* **2006**, *12*(12), 1365-1371. http://dx.doi.org/10.1038/nm1511

[92]   Cardoso, F.L.; Kittel, A.; Veszelka, S.; Palmela, I.; Tóth, A.; Brites, D.; Deli, M.A.; Brito, M.A. Exposure to lipopolysaccharide and/or unconjugated bilirubin impair the integrity and function of brain microvascular endothelial cells. *PLoS One.,* **2012**, *7*(5), e35919. http://dx.doi.org/10.1371/journal.pone.0035919

[93]   Block, M.L.; Zecca, L.; Hong, J.S. Microglia-mediated neuro-toxicity: uncovering the molecular mechanisms. *Nat. Rev. Neurosci.,* **2007**, *8*(1), 57-69. http://dx.doi.org/10.1038/nrn2038

[94]   Lehnardt, S. Innate immunity and neuroinflammation in the CNS: the role of microglia in Toll-like receptor-mediated neuronal injury. *Glia.,* **2010**, *58*(3), 253-263. DOI: 10.1002/glia.20928

[95]   Rock, R.B.; Gekker, G.; Hu, S.; Sheng, W.S.; Cheeran, M.; Lokensgard, J.R.; Peterson, P.K. Role of microglia in central nervous system infections. *Clin. Microbiol. Rev.* **2004**, *17*(4), 942-964. http://dx.doi.org/10.1128/CMR.17.4.942-964.2004

[96]   Ransohoff, R.M.; Brown, M.A. Innate immunity in the central nervous system. *J. Clin. Invest.,* **2012**, *122*(4), 1164-1171. http://dx.doi.org/10.1172/JCI58644

[97]   Saijo, K.; Glass, C.K. Microglial cell origin and phenotypes in health and disease. *Nat. Rev. Immunol.,* **2011**, *11*(11), 775-787. http://dx.doi.org/10.1038/nri3086

[98]   Gallego, C.; Golenbock, D.; Gomez, M.A.; Saravia, N.G. Toll-like receptors participate in macrophage activation and intracellular control of Leishmania (Viannia) panamensis. *Infect. Immun.,* **2011**, *79*(7), 2871-2879. http://dx.doi.org/10.1128/IAI.01388-10

[99]   Lucas, K.; Maes, M. Role of the Toll Like Receptor (TLR) Radical Cycle in Chronic Inflammation: Possible Treatments Targeting the TLR4 Pathway. *Mol. Neurobiol.,* **2003**, *48*(1), 190-204. http://dx.doi.org/10.1007/s12035-013-8425-7

[100]   Chakravarty, S.; Herkenham, M. Toll-like receptor 4 on nonhematopoietic cells sustains CNS inflammation during endotoxemia, independent of systemic cytokines. *J. Neurosci.,* **2005**, *25*(7), 1788-1796. http://dx.doi.org/10.1523/JNEUROSCI.4268-04.2005

[101]   Lehnardt, S.; Schott, E.; Trimbuch, T.; Laubisch, D.; Krueger, C.; Wulczyn, G.; Nitsch, R.; Weber, J.R. A vicious cycle involving release of heat shock protein 60 from injured cells and activation of toll-like receptor 4 mediates neurodegeneration in the CNS. *J. Neurosci.,* **2008**, *28*(10), 2320-2331. http://dx.doi.org/10.1523/JNEUROSCI.4760-07.2008

[102]   Mallard, C. 2012. Innate immune regulation by toll-like receptors in the brain. *ISRN Neurol.,* **2012**, *2012*, 701950. PMID: 23097717

[103]   Gow, J.W.; Hagan, S.; Herzyk, P.; Cannon, C.; Behan, P.O.; Chaudhuri, A. A gene signature for post-infectious chronic fatigue syndrome. *BMC Med. Genom,* **2009**, *2*, 38. http://dx.doi.org/10.1186/1755-8794-2-38

[104]   Light, A.R.; White, A.T.; Hughen. R.W.; Light, K.C. Moderate exercise increases expression for sensory, adrenergic, and immune genes in chronic fatigue syndrome patients but not in normal subjects. *J. Pain.,* **2009**, *10* (10), 1099-1112. http://dx.doi.org/10.1016/j.jpain.2009.06.003

[105]   Rubartelli, A.; Lotze, M.T. Inside, outside, upside down: damage-associated molecular-pattern molecules (DAMPs) and redox. *Trends Immunol.,* **2007**, *28*(10), 429-436. http://dx.doi.org/10.1016/j.it.2007.08.004

[106]   Lotze, M.T.; Zeh, H.J.; Rubartelli, A.; Sparvero, L.J.; Amoscato, A.A.; Washburn, N.R.; Devera, M.E.; Liang, X.; Tör, M.; Billiar, T. The grateful dead: damage-associated molecular pattern molecules and reduction/oxidation regulate immunity. *Immunol. Rev.,* **2007**, *220*, 60-81. http://dx.doi.org/10.1111/j.1600-065X.2007.00579.x

[107]   Uchida, K. Redox-derived damage-associated molecular patterns: Ligand function of lipid peroxidation adducts. *Redox Biol.,* **2013**, *1*(1), 94-96. http://dx.doi.org/10.1016/j.redox.2012.12.005

[108]   Miller, Y.I.; Choi, S.H.; Wiesner, P.; Fang, L.; Harkewicz, R.; Hartvigsen, K.; Boullier, A.; Gonen, A.; Diehl, C.J.; Que, X.; Montano, E.; Shaw, P.X.; Tsimikas, S.; Binder, C.J.; Witztum, J.L. Oxidation-specific epitopes are danger-associated molecular patterns recognized by pattern recognition receptors of innate immunity. *Circ. Res.,* **2011**, *108*(2), 235-248. http://dx.doi.org/10.1161/CIRCRESAHA.110.223875

[109]   Zhou, R.; Yazdi, A.S.; Menu, P.; Tschopp, J. A role for mitochondria in NLRP3 inflammasome activation. *Nature,* **2011**, *469*(7329), 221-5. http://dx.doi.org/10.1038/nature09663

[110]   Bonar, S.L.; Brydges, S.D., Mueller, J.L., McGeough, M.D., Pena, C., Chen, D., Grimston, S.K., Hickman-Brecks, C.L., Ravindran, S., McAlinden, A., Novack, D.V., Kastner, D.L., Civitelli, R., Hoffman, H.M., Mbalaviele, G. Constitutively activated NLRP3 inflammasome causes inflammation and abnormal skeletal development in mice. *PLoS One.,* **2012**, *7*(4), e35979. http://dx.doi.org/10.1371/journal.pone.0035979

[111]   Inoue, M.; Shinohara, M.L. NLRP3 Inflammasome and MS/EAE. *Autoimmune Dis.,* **2013**, *2013*, 859145. PMID: 23365725

[112]   Miao, E.A.; Leaf, I.A.; Treuting, P.M.; Mao, D.P.; Dors, M.; Sarkar, A.; Warren, S.E.; Wewers, M.D.; Aderem, A. Caspase-1-induced pyroptosis is an innate immune effector mechanism against intracellular bacteria. *Nat. Immunol.,* **2010**, *11*(12), 1136-1142. http://dx.doi.org/10.1038/ni.1960

[113]   Maes, M.; Mihaylova, I.; Leunis, J.C. In chronic fatigue syndrome, the decreased levels of omega-3 poly-unsaturated fatty acids are related to lowered serum zinc and defects in T cell activation. *Neuro-Endocrinol. Lett.,* **2005**, *26*(6), 745-751. PubMed ID: 16380690

[114]   Tai, C.C., Ding, S.T. N-3 polyunsaturated fatty acids regulate lipid metabolism through several inflammation mediators: mechanisms and implications for obesity prevention. *J. Nutr. Biochem.,* **2010**, *21*(5), 357-363. http://dx.doi.org/10.1016/j.jnutbio.2009.09.010

[115]   Behan, P.O.; Behan, W.M.; Horrobin, D. Effect of high doses of essential fatty acids on the postviral fatigue syndrome. *Acta Neurol. Scand.,* **1990**, *82*(3), 209-216. http://dx.doi.org/10.1111/j.1600-0404.1990.tb04490.x

[116]   Puri, B.K., Holmes, J., Hamilton, G. Eicosapentaenoic acid-rich essential fatty acid supplementation in chronic fatigue syndrome associated with symptom remission and structural brain changes. *Int. J. Clin. Pract.,* **2004**, *58*(3), 297-299. http://dx.doi.org/10.1111/j.1368-5031.2004.00073.x

[117]   Narayanan, A.; Popova, T.; Turell, M.; Kidd, J.; Chertow, J.; Popov, S.G.; Bailey, C.; Kashanchi, F.; Kehn-Hall, K. Alteration in superoxide dismutase 1 causes oxidative stress and p38 MAPK activation following RVFV infection. *PLoS One,* **2011**, *6*(5), e20354. http://dx.doi.org/10.1371/journal.pone.0020354

[118]   Song, Y.; Leonard, S.W.; Traber, M.G.; Ho, E. Zinc deficiency affects DNA damage, oxidative stress, antioxidant defenses, and DNA repair in rats. *J. Nutr.,* **2009**, *139*(9), 1626-1631. http://dx.doi.org/10.3945/jn.109.106369

