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EDITORIAL

## The 4I Hypothesis: A Neuro-Immunological Explanation for Characteristic Symptoms of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome

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abnormalities: inflammation, (Th2-predominated) immune activation, immunosuppression, and immune dysfunction. The potential direct sequels of these abnormalities, e.g. increased oxidative and nitrosative stress, (re)activating or chronic infections, and their possible indirect consequences, e.g. mitochondrial dysfunction, hypothalamic-pituitary-adrenal axis (HPA) axis hypofunction, and cardiovascular dysregulation, can plausibly explain various distinctive symptoms of ME/CFS, e.g. “exhaustion”, (muscle) weakness, pain, cognitive deficits, a flu-like feeling, and post-exertional “malaise”.

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### ABSTRACT

Characteristic symptoms of Myalgic Encephalomyelitis (ME) are (muscle) weakness, muscle pain, cognitive deficits, neurological abnormalities, but above all post-exertional malaise: a long-lasting increase of symptoms after a minor exertion. In contrast, Chronic Fatigue Syndrome (CFS) is primarily defined by “chronic fatigue”. Since “chronic fatigue” is not mandatory for the diagnosis ME, and post-exertional malaise and cognitive deficits are not obligatory for the diagnosis CFS, the case criteria for ME and CFS define two distinct, partly overlapping nosological entities. ME and CFS are considered to be enigmatic diseases, qualified by some authors as “medically unexplained syndromes” of “functional syndromes”. However, specific abnormalities consistently observed over the years and their direct and indirect sequels can plausibly explain characteristic symptoms, e.g. “exhaustion” and pain. Abnormalities established repetitively incorporate immunological aberrations (inflammation, immune activation, immunosuppression, and immune dysfunction), persistent and/or reactivating infections, gastrointestinal dysbiosis, oxidative and nitrosative stress, mitochondrial dysfunction, a (prolonged) deviant response to exertion and orthostatic stress, circulatory deficits, and neurological abnormalities. This article depicts the 4I hypothesis, an explanatory model for ME (CFS) with a central role for four types of immunological

**Key words:** Myalgic Encephalomyelitis; Chronic fatigue syndrome; Immunology; Neurology; Oxidative stress; Nitrosative stress; Pathophysiology; Hypothesis; Post-exertional malaise

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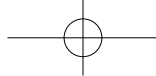
### CORE TIPS

ME and CFS are two distinct, partially overlapping diagnostic entities.

More consistent findings in ME and/or CFS relate to four types of immunological abnormalities: inflammation (I1), (Th2-dominated) immune activation (I2), immunosuppression (I3), and immune dysfunction (I4).

The potential sequels of these abnormalities encompass oxidative/nitrosative stress and infections.

The possible indirect consequences of the immunological aberrations and increased oxidative/nitrosative stress include neurological



## Twisk FNM. 4I hypothesis for Myalgic Encephalomyelitis

abnormalities, mitochondrial dysfunction and cardiovascular disturbances.

The direct and indirect sequels of the immunological abnormalities can plausibly explain distinctive symptoms of ME/CFS, e.g. cognitive deficits, and post-exertional “malaise”: a prolonged aggravation of characteristic symptoms, e.g. cognitive impairments and pain, after a minor exertion.

## INTRODUCTION

Although ME and CFS are often used interchangeably, the case criteria for ME<sup>[1]</sup> and CFS<sup>[2]</sup> define two distinct, partially overlapping diagnostic entities<sup>[3]</sup>. The diagnosis ME requires specific neurological/neurocognitive and immunological symptoms and energy production and/or transport impairment, but the distinctive feature of ME is post-exertional malaise or “neuro-immune exhaustion”: ‘a pathological inability to produce sufficient energy on demand’ resulting into symptom exacerbation, e.g. flu-like symptoms and pain, after minor exertion<sup>[1]</sup>. The distinctive feature of CFS<sup>[2]</sup> on the other hand is (unexplained) chronic fatigue, which should be accompanied by at least four out of a eight symptoms, e.g. sore throat, unrefreshing sleep, and headaches. While post-exertional malaise is not obligatory for CFS<sup>[2]</sup>, “fatigue” is not mandatory for the diagnosis ME<sup>[1]</sup>. The distinction between patients with post-exertional malaise and without post-exertional malaise seems to be reflected by specific immunological differences<sup>[4,5]</sup>. Although ME and CFS criteria select partially overlapping, partially disjoint patient groups, the majority of the research into ME/CFS in the last decades has been conducted in patients selected by CFS criteria<sup>[2]</sup>. However, since many optional symptoms of CFS are mandatory for the diagnosis ME, the CFS criteria also apply to a substantial ME patient subgroup reporting “fatigue” (Figure 1). In conclusion, while ME is a neuro-immunological disease in nature<sup>[1]</sup>, the CFS criteria<sup>[2]</sup> select a heterogeneous patient population of people with self-reported “chronic fatigue”.