[119]   Kaur, S.; Nehru, B. Alteration in glutathione homeostasis and oxidative stress during the sequelae of trimethyltin syndrome in rat brain. *Biol. Trace. Elem. Res.,* **2013**, *153*(1-3), 299-308. http://dx.doi.org/10.1007/s12011-013-9676-x

[120]   Belrose, J.C.; Xie, Y.F.; Gierszewski, L.J.; MacDonald, J.F.; Jackson, M.F. Loss of glutathione homeostasis associated with neuronal senescence facilitates TRPM2 channel activation in cultured hippocampal pyramidal neurons. *Mol. Brain,* **2012**, *5*, 11. http://dx.doi.org/10.1186/1756-6606-5-11

[121]   Federico, A.; Cardaioli, E.; Da Pozzo, P.; Formichi, P.; Gallus, G.N.; Radi, E. Mitochondria, oxidative stress and neurodegeneration. *J. Neurol. Sci.,* **2012**, *322* (1-2), 254-262. http://dx.doi.org/10.1016/j.jns.2012.05.030

[122]   Sofic, E.; Lange, K.W.; Jellinger, K.; Riederer, P. Reduced and oxidized glutathione in the *substantia nigra* of patients with

Parkinson's disease. *Neurosci. Lett.,* **1992**, *142*(2), 128-130. http://dx.doi.org/10.1016/0304-3940(92)90355-B

[123]　Cristalli, D.O., Arnal, N., Marra FA, de Alaniz MJ, Marra CA. Peripheral markers in neurodegenerative patients and their first-degree relatives. *J. Neurol. Sci.,* **2012**, *314*(1-2), 48-56. http://dx.doi.org/10.1016/j.jns.2011.11.001

[124]　Klepac, N.; Relja, M.; Klepac, R.; Hećimović, S.; Babić, T.; Trkulja, V. Oxidative stress parameters in plasma of Huntington's disease patients, asymptomatic Huntington's disease gene carriers and healthy subjects: a cross-sectional study. *J. Neurol.,* **2007**, *254*(12), 1676-1683. http://dx.doi.org/10.1007/s00415-007-0611-y

[125]　Babu, G.N.; Kumar, A.; Chandra, R.; Puri, S.K.; Singh, R.L.; Kalita, J.; Misra, U.K. Oxidant-antioxidant imbalance in the erythrocytes of sporadic amyotrophic lateral sclerosis patients correlates with the progression of disease. *Neurochem. Int.,* **2008**, *52*(6), 1284-1289. http://dx.doi.org/10.1016/j.neuint.2008.01.009

[126]　Parathath, S.R.; Parathath, S.; Tsirka, S.E. Nitric oxide mediates neurodegeneration and breakdown of the blood-brain barrier in tPA-dependent excitotoxic injury in mice. *J. Cell Sci.* **2006**, *119*, 339-349. http://dx.doi.org/10.1242/jcs.02734

[127]　Lochhead, J.J.; McCaffrey, G.; Quigley, C.E.; Finch, J.; DeMarco, K.M.; Nametz, N.; Davis, T.P. Oxidative stress increases blood-brain barrier permeability and induces alterations in occludin during hypoxia-reoxygenation. *J. Cereb. Blood Flow Metab.,* **2010**, *30* (9), 1625-1636. http://dx.doi.org/10.1038/jcbfm.2010.29

[128]　Yu, L.C., Wang, J.T., Wei, S.C., Ni, Y.H. Host-microbial inter-actions and regulation of intestinal epithelial barrier function: From physiology to pathology. *World J. Gastrointest. Pathophysiol.,* **2012**, *3*(1), 27-43. doi: 10.4291/wjgp.v3.i1.27.

[129]　Han, X., Fink, M.P., Delude, R.L. Proinflammatory cytokines cause NO*-dependent and -independent changes in expression and localization of tight junction proteins in intestinal epithelial cells. *Shock,* **2003**, *19*(3), 229-237. PMID: 12630522.

[130]　Fujinami, R.S.; Oldstone, M.B. Amino acid homology between the encephalitogenic site of myelin basic protein and virus: mechanism for autoimmunity. *Science,* **1985**, *230*(4729), 1043-1045. http://dx.doi.org/10.1126/science.2414848

[131]　Lang, H.L.E.; Jacobsen, H.; Ikemizu, S.; Andersson, C.; Harlos, K.; Madsen, L.; Hjorth, P.; Sondergaard, L.; Svejgaard, A.; Wucherpfennig, K.; Stuart, D.I.; Bell, J.I.; Jones, E.Y.; Fugger, L. A functional and structural basis for TCR cross-reactivity in multiple sclerosis. *Nat. Immunol.,* **2002**, *3*, 940-943. http://dx.doi.org/10.1038/ni835

[132]　Zajac, A.J.; Muller, D.; Pederson, K.; Frelinger, J.A.; Quinn, D.G. Natural killer cell activity in lymphocytic choriomeningitis virus-infected beta 2-microglobulin-deficient mice. *Int. Immunol.,* **1995**, *7*, 1545-1556. http://dx.doi.org/10.1093/intimm/7.10.1545

[133]　Al-Sadi, R.; Ye, D.; Said, H.M.; Ma, T.Y. IL-1beta-induced increase in intestinal epithelial tight junction permeability is mediated by MEKK-1 activation of canonical NF-kappaB pathway. *Am. J. Pathol.,* **2010**, *177*(5), 2310-2322. http://dx.doi.org/10.2353/ajpath.2010.100371

[134]　Maes, M.; Leunis, J.C. Normalization of leaky gut in chronic fatigue syndrome (CFS) is accompanied by a clinical improvement: effects of age, duration of illness and the translocation of LPS from gram-negative bacteria. *Neuro-Endocrinol. Lett.,* **2008**, *29*(6), 902-910. PMID: 19112401.

[135]　Wucherpfennig, K.W.; Ota, K.; Endo, N.; Seidman, J.G.; Rosenzweig, A.; Weiner, H.L.; Hafler, D.A. Shared human T cell receptor Vb usage to immunodominant regions of myelin basic protein. *Science,* **1990**, *248*, 1016-1019. PMID: 1693015.

[136]　Ericsson, A.; Arias, C.; Sawchenko, P.E. Evidence for an intra-medullary prostaglandin-dependent mechanism in the activation of stress-related neuroendocrine circuitry by intravenous interleukin-1. *J. Neurosci.,* **1997**, *17*(18), 7166-7179. http://dx.doi.org/10.1111/j.1460-9568.2008.06296.x

[137]　Marty, V.; El Hachmane M.; Amédée, T. Dual modulation of synaptic transmission in the nucleus tractus solitarius by prostaglandin E2 synthesized downstream of IL-1beta. *Eur. J. Neurosci.,* **2008**, *27*(12), 3132-3150. http://dx.doi.org/10.1016/S0306-4522(98)00368-6

[138]　Konsman, J.P.; Kelley, K.; Dantzer, R. Temporal and spatial relationships between lipopolysaccharide-induced expression of Fos, interleukin-1beta and inducible nitric oxide synthase in rat brain. *Neuroscience,* **1999**, *89*(2), 535-548.

[139]　Bechmann, I.; Galea, I.; Perry, V.H. What is the blood-brain barrier (not)? *Trends Immunol.,* **2007**, *28* (1), 5-11. http://dx.doi.org/10.1016/j.it.2006.11.007

[140]　Lacroix, S.; Feinstein, D.; Rivest, S. The bacterial endotoxin lipopolysaccharide has the ability to target the brain in upregulating its membrane CD14 receptor within specific cellular populations. *Brain Pathol.,* **1998**, *8*(4), 625-640. PubMed ID: 9804372

[141]　Laflamme, N.; Lacroix, S.; Rivest, S. An essential role of interleukin-1beta in mediating NF-kappaB activity and COX-2 transcription in cells of the blood-brain barrier in response to a systemic and localized inflammation but not during endotoxemia. *J. Neurosci.,* **1999**, *19*(24), 10923-10930. PMID: 10594073.

[142]　Ching, S.; Zhang, H.; Belevych, N.; He, L.; Lai, W.; Pu, X.A.; Jaeger, L.B.; Chen, Q.; Quan, N. Endothelial-specific knockdown of interleukin-1 (IL-1) type 1 receptor differentially alters CNS responses to IL-1 depending on its route of administration. *J. Neurosci.,* **2007**, *27*(39), 10476-10486. http://dx.doi.org/10.1523/JNEUROSCI.3357-07.2007

[143]　Quan, N.; Banks, W.A. Brain-immune communication pathways. *Brain Behav. Immun.,* **2007**, *21*(6), 727-735. http://dx.doi.org/10.1016/j.bbi.2007.05.005

[144]　Goehler, L.E.; Erisir, A., Gaykema, R.P. Neural-immune interface in the rat area postrema. *Neuroscience,* **2006**, *140*(4), 1415-1434. http://dx.doi.org/10.1016/j.neuroscience.2006.03.048

[145]　Marvel, F.A.; Chen, C.C.; Badr, N.; Gaykema, R.P.; Goehler, L.E. Reversible inactivation of the dorsal vagal complex blocks lipopolysaccharide-induced social withdrawal and c-Fos expression in central autonomic nuclei. *Brain Behav. Immun.,* **2004**, *18*(2), 123-134. http://dx.doi.org/10.1016/j.bbi.2003.09.004

[146]　Johnson, J.D.; Cortez, V.; Kennedy, S.L.; Foley, T.E.; Hanson, H.3rd.; Fleshner, M. Role of central beta-adrenergic receptors in regulating proinflammatory cytokine responses to a peripheral bacterial challenge. *Brain Behav. Immun.,* **2008**, *22*(7), 1078-1086. http://dx.doi.org/10.1016/j.bbi.2008.03.007

[147]　Watkins, L.R.; Maier, S.F.; Goehler, L.E. Cytokine-to-brain communication: a review & analysis of alternative mechanisms. *Life Sci.,* **1995**, *57*(11), 1011-1126. DOI: 10.1016/0024-3205(95)02047-M

[148]　Tracey, K.J. The inflammatory reflex. *Nature,* **2002**, *420*(6917), 853-859. http://dx.doi.org/10.1038/nature01321

[149]　Watkins, L.R., Goehler, L.E., Relton, J.K., Tartaglia, N., Silbert, L., Martin, D., Maier, S.F. Blockade of interleukin-1 induced hyperthermia by subdiaphragmatic vagotomy: evidence for vagal mediation of immune-brain communication. *Neurosci. Lett.,* **1995**, *183*(1-2), 27-31. http://dx.doi.org/10.1016/0304-3940(94)11105-R

[150]　Johnston, G.R.; Webster, N.R. Cytokines and the immuno-modulatory function of the vagus nerve. *Br. J. Anaesth.* **2009**, *102*(4), 453-462. http://dx.doi.org/10.1093/bja/aep037

[151]　Balasubramaniam, B.; Carter, D.A.; Mayer, E.J.; Dick, A.D. Microglia derived IL-6 suppresses neurosphere generation from adult human retinal cell suspensions. *Exp. Eye Res.,* **2009**, *89*(5), 757-766. http://dx.doi.org/10.1016/j.exer.2009.06.019

[152]　Dheen, S.T.; Jun, Y.; Yan, Z.; Tay, S.S.; Ling, E.A. Retinoic acid inhibits expression of TNF-alpha and iNOS in activated rat microglia. *Glia.,* **2005**, *50*(1), 21-31. http://dx.doi.org/10.1002/glia.20153

[153]　Bi, X.L.; Yang, J.Y.; Dong, Y.X.; Wang, J.M.; Cui, Y.H.; Ikeshima, T.; Zhao, Y.Q.; Wu, C.F. Resveratrol inhibits nitric oxide and TNF-alpha production by lipopolysaccharide-activated microglia. *Int. Immunopharmacol.,* **2005**, *5*(1), 185-193. http://dx.doi.org/10.1016/j.intimp.2004.08.008

[154]　Moss, D.W.; Bates, T.E.. Activation of murine microglial cell lines by lipopolysaccharide and interferon-gamma causes NO-mediated decreases in mitochondrial and cellular function. *Eur. J. Neurosci.,* **2001**, *13*(3), 529-538. http://dx.doi.org/10.1046/j.1460-9568.2001.01418.x

[155]　Liu, B.; Gao, H.M. Wang, J.Y.; Jeohn, G.H.; Cooper, C.L. Hong JS. Role of nitric oxide in inflammation-mediated neurodegeneration. *Ann. N. Y. Acad. Sci.,* **2002**, *962*, 318-331. http://dx.doi.org/10.1111/j.1749-6632.2002.tb04077.x

[156]　Colton, C.A.; Gilbert, D.L. Production of superoxide anions by a CNS macrophage, the microglia. *FEBS Lett.,* **1987**, *223*(2), 284-288. http://dx.doi.org/10.1016/0014-5793(87)80305-8

[155]　Mao, H.; Liu, B. Synergistic microglial reactive oxygen species generation induced by pesticides lindane and dieldrin. *Neuroreport,*

2008, *19*(13), 1317-1320. http://dx.doi.org/10.1097/WNR.0b013 e32830b3677

[156]   Pascual, O.; Ben, A. S.; Rostaing, P.; Triller, A.; Bessis, A. Microglia activation triggers astrocyte-mediated modulation of excitatory neurotransmission. *Proc. Natl. Acad. Sci. U. S. A.,* **2012**, *109*(4), E197-E205. http://dx.doi.org/10.1073/pnas.1111098109

[157]   Colangelo, A.M.; Cirillo, G.; Lavitrano, M.L.; Alberghina, L.; Papa, M. Targeting reactive astrogliosis by novel biotechnological strategies. *Biotechnol. Adv.,* **2012**, *30* (1), 261-271. http://dx.doi. org/10.1016/j.biotechadv.2011.06.016

[158]   Bezzi, P.; Volterra, A. A neuron-glia signalling network in the active brain. *Curr. Opin. Neurobiol.,* **2001**, *11*(3), 387-394. http:// dx.doi.org/10.1016/S0959-4388(00)00223-3

[159]   Font-Nieves, M.; Sans-Fons, M.G.; Gorina, R.; Bonfill-Teixidor, E.; Salas-Pérdomo, A.; Márquez-Kisinousky, L.; Santalucia, T.; Planas, A.M. Induction of COX-2 enzyme and down-regulation of COX-1 expression by lipopolysaccharide (LPS) control prostaglandin E2 production in astrocytes. *J. Biol. Chem.,* **2012**, *287*(9), 6454-6468. http://dx.doi.org/10.1074/jbc.M111.327874

[160]   Möller, T. Neuroinflammation in Huntington's disease. *J. Neural. Transm.,* **2012**, *117*(8), 1001-1008. http://dx.doi.org/10.1007/ s00702-010-0430-7

[161]   Graeber, M.B; Li, W.; Rodriguez, M.L. Role of microglia in CNS inflammation. *FEBS Lett.,* **2011**, *585*(23), 3798-3805. http://dx.doi. org/10.1016/j.febslet.2011.08.033

[162]   Block, M.L.; Hong, J.S. Chronic microglial activation and progressive dopaminergic neurotoxicity. *Biochem. Soc. Trans.,* **2007**, *35*(Pt 5), 1127-1132. DOI: 10.1042/BST0351127

[163]   Sofroniew, M.V.; Vinters, H.V. Astrocytes: biology and pathology. *Acta. Neuropathol.,* **2010**, *119*(1), 7-35. http://dx.doi.org/10.1007/ s00401-009-0619-8

[164]   Kuboyama, K.; Harada, H.; Tozaki-Saitoh, H.; Tsuda, M.; Ushijima, K.; Inoue, K. Astrocytic P2Y(1) receptor is involved in the regulation of cytokine/chemokine transcription and cerebral damage in a rat model of cerebral ischemia. *J. Cereb. Blood Flow Metab.,* **2011**, *31*(9), 1930-1941. http://dx.doi.org/10.1038/ jcbfm.2011.49

[165]   McGraw, J.; Hiebert, G.W.; Steeves, J.D. Modulating astrogliosis after neurotrauma. *J. Neurosci. Res.,* **2001**, *63* (2), 109-115. http://dx.doi.org/10.1002/1097-4547(20010115)63:2<109::AID-JNR1002>3.0.CO;2-J

[166]   North, R.A.; Verkhratsky, A. Purinergic transmission in the central nervous system. *Pflugers. Arch.,* **2006**, *452*(5), 479-485. http://dx.doi.org/10.1007/s00424-006-0060-y

[167]   Rodríguez, J.J.; Olabarria, M.; Chvatal, A.; Verkhratsky, A. Astroglia in dementia and Alzheimer's disease. *Cell. Death Differ.,* **2009**, *16*(3), 378-385. http://dx.doi.org/10.1038/cdd.2008.172

[168]   Li, L.; Lundkvist, A.; Andersson, D.; Wilhelmsson, U.; Nagai, N.; Pardo, A.C.; Nodin, C.; Ståhlberg, A.; Aprico, K.; Larsson, K.; Yabe, T.; Moons, L.; Fotheringham, A.; Davies, I.; Carmeliet, P.; Schwartz, J.P.; Pekna, M.; Kubista, M.; Blomstrand, F.; Maragakis, N.; Nilsson, M.; Pekny, M. Protective role of reactive astrocytes in brain ischemia. *J. Cereb. Blood Flow Metab.,* **2008**, *28*(3), 468-481. http://dx.doi.org/10.1038/sj.jcbfm.9600546

[169]   Adzemovic, M.Z.; Zeitelhofer, M.; Eriksson, U.; Olsson, T.; Nilsson, I. Imatinib ameliorates neuroinflammation in a rat model of multiple sclerosis by enhancing blood-brain barrier integrity and by modulating the peripheral immune response. *PLoS One.,* **2013**, *8*(2), e56586. http://dx.doi.org/10.1371/journal.pone.0056586

[170]   Olajide, O.A.; Bhatia, H.S.; de Oliveira, A.C.; Wright, C.W.; Fiebich, B.L. Inhibition of Neuroinflammation in LPS-Activated Microglia by Cryptolepine. Evid Based Complement *Alter. Med.,* **2013**, *2013*, 459723. DOI: 10.1155/2013/459723

[171]   Franke, H.; Verkhratsky, A.; Burnstock, G.; Illes, P. Patho-physiology of astroglial purinergic signalling. *Purinergic Signal.,* **2012**, *8* (3), 629-657. http://dx.doi.org/10.1007/s11302-012-9300-0

[172]   Philips, T.; Robberecht, W. Neuroinflammation in amyotrophic lateral sclerosis: role of glial activation in motor neuron disease. *Lancet Neurol.,* **2011**, *10*(3), 253-63. http://dx.doi.org/10.1016/ S1474-4422(11)70015-1

[173]   Hirsch, E.C.; Vyas, S.; Hunot, S. Neuroinflammation in Parkinson's disease. *Parkinsonism Relat. Disord.,* **2012**, *18* Suppl 1, S210-2. http://dx.doi.org/10.1016/S1353-8020(11)70065-7

[174]   Sekiyama, K.; Sugama, S.; Fujita, M.; Sekigawa, A.; Takamatsu, Y.; Waragai, M.; Takenouchi, T.; Hashimoto, M. Neuro-inflammation in Parkinson's Disease and Related Disorders: A Lesson from Genetically Manipulated Mouse Models of α-Synucleinopathies. *Parkinsons Dis.,* **2012**, 2012 (2012), ID 271732. PMID: 22550610

[175]   Krause, D.L.; Müller, N. Neuroinflammation, microglia and implications for anti-inflammatory treatment in Alzheimer's disease. *Int. J. Alzheimers Dis.* **2010**, 2010, 732806. DOI: 10.4061/ 2010/732806

[176]   Hensley, K. Neuroinflammation in Alzheimer's disease: mechanisms, pathologic consequences, and potential for therapeutic manipulation. *J. Alzheimers Dis.,* **2010**, *21*(1), 1-14. DOI: 10.3233/JAD-2010-1414

[177]   Lewis, C.A.; Manning, J.; Rossi, F.; Krieger, C. The Neuro-inflammatory Response in ALS: The Roles of Microglia and T Cells. *Neurol. Res. Int.,* **2012**, *2012*, 803701. http://dx.doi.org/ 10.1155/2012/803701

[178]   Frank-Cannon, T.C.; Alto, L.T.; McAlpine, F.E.; Tansey, M.G. Does neuroinflammation fan the flame in neurodegenerative diseases? *Mol. Neurodegener.,* **2009**, *4*, 47. http://dx.doi.org/10.1186/1750-1326-4-47

[179]   Nataf, S. Neuroinflammation responses and neurodegeneration in multiple sclerosis. *Rev. Neurol. (Paris).,* **2009**, *165*(12), 1023-1028. http://dx.doi.org/10.1016/j.neurol.2009.09.012

[180]   Agius, L.M. Neuroinflammation as the proximate cause of signature pathogenic pattern progression in amyotrophic lateral sclerosis, AIDS, and multiple sclerosis. *Pathol. Res. Int.,* **2012**, *2012*, 169270. DOI: 10.1155/2012/169270

[181]   Pacher, P.; Beckman, J.S.; Liaudet, L. Nitric oxide and peroxynitrite in health and disease. *Physiol. Rev.,* **2007**, *87*(1), 315-424. http://dx.doi.org/10.1152/physrev.00029.2006

[182]   Iadecola, C.; Zhang, F.; Xu, S.; Casey, R.; Ross, M.E. Inducible nitric oxide synthase gene expression in brain following cerebral ischemia. *J. Cereb. Blood Flow Metab.,* **1995**, *15*(3), 378-384. http://dx.doi.org/10.1038/jcbfm.1995.47

[183]   Yu, Z.; Kone, B.C. Hypermethylation of the inducible nitric-oxide synthase gene promoter inhibits its transcription. *J. Biol. Chem.,* **2004**, *279*(45), 46954-46961. http://dx.doi.org/10.1074/jbc. M407192200

[184]   Prast, H.; Tran, M.H.; Fischer, H.; Philippu, A. Nitric oxide-induced release of acetylcholine in the nucleus accumbens: role of cyclic GMP, glutamate, and GABA. *J. Neurochem.,* **1998**, 71(1), 266-273. http://dx.doi.org/10.1046/j.1471-4159.1998.71010266.x

[185]   Getting, S.J.; Segieth, J.; Ahmad, S.; Biggs, C.S.; Whitton, P.S. Biphasic modulation of GABA release by nitric oxide in the hippocampus of freely moving rats *in vivo*. *Brain Res.,* **1996**, *717*(1-2), 196-199. http://dx.doi.org/10.1016/0006-8993(96)00127-8

[186]   Boulton, C.L.; Southam, E.; Garthwaite, J. Nitric oxide-dependent long-term potentiation is blocked by a specific inhibitor of soluble guanylyl cyclase. *Neurosci.,* **1995**, 69(3), 699-703. http://dx.doi. org/10.1016/0306-4522(95)00349-N

[187]   Schuman, E.M.; Madison, D.V. A requirement for the intercellular messenger nitric oxide in long-term potentiation. *Science.,* **1991**, 254(5037), 1503-1506. http://dx.doi.org/10.1126/science.1720572

[188]   Sequeira, S.M.; Ambrósio, A.F.; Malva, J.O.; Carvalho, A.P.; Carvalho, C.M. Modulation of glutamate release from rat hippocampal synaptosomes by nitric oxide. *Nitric Oxide,* **1997**, *1*(4), 315-329. http://dx.doi.org/10.1006/niox.1997.0144

[189]   Feldman, S.; Weidenfeld, J. Involvement of endogenous glutamate in the stimulatory effect of norepinephrine and serotonin on the hypothalamo-pituitary-adrenocortical axis. *Neuroendocrinology.,* **2004**, *79*(1), 43-53. http://dx.doi.org/10.1159/000076044

[190]   Kolo, L.L.; Westfall, T.C.; Macarthur, H. Nitric oxide decreases the biological activity of norepinephrine resulting in altered vascular tone in the rat mesenteric arterial bed. *Am. J. Physiol. Heart Circ. Physiol.,* **2004**, *286*(1), H296-H303. http://dx.doi.org/ 10.1152/ajpheart.00668.2003

[191]   Shelkovnikov S.; Merlic, C.A.; Gonick, H.C. Influence of nitric oxide donors and peroxynitrite on the contractile effect and concentration of norepinephrine. *Life Sci.,* **2004**, *74* (23), 2919-2928. http://dx.doi.org/10.1016/j.lfs.2003.11.006

[192]   Dawson, V.L.; Dawson, T.M.; London, E.D.; Bredt, D.S.; Snyder, S.H. Nitric oxide mediates glutamate neurotoxicity in primary cortical cultures. *Proc. Natl. Acad. Sci. U. S. A.,* **1991**, *88*(14), 6368-6371. http://dx.doi.org/10.1073/pnas.88.14.6368

[193]   Pryor, W.A.; Squadrito, G.L. The chemistry of peroxynitrite: a product from the reaction of nitric oxide with superoxide. *Am. J. Physiol.,* **1995**, *268*(5 Pt 1), L699-L722. PubMed ID: 7762673

[194]   Szabó, C. The pathophysiological role of peroxynitrite in shock, inflammation, and ischemia-reperfusion injury. *Shock.,* **1996**, *6*(2), 79-88. http://dx.doi.org/10.1097/00024382-199608000-00001

[195]   Nathan, C. Specificity of a third kind: reactive oxygen and nitrogen intermediates in cell signaling. *J. Clin. Invest.,* **2003**, *111*(6), 769-778. http://dx.doi.org/10.1172/JCI200318174

[196]   Beckman, J.S. Ischaemic injury mediator. *Nature,* **1990**, *345*(6270), 27-28. http://dx.doi.org/10.1038/345027b0

[197]   Beckman, J.S.; Beckman, T.W.; Chen, J.; Marshall, P.A.; Freeman, B.A. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. *Proc. Natl. Acad. Sci. U. S. A.,* **1990**, *87*(4), 1620-1624. http://dx.doi.org/10.1073/pnas.87.4.1620

[198]   Beckman, J.S.; Koppenol, W.H. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. *Am. J. Physiol.,* **1996**, *271*(5 Pt 1), C1424-C1437. PubMed ID: 8944624

[199]   Denicola, A.; Souza, J.M.; Radi, R. Diffusion of peroxynitrite across erythrocyte membranes. *Proc. Natl. Acad. Sci. U. S. A.,* **1998**, *95*(7), 3566-3571. http://dx.doi.org/10.1073/pnas.95.7.3566

[200]   Aulak, K.S.; Miyagi, M.; Yan, L.; West, K.A.; Massillon, D.; Crabb, J.W.; Stuehr, D.J. Proteomic method identifies proteins nitrated *in vivo* during inflammatory challenge. *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98*(21), 12056-12061. http://dx.doi.org/10.1073/pnas.221269198

[201]   Gow, A.J.; Farkouh, C.R.; Munson, D.A.; Posencheg, M.A.; Ischiropoulos, H. Biological significance of nitric oxide-mediated protein modifications. *Am. J. Physiol. Lung Cell. Mol. Physiol.,* **2004**, *287*(2), L262-L268. http://dx.doi.org/10.1152/ajplung.00295.2003

[202]   Kanski, J.; Behring, A.; Pelling, J.; Schöneich, C. Proteomic identification of 3-nitrotyrosine-containing rat cardiac proteins: effects of biological aging. *Am. J. Physiol. Heart. Circ. Physiol.,* **2005**, *288*(1), H371-H381. http://dx.doi.org/10.1152/ajpheart.01030.2003

[203]   Kanski, J.; Hong, S.J.; Schöneich, C. Proteomic analysis of protein nitration in aging skeletal muscle and identification of nitrotyrosine-containing sequences *in vivo* by nanoelectrospray ionization tandem mass spectrometry. *J. Biol. Chem.* **2005**, *280*(25), 24261-24266. http://dx.doi.org/10.1074/jbc.M501773200

[204]   Boccini, F.; Herold, S. Mechanistic studies of the oxidation of oxyhemoglobin by peroxynitrite. *Biochemistry.,* **2004**, *43*(51), 16393-16404. http://dx.doi.org/10.1021/bi0482250

[205]   Herold, S.; Exner, M.; Boccini, F. The mechanism of the peroxynitrite-mediated oxidation of myoglobin in the absence and presence of carbon dioxide. *Chem. Res. Toxicol.,* **2003**, *16*(3), 390-402. http://dx.doi.org/10.1021/tx025595l

[206]   Thomson, L.; Trujillo, M.; Telleri, R.; Radi, R. Kinetics of cytochrome c2+ oxidation by peroxynitrite: implications for superoxide measurements in nitric oxide-producing biological systems. *Arch. Biochem. Biophys.,* **1995**, *319*(2), 491-497.

[207]   Hühmer, A.F.; Nishida, C.R.; Ortiz, de Montellano, P.R.; Schöneich, C. Inactivation of the inducible nitric oxide synthase by peroxynitrite. *Chem. Res. Toxicol.,* **1997**, *10*(5), 618-626.

[208]   Castro, L.; Rodriguez, M.; Radi, R. Aconitase is readily inactivated by peroxynitrite, but not by its precursor, nitric oxide. *J. Biol. Chem.,* **1994**, *269*(47), 29409-29415. PubMed ID: 7961920

[209]   Keyer, K.; Imlay, J.A. Inactivation of dehydratase [4Fe-4S] clusters and disruption of iron homeostasis upon cell exposure to peroxynitrite. *J. Biol. Chem.* **1997**, *272*(44), 27652-27659. http://dx.doi.org/10.1074/jbc.272.44.27652

[210]   Zou, M.H.; Cohen, R.; Ullrich, V. Peroxynitrite and vascular endothelial dysfunction in diabetes mellitus. *Endothelium.,* **2004**, *11*(2), 89-97. http://dx.doi.org/10.1080/10623320490482619

[211]   Crow, J.P.; Beckman, J.S.; McCord, J.M. Sensitivity of the essential zinc-thiolate moiety of yeast alcohol dehydrogenase to hypochlorite and peroxynitrite. *Biochemistry.,* **1995**, *34*(11), 3544-3552. http://dx.doi.org/10.1021/bi00011a008

[212]   Radi, R.; Beckman, J.S.; Bush, K.M.; Freeman, B.A. Peroxynitrite oxidation of sulfhydryls. The cytotoxic potential of superoxide and nitric oxide. *J. Biol. Chem.,* **1991**, *266*(7), 4244-4250. PubMed ID: 1847917

[213]   Pacher, P.; Obrosova, I.G.; Mabley, J.G.; Szabó, C. Role of nitrosative stress and peroxynitrite in the pathogenesis of diabetic complications. Emerging new therapeutical strategies. *Curr. Med. Chem.,* **2005**, *12*(3), 267-275. http://dx.doi.org/10.2174/0929867053363207

[214]   Rork, T.H.; Hadzimichalis, N.M.; Kappil, M.A.; Merrill, G.F. Acetaminophen attenuates peroxynitrite-activated matrix metalloproteinase-2-mediated troponin I cleavage in the isolated guinea pig myocardium. *J. Mol. Cell. Cardiol.,* **2006**, *40*(4), 553-561. http://dx.doi.org/10.1016/j.yjmcc.2006.01.010

[215]   Wang, W.; Sawicki, G.; Schulz, R. Peroxynitrite-induced myocardial injury is mediated through matrix metalloproteinase-2. *Cardiovasc. Res.* **2002**, *53*(1), 165-174. http://dx.doi.org/10.1016/S0008-6363(01)00445-X

[216]   Gürsoy-Ozdemir, Y.; Can, A.; Dalkara, T. Reperfusion-induced oxidative/nitrative injury to neurovascular unit after focal cerebral ischemia. *Stroke.,* **2004**, *35*(6), 1449-1453. http://dx.doi.org/10.1161/01.STR.0000126044.83777.f4

[217]   Ischiropoulos, H.; Zhu, L.; Chen, J.; Tsai, M.; Martin, J.C.; Smith, C.D.; Beckman, J.S. Peroxynitrite-mediated tyrosine nitration catalyzed by superoxide dismutase. *Arch. Biochem. Biophys.,* **1992**, *298*(2), 431-437. http://dx.doi.org/10.1016/0003-9861(92)90431-U

[218]   MacMillan-Crow, L.A.; Thompson, J.A. Tyrosine modifications and inactivation of active site manganese superoxide dismutase mutant (Y34F) by peroxynitrite. *Arch. Biochem. Biophys.,* **1999**, *366*(1), 82-88. http://dx.doi.org/10.1006/abbi.1999.1202

[219]   Yamakura, F.; Taka, H.; Fujimura, T.; Murayama, K. Inactivation of human manganese-superoxide dismutase by peroxynitrite is caused by exclusive nitration of tyrosine 34 to 3-nitrotyrosine. *J. Biol. Chem.,* **1998**, *273*(23), 14085-14089. http://dx.doi.org/10.1074/jbc.273.23.14085

[220]   Aykaç-Toker, G.; Bulgurcuoğlu, S.; Koçak-Toker, N. Effect of peroxynitrite on glutaredoxin. *Hum. Exp. Toxicol.,* **2001**, *20*(7), 373-376. http://dx.doi.org/10.1191/096032701680350578

[221]   Trujillo, M.; Folkes, L.; Bartesaghi, S.; Kalyanaraman, B.; Wardman, P.; Radi, R. Peroxynitrite-derived carbonate and nitrogen dioxide radicals readily react with lipoic and dihydrolipoic acid. *Free Radic. Biol. Med.,* **2005**, *39*(2), 279-288. http://dx.doi.org/10.1016/j.freeradbiomed.2005.03.014

[222]   Cuzzocrea, S.; Zingarelli, B.; O'Connor, M.; Salzman, A.L.; Szabó, C. Effect of L-buthionine-(S,R)-sulphoximine, an inhibitor of gamma-glutamylcysteine synthetase on peroxynitrite- and endotoxic shock-induced vascular failure. *Br. J. Pharmacol.,* **1998**, *123*(3), 525-537. DOI: 10.1038/sj.bjp.0701612

[223]   Arteel, G.E.; Briviba, K.; Sies, H. Protection against peroxynitrite. *FEBS Lett.,* **1999**, *445* (2-3), 226-230. http://dx.doi.org/10.1016/S0014-5793(99)00073-3

[224]   Cuzzocrea, S.; Costantino, G.; Mazzon, E.; Caputi, A.P. Protective effect of N-acetylcysteine on multiple organ failure induced by zymosan in the rat. *Crit. Care Med.,* **1999**, *27*(8), 1524-1532. http://dx.doi.org/10.1097/00003246-199908000-00021

[225]   Jonnala, R.R.; Buccafusco, J.J. Inhibition of nerve growth factor signaling by peroxynitrite. *J. Neurosci. Res.,* **2001**, *63*(1), 27-34. http://dx.doi.org/10.1002/1097-4547(20010101)63:1<27::AID-JNR4>3.3.CO;2-R

[226]   Good, P.F.; Werner, P.; Hsu, A.; Olanow, C.W.; Perl, D.P. Evidence of neuronal oxidative damage in Alzheimer's disease. *Am. J. Pathol.,* **1996**, *149*(1), 21-28. PMCID: PMC1865248

[227]   Smith, M.A.; Richey Harris, P.L.; Sayre, L.M.; Beckman, J.S.; Perry, G. Widespread peroxynitrite-mediated damage in Alzheimer's disease. *J. Neurosci.,* **1997**, *17*(8), 2653-2657. PMID: 9092586

[228]   Good, P.F.; Hsu, A.; Werner, P.; Perl, D.P.; Olanow, C.W. Protein nitration in Parkinson's disease. *J. Neuropathol. Exp. Neurol.,* **1998**, *57*(4), 338-342. http://dx.doi.org/10.1097/00005072-199804000-00006

[229]   Cookson, M.R.; Shaw, P.J.. Oxidative stress and motor neurone disease. *Brain Pathol.,* **1999**, *9*(1), 165-186. http://dx.doi.org/10.1111/j.1750-3639.1999.tb00217.x

[230]   Schmidt, P.; Youhnovski, N.; Daiber, A.; Balan, A.; Arsic, M.; Bachschmid, M.; Przybylski, M.; Ullrich, V. Specific nitration at tyrosine 430 revealed by high resolution mass spectrometry as basis for redox regulation of bovine prostacyclin synthase. *J. Biol. Chem.,* **2003**, *278*(15), 12813-12819. http://dx.doi.org/10.1074/jbc.M208080200

[231]   Birnboim, H.C.; Lemay, A.M.; Lam, D.K.; Goldstein, R.; Webb, J.R. Cutting edge: MHC class II-restricted peptides containing the inflammation-associated marker 3-nitrotyrosine evade central tolerance and elicit a robust cell-mediated immune response. *J. Immunol.,* **2003**, 171(2), 528-532. PMID:12847213

[232]   Ohmori, H.; Kanayama, N. Immunogenicity of an inflammation-associated product, tyrosine nitrated self-proteins. *Autoimmun. Rev.,* **2005**, *4*(4), 224-229. http://dx.doi.org/10.1016/j.autrev.2004. 11.011

[233]   Hsu, H.C.; Zhou, T.; Kim, H.; Barnes, S.; Yang, P.; Wu, Q.; Zhou, J.; Freeman, B.A.; Luo, M.; Mountz, J.D. Production of a novel class of polyreactive pathogenic autoantibodies in BXD2 mice causes glomerulonephritis and arthritis. *Arthritis Rheum.,* **2006**, *54*(1), 343-355. http://dx.doi.org/10.1002/art.21550

[234]   Khan, F., Ali, R. Antibodies against nitric oxide damaged poly L-tyrosine and 3-nitrotyrosine levels in systemic lupus erythematosus. *J. Biochem. Mol. Biol.,* **2006**, *39* (2), 189-196. http://dx.doi.org/ 10.5483/BMBRep.2006.39.2.189

[235]   Radi, R.; Beckman, J.S.; Bush, K.M.; Freeman, B.A. Peroxynitrite-induced membrane lipid peroxidation: the cytotoxic potential of superoxide and nitric oxide. *Arch. Biochem. Biophys.,* **1991**, 288(2), 481-487. http://dx.doi.org/10.1016/0003-9861(91)90224-7

[236]   Shi, H.; Noguchi, N.; Xu, Y.; Niki, E. Formation of phospholipid hydroperoxides and its inhibition by alpha-tocopherol in rat brain synaptosomes induced by peroxynitrite. *Biochem. Biophys. Res. Commun.,* **1999**, *257*(3), 651-656. http://dx.doi.org/10.1006/ bbrc.1999.0434

[237]   Smith, K.J.; Kapoor, R.; Felts, P.A. Demyelination: the role of reactive oxygen and nitrogen species. *Brain Pathol.,* **1999**, *9*(1), 69-92. http://dx.doi.org/10.1111/j.1750-3639.1999.tb00212.x

[238]   van der Veen, R.C.; Roberts, L.J. Contrasting roles for nitric oxide and peroxynitrite in the peroxidation of myelin lipids. *J. Neuroimmunol.,* **1999**, *95*(1-2), 1-7. http://dx.doi.org/10.1016/ S0165-5728(98)00239-2

[239]   Burney, S., Caulfield, J.L., Niles, J.C., Wishnok, J.S., Tannenbaum, S.R., 1999. The chemistry of DNA damage from nitric oxide and peroxynitrite. *Mutat. Res., 424* (1-2), 37-49. http://dx.doi.org/ 10.1016/S0027-5107(99)00006-8

[240]   Niles, J.C.; Wishnok, J.S.; Tannenbaum, S.R. Peroxynitrite-induced oxidation and nitration products of guanine and 8-oxoguanine: structures and mechanisms of product formation. *Nitric Oxide.,* **2006**, *14*(2), 109-121. http://dx.doi.org/10.1016/ j.niox.2005.11.001

[241]   Yu, H.; Venkatarangan, L.; Wishnok, J.S.; Tannenbaum, S.R. Quantitation of four guanine oxidation products from reaction of DNA with varying doses of peroxynitrite. *Chem. Res. Toxicol.,* **2005**, *18*(12), 1849-1857. http://dx.doi.org/10.1021/tx050146h

[242]   Buchczyk, D.P.; Grune, T.; Sies, H.; Klotz, L.O. Modifications of glyceraldehyde-3-phosphate dehydrogenase induced by increasing concentrations of peroxynitrite: early recognition by 20S proteasome. *Biol. Chem.,* **2003**, *384* (2), 237-241. DOI: 10.1515/ BC.2003.026

[243]   Souza, J.M.; Radi, R. Glyceraldehyde-3-phosphate dehydrogenase inactivation by peroxynitrite. *Arch. Biochem. Biophys.,* **1998**, *360*(2), 187-194. http://dx.doi.org/10.1006/abbi.1998.0932

[244]   Konorev, E.A.; Hogg, N.; Kalyanaraman, B. Rapid and irreversible inhibition of creatine kinase by peroxynitrite. *FEBS Lett.,* **1998**, *427*(2), 171-174. http://dx.doi.org/10.1016/S0014-5793(98)00413-X

[245]   Brown, G.C.; Borutaite, V. Inhibition of mitochondrial respiratory complex I by nitric oxide, peroxynitrite and S-nitrosothiols. *Biochim. Biophys. Acta.,* **2004**, *1658*(1-2), 44-49. http://dx.doi.org/ 10.1016/j.bbabio.2004.03.016

[246]   Bolaños, J.P.; Heales, S.J.; Land, J.M.; Clark, J.B. Effect of peroxynitrite on the mitochondrial respiratory chain: differential susceptibility of neurones and astrocytes in primary culture. *J. Neurochem.,* **1995**, *64*(5), 1965-1972. http://dx.doi.org/10.1046/ j.1471-4159.1995.64051965.x

[247]   Pearce, L.L.; Kanai, A.J.; Epperly, M.W.; Peterson, J. Nitrosative stress results in irreversible inhibition of purified mitochondrial complexes I and III without modification of cofactors. *Nitric Oxide.,* **2005**, *13*(4), 254-263. http://dx.doi.org/10.1016/j.niox. 2005.07.010

[248]   Radi, R.; Cassina, A.; Hodara, R. Nitric oxide and peroxynitrite interactions with mitochondria. *Biol. Chem.,* **2002**, *383* (3-4), 401-409. http://dx.doi.org/10.1515/BC.2002.044

[249]   Radi, R., Cassina, A., Hodara, R., Quijano, C., Castro, L. Peroxynitrite reactions and formation in mitochondria. *Free Radic. Biol. Med.,* **2002b**, *33* (11), 1451-1464. http://dx.doi.org/10.1016/ S0891-5849(02)01111-5

[250]   Lopez, C.J.; Qayyum, I.; Mishra, O.P.; Delivoria-Papadopoulos, M. Effect of nitration on protein tyrosine phosphatase and protein phosphatase activity in neuronal cell membranes of newborn piglets. *Neurosci. Lett.,* **2005**, *0*(2), 78-81. http://dx.doi.org/ 10.1016/j.neulet.2005.04.089

[251]   Takakura, K.; Beckman, J.S.; MacMillan-Crow, L.A.; Crow, J.P. Rapid and irreversible inactivation of protein tyrosine phosphatases PTP1B, CD45, and LAR by peroxynitrite. *Arch. Biochem. Biophys.,* **1999**, *369*(2), 197-207. http://dx.doi.org/10.1006/ abbi.1999.1374

[252]   Cassina, A.M.; Hodara, R.; Souza, J.M.; Thomson, L.; Castro, L.; Ischiropoulos, H.; Freeman, B.A.; Radi, R. Cytochrome c nitration by peroxynitrite. *J. Biol. Chem.,* **2000**, *275*(28), 21409-21415. http://dx.doi.org/10.1074/jbc.M909978199

[253]   Jang, B.; Han, S. Biochemical properties of cytochrome c nitrated by peroxynitrite. *Biochimie.,* **2006**, *88*(1), 53-58. http://dx.doi.org/ 10.1016/j.biochi.2005.06.016

[254]   Han, D.; Canali, R.; Garcia, J.; Aguilera, R.; Gallaher, T.K.; Cadenas, E. Sites and mechanisms of aconitase inactivation by peroxynitrite: modulation by citrate and glutathione. *Biochemistry.,* **2005**, *44*(36), 11986-11996. http://dx.doi.org/10.1021/bi0509393

[255]   Forsmark-Andrée, P.; Persson, B.; Radi, R.; Dallner, G.; Ernster, L. Oxidative modification of nicotinamide nucleotide transhydrogenase in submitochondrial particles: effect of endogenous ubiquinol. *Arch. Biochem. Biophys.,* **1996**, *336*(1), 113-120. http://dx.doi.org/ 10.1006/abbi.1996.0538

[256]   Scarlett, J.L.; Packer, M.A.; Porteous, C.M.; Murphy, M.P. Alterations to glutathione and nicotinamide nucleotides during the mitochondrial permeability transition induced by peroxynitrite. *Biochem. Pharmacol.,* **1996**, *52*(7), 1047-1055. http://dx.doi.org/ 10.1016/0006-2952(96)99426-5

[257]   Vieira, H.L.; Belzacq, A.S.; Haouzi, D.; Bernassola, F.; Cohen, I.; Jacotot, E.; Ferri, K.F.; El Hamel, C.; Bartle, L.M.; Melino, G.; Brenner, C.; Goldmacher, V.; Kroemer, G. The adenine nucleotide translocator: a target of nitric oxide, peroxynitrite, and 4-hydroxynonenal. *Oncogene.,* **2001**, *20*(32), 4305-4316. http://dx. doi.org/10.1038/sj.onc.1204575

[258]   Szabó, C.; Zingarelli, B.; O'Connor, M.; Salzman, A.L. DNA strand breakage, activation of poly (ADP-ribose) synthetase, and cellular energy depletion are involved in the cytotoxicity of macrophages and smooth muscle cells exposed to peroxynitrite. *Proc. Natl. Acad. Sci. U. S. A.,* **1996**, *93*(5), 1753-1758. http://dx. doi.org/10.1073/pnas.93.5.1753

[259]   Liaudet, L. Poly(adenosine 5'-diphosphate) ribose polymerase activation as a cause of metabolic dysfunction in critical illness. *Curr. Opin. Clin. Nutr. Metab. Care,* **2002**, *5*(2), 175-184. PubMed ID: 11844985

[260]   Liaudet, L.; Szabó, G.; Szabó, C. Oxidative stress and regional ischemia-reperfusion injury: the peroxynitrite-poly(ADP-ribose) polymerase connection. *Coron. Artery Dis.,* **2003**, *14* (2), 115-122. http://dx.doi.org/10.1097/00019501-200304000-00004

[261]   Szabó, C. Multiple pathways of peroxynitrite cytotoxicity. *Toxicol. Lett.,* **2003**, 140-141, 105-112. http://dx.doi.org/10.1016/S0378-4274(02)00507-6

[262]   Ha, H.C.; Snyder, S.H. Poly(ADP-ribose) polymerase is a mediator of necrotic cell death by ATP depletion. *Proc. Natl. Acad. Sci. U. S. A.,* **1999**, *96*(24), 13978-13982. http://dx.doi.org/10.1073/pnas. 96.24.13978

[263]   Liaudet, L.; Oddo, M. Role of poly(adenosine diphosphate-ribose) polymerase 1 in septic peritonitis. *Curr. Opin. Crit. Care,* **2003**, 9(2), 152-158. http://dx.doi.org/10.1097/00075198-200304000-00012

[264]   Griendling, K.K.; Sorescu, D.; Ushio-Fukai, M. NAD(P)H oxidase: role in cardiovascular biology and disease. *Circ. Res.,* **2000**, *86*(5), 494-501. http://dx.doi.org/10.1161/01.RES.86.5.494

[265]   Ohtsu, H.; Frank, G.D.; Utsunomiya, H.; Eguchi, S. Redox-dependent protein kinase regulation by angiotensin II: mechanistic insights and its pathophysiology. *Antioxid. Redox. Signal.,* **2005**, *7*(9-10), 1315-1326. http://dx.doi.org/10.1089/ars.2005.7.1315

[266]   Paravicini, T.M.; Touyz, R.M. Redox signaling in hypertension. *Cardiovasc. Res.,* **2006**, *71*(2), 247-258. http://dx.doi.org/10.1016/ j.cardiores.2006.05.001

[267]   Tonks, N.K. Protein tyrosine phosphatases: from genes, to function, to disease. *Nat. Rev. Mol. Cell. Biol.*, **2006**, *7*(11), 833-846. http://dx.doi.org/10.1038/nrm2039

[268]   Mallozzi, C.; Di Stasi, A.M.; Minetti, M. Peroxynitrite modulates tyrosine-dependent signal transduction pathway of human erythrocyte band 3. *FASEB J.*, **1997**, *11*(14), 1281-1290. PubMed ID: 9409547

[269]   Gow, A.J.; Duran, D.; Malcolm, S.; Ischiropoulos, H. Effects of peroxynitrite-induced protein modifications on tyrosine phosphorylation and degradation. *FEBS Lett.*, **1996**, *385*(1-2), 63-66.

[270]   Kong, S.K.; Yim, M.B.; Stadtman, E.R.; Chock, P.B. Peroxynitrite disables the tyrosine phosphorylation regulatory mechanism: Lymphocyte-specific tyrosine kinase fails to phosphorylate nitrated cdc2(6-20)NH2 peptidDi Stasi e. *Proc. Natl. Acad. Sci. U. S. A.*, **1996**, *93*(8), 3377-3382.

[271]   Brito, C.; Naviliat, M.; Tiscornia, A.C.; Vuillier, F.; Gualco, G.; Dighiero, G.; Radi, R.; Cayota, A.M. Peroxynitrite inhibits T lymphocyte activation and proliferation by promoting impairment of tyrosine phosphorylation and peroxynitrite-driven apoptotic death. *J. Immunol.*, **1999**, *162*(6), 3356-3366. PubMed ID: 10092790

[272]   Maccaglia, A.; Mallozzi, C.; Minetti, M. Differential effects of quercetin and resveratrol on Band 3 tyrosine phosphorylation signalling of red blood cells. *Biochem. Biophys. Res. Commun.*, **2003**, *305*(3), 541-547. http://dx.doi.org/10.1016/S0006-291X(03)00762-9

[273]   Di Stasi, A.M.; Mallozzi, C.; Macchia, G.; Petrucci, T.C.; Minetti, M. Peroxynitrite induces tryosine nitration and modulates tyrosine phosphorylation of synaptic proteins. *J. Neurochem.*, **1999**, *73*(2), 727-735. http://dx.doi.org/10.1046/j.1471-4159.1999.0730727.x

[274]   Li, X.; De Sarno, P.; Song, L.; Beckman, J.S.; Jope, R.S. Peroxynitrite modulates tyrosine phosphorylation and phosphoinositide signalling in human neuroblastoma SH-SY5Y cells: attenuated effects in human 1321N1 astrocytoma cells. *Biochem. J.*, **1998**, *331*(Pt 2), 599-606. PubMed ID: 9531503

[275]   Dong, C.; Davis, R.J.; Flavell, R.A. MAP kinases in the immune response. *Annu. Rev. Immunol.*, **2001**, *20*, 55-72. http://dx.doi.org/10.1146/annurev.immunol.20.091301.131133

[276]   Kang, K.W.; Choi, S.H.; Kim, S.G. Peroxynitrite activates NF-E2-related factor 2/antioxidant response element through the pathway of phosphatidylinositol 3-kinase: the role of nitric oxide synthase in rat glutathione S-transferase A2 induction. *Nitric Oxide.*, **2002**, *7*(4), 244-253. http://dx.doi.org/10.1016/S1089-8603(02)00117-9

[277]   Qi, M.; Elion, E.A. MAP kinase pathways. *J. Cell. Sci.*, **2005**, *118*(Pt 16), 3569-3572. http://dx.doi.org/10.1242/jcs.02470

[278]   Andreone, T.L.; O'Connor, M.; Denenberg, A.; Hake, P.W.; Zingarelli, B. Poly(ADP-ribose) polymerase-1 regulates activation of activator protein-1 in murine fibroblasts. *J. Immunol.*, **2003**, *170*(4), 2113-2120. PubMed ID: 12574383

[279]   Go, Y.M.; Patel, R.P.; Maland, M.C.; Park, H.; Beckman, J.S.; Darley-Usmar, V.M.; Jo, H. Evidence for peroxynitrite as a signaling molecule in flow-dependent activation of c-Jun NH(2)-terminal kinase. *Am. J. Physiol.*, **1999**, *277*(4 Pt 2), H1647-H1653. PubMed ID: 10516206

[280]   Jope, R.S.; Zhang, L.; Song, L. Peroxynitrite modulates the activation of p38 and extracellular regulated kinases in PC12 cells. *Arch. Biochem. Biophys.*, **2000**, *376*(2), 365-370. http://dx.doi.org/10.1006/abbi.2000.1728

[281]   Kaji, T.; Kaieda, I.; Hisatsune, T.; Kaminogawa, S. 3-Morpholinosydnonimine hydrochloride induces p53-dependent apoptosis in murine primary neural cells: a critical role for p21(ras)-MAPK-p19(ARF) pathway. *Nitric Oxide*, **2002**, *6*(2), 125-134. DOI: 10.1006/niox.2001.0389

[282]   Zouki, C.; Zhang, S.L.; Chan, J.S.; Filep, J.G. Peroxynitrite induces integrin-dependent adhesion of human neutrophils to endothelial cells *via* activation of the Raf-1/MEK/Erk pathway. *FASEB J.*, **2001**, *15*(1), 25-27. PubMed ID: 11099490

[283]   Mallozzi, C.; Di Stasi, A.M.; Minetti, M. Activation of src tyrosine kinases by peroxynitrite. *FEBS Lett.*, **1999**, *456*(1), 201-206. http://dx.doi.org/10.1016/S0014-5793(99)00945-X

[284]   Mallozzi, C.; Di Stasi, M.A.; Minetti, M. Peroxynitrite-dependent activation of src tyrosine kinases lyn and hck in erythrocytes is under mechanistically different pathways of redox control. *Free Radic. Biol. Med.*, **2001**, *30*(10), 1108-1117. http://dx.doi.org/10.1016/S0891-5849(01)00509-3

[285]   Mallozzi, C.; De Franceschi, L.; Brugnara, C.; Di Stasi, A.M. Protein phosphatase 1alpha is tyrosine-phosphorylated and inactivated by peroxynitrite in erythrocytes through the src family kinase fgr. *Free Radic. Biol. Med.*, **2005**, *38*(12), 1625-1636. http://dx.doi.org/10.1016/j.freeradbiomed.2005.02.021

[286]   Hainaut, P.; Milner, J. Redox modulation of p53 conformation and sequence-specific DNA binding *in vitro*. *Cancer Res.*, **1993**, *53*(19), 4469-4473. PMID: 8402615.

[287]   Buizza, L.; Cenini, G.; Lanni, C.; Ferrari-Toninelli, G.; Prandelli, C.; Govoni, S.; Buoso, E.; Racchi, M.; Barcikowska, M.; Styczynska, M.; Szybinska, A.; Butterfield, D.A.; Memo, M.; Uberti, D. Conformational altered p53 as an early marker of oxidative stress in Alzheimer's disease. *PLoS One.*, **2012**, *7*(1), e29789. http://dx.doi.org/10.1371/journal.pone.0029789

[288]   Buizza, L.; Prandelli, C.; Bonini, S.A.; Delbarba, A.; Cenini, G.; Lanni, C.; Buoso, E.; Racchi, M.; Govoni, S.; Memo, M.; Uberti, D. Conformational altered p53 affects neuronal function: relevance for the response to toxic insult and growth-associated protein 43 expression. *Cell. Death Dis.*, **2013**, *4*, e484. http://dx.doi.org/10.1038/cddis.2013.13

[289]   Cobbs, C.S.; Whisenhunt, T.R.; Wesemann, D.R.; Harkins, L.E.; Van Meir, E.G.; Samanta, M. Inactivation of wild-type p53 protein function by reactive oxygen and nitrogen species in malignant glioma cells. *Cancer Res.*, **2003**, *63*(24), 8670-8673. PMID: 14695179

[290]   Platt, D.H.; Bartoli, M.; El-Remessy, A.B.; Al-Shabrawey, M.; Lemtalsi, T.; Fulton, D.; Caldwell, R.B. Peroxynitrite increases VEGF expression in vascular endothelial cells *via* STAT3. *Free Radic. Biol. Med.*, **2005**, *39*(10), 1353-1361. http://dx.doi.org/10.1016/j.freeradbiomed.2005.06.015

[291]   He, G.; Yu, G.Y.; Temkin, V.; Ogata, H.; Kuntzen, C.; Sakurai, T.; Sieghart, W.; Peck-Radosavljevic, M.; Leffert, H.L.; Karin, M. Hepatocyte IKKbeta/NF-kappaB inhibits tumor promotion and progression by preventing oxidative stress-driven STAT3 activation. *Cancer Cell*, **2010**, *17*(3), 286-297. http://dx.doi.org/10.1016/j.ccr.2009.12.048

[292]   Bartoli, M.; Platt, D.H.; Lemtalsi, T.; El Remessy, A.; Rojas, M.; Caldwell, R.W.; Caldwell, R.B. Molecular Mechanisms of Oxidative Stress-induced STAT3 Activation in Endothelial Cells. *FASEB J.*, **2006**, 20 (Meeting Abstract Supplement) A703.

[293]   Martindale, J.L.; Holbrook, N.J. Cellular response to oxidative stress: signaling for suicide and survival. *J. Cell. Physiol.*, **2002**, *192*(1), 1-15. http://dx.doi.org/10.1002/jcp.10119

[294]   Lee, H.; Herrmann, A.; Deng, J.H.; Kujawski, M.; Niu, G.; Li, Z.; Forman, S.; Jove, R.; Pardoll, D.M.; Yu, H. Persistently activated Stat3 maintains constitutive NF-jB activity in tumors. *Cancer Cell.*, **2009**, *15*(4), 283-293. http://dx.doi.org/10.1016/j.ccr.2009.02.015

[295]   Grivennikov, S.I.; Karin, M. Dangerous liaisons: STAT3 and NF-kappaB collaboration and crosstalk in cancer. *Cytokine Growth Factor Rev.*, **2010**, *21*(1), 11-19. http://dx.doi.org/10.1016/j.cytogfr.2009.11.005

[296]   Ak, P.; Levine, A.J. P53 and NF-jB: different strategies for responding to stress lead to a functional antagonism. *FASEB J.*, **2010**, *24*(10), 3643-3652. http://dx.doi.org/10.1096/fj.10-160549

[297]   Johnson, R.F.; Witzel, II.; Perkins, N.D. P53-dependent regulation of mitochondrial energy production by the RelA subunit of NF-jB. *Cancer Res.*, **2011**, *71*(16), 5588-5597. http://dx.doi.org/10.1158/0008-5472.CAN-10-4252

[298]   Mauro, C.; Leow, S.C.; Anso, E.; Rocha, S.; Thotakura, A.K.; Tornatore, L.; Moretti, M.; De Smaele, E.; Beg, A.A.; Tergaonkar, V.; Chandel, N.S.; Franzoso, G. NF-jB controls energy homeostasis and metabolic adaptation by upregulating mitochondrial respiration. *Nat. Cell. Biol.*, **2011**, *13*(10), 1272-1279. http://dx.doi.org/10.1038/ncb2324

[299]   Assaily, W. Benchimol, S. Differential utilization of two ATP-generating pathways is regulated by p53. *Cancer Cell.*, **2006**, *10* (1), 4-6. http://dx.doi.org/10.1016/j.ccr.2006.06.014

[300]   Park, J.Y.; Wang, P.Y.; Matsumoto, T.; Sung, H.J.; Ma, W.; Choi, J.W.; Anderson, S.A.; Leary, S.C.; Balaban, R.S.; Kang, J.G.; Hwang, P.M. P53 improves aerobic exercise capacity and augments skeletal muscle mitochondrial DNA content. *Circ. Res.*, **2009**, *105*(7), 705-712. http://dx.doi.org/10.1161/CIRCRESAHA.109.205310

[301]   Saleem, A.; Adhihetty, P.J.; Hood, D.A. Role of p53 in mitochondrial biogenesis and apoptosis in skeletal muscle. *Physiol.*

Genomics., **2009**, *37*(1), 58-66. http://dx.doi.org/10.1152/physiolgenomics.90346.2008

[302]   Kawauchi, K.; Araki, K.; Tobiume, K.; Tanaka, N. P53 regulates glucose metabolism through an IKK-NF-kappaB pathway and inhibits cell transformation. *Nat. Cell. Biol.,* **2008**, *10*(5), 611-618. http://dx.doi.org/10.1038/ncb1724

[303]   Johnson, R.F.; Perkins, N.D. Nuclear factor-jB, p53, and mitochondria: regulation of cellular metabolism and the Warburg effect. *Trends. Biochem. Sci.,* **2012**, *37*(8), 317-324. http://dx.doi.org/10.1016/j.tibs.2012.04.002

[304]   Zhang, C.; Lin, M.; Wu, R.; Wang, X.; Yang, B.; Levine, A.J.; Hu, W.; Feng, Z. Parkin, a p53 target gene, mediates the role of p53 in glucose metabolism and the Warburg effect. *PNAS.,* **2011**, *108*(39), 16259-16264. http://dx.doi.org/10.1073/pnas.1113884108

[305]   Morris, G.; Maes, M. Increased nuclear factor-κB and loss of p53 are key mechanisms in Myalgic Encephalomyelitis/chronic fatigue syndrome (ME/CFS). *Med. Hypotheses.,* **2012**, *79*(5), 607-613. http://dx.doi.org/10.1016/j.mehy.2012.07.034

[306]   Ma, W.; Sung, H.J.; Park, J.Y.; Matoba, S.; Hwang, P.M. A pivotal role for p53: balancing aerobic respiration and glycolysis. *J. Bioenerg. Biomembr.,* **2007**, *39*(3), 243-246. http://dx.doi.org/10.1007/s10863-007-9083-0

[307]   Bensaad, K.; Vousden, K.H. P53: new roles in metabolism. *Trends. Cell. Biol.,* **2007**, *17*, 286-291. http://dx.doi.org/10.1016/j.tcb.2007.04.004

[308]   Olovnikov, I.A.; Kravchenko, J.E.; Chumakov, P.M. Homeostatic functions of the p53 tumor suppressor: regulation of energy metabolism and antioxidant defense. *Semin. Cancer Biol.,* **2009**, *19*(1), 32-41. http://dx.doi.org/10.1016/j.semcancer.2008.11.005