## SYMPTOMS

Notwithstanding the debate about the distinction between ME and CFS<sup>[6-8]</sup> and definitional criteria of ME and CFS<sup>[9]</sup>, including obligatory symptoms, many patients with ME/CFS experience a plethora of symptoms<sup>[3]</sup>, which differ inter-individually and seem to fluctuate in number and severity within an individual over time as a consequence of daily activity<sup>[10]</sup>. Symptoms experienced by

substantial patient subgroups are: post-exertional malaise, “fatigue”/ lack of energy, muscle weakness, (muscle/joint) pain, cognitive impairment (“brain fog”), a flu-like feeling, sleep dysfunction (“unrefreshing sleep”), hypersensitivity to food, light, sound and odours (“central sensitisation”), stress intolerance, orthostatic intolerance and depression<sup>[9]</sup> (Table 1). Various characteristic symptoms can be assessed objectively using well-accepted methods<sup>[3]</sup>, e.g. neurocognitive tests, while other symptoms due to their nature, e.g. (muscle) pain, cannot be assessed objectively.

**Table 1** Characteristic symptoms present in many patients with ME/CFS.

Symptom	References
“Fatigue”: a lack of energy resources needed for basic daily functioning, “brain fog”, post-exertional “fatigue”, a “wired feeling” when exhausted, and a flu-like feeling *1	[9,11]
Infectious type symptoms	[5,12]
Muscle weakness/fatigability *1	[13,14]
Widespread muscle and/or joint pain	[15,16]
Orthostatic intolerance	[17,18]
Depression	[19,20]
Headaches	[21,22]
Hyperalgesia and hypersensitivity to light, sound etc.	[21,23]
Neurocognitive impairment	[19,24]
Sleep disturbances	[19,25]
Stress intolerance	[26,27]
Abdominal pain and discomfort	[28,29]
Post-exertional malaise	[30,31]

\*1 A clear distinction should be made between the perception of “fatigue”, a feeling, and “(muscle) fatigability”: a (prolonged) decline of muscle power after repeated muscle contractions, which can be assessed objectively using dynamometers.

## ABNORMALITIES

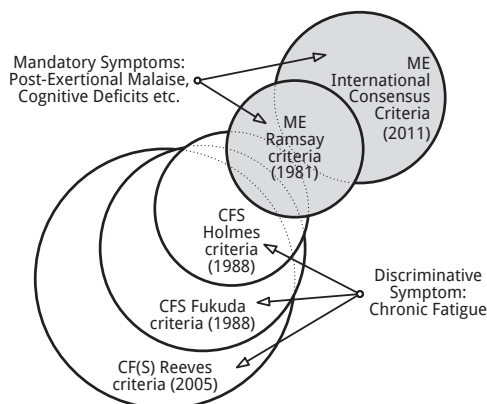
Partly due to the heterogeneity<sup>[32]</sup> of the CFS<sup>[2]</sup> patient population and the variety of methods employed and samples investigated, research into ME/CFS has yielded contradictory results. However, various typical aberrations (Table 2) have been observed repetitively in the ME/CFS patient population or subgroups thereof<sup>[1,33]</sup>. Several abnormalities are confirmed by differential gene expression<sup>[34-37]</sup>.

### Onset

Contrary to gradual onset ME/CFS, sudden-onset ME/CFS is often preceded by a (viral) infection/“flu-like” illness<sup>[19,95]</sup>. The onset is

**Table 2** Abnormalities in ME/CFS patients or major patient subgroups.

Abnormality	References
Immunological aberrations :	[38-41]
• inflammation,	
• (Th2-dominated) immune activation,	
• immunosuppression and	
• immune dysfunction;	
which seem consistent with an infectious state;	[42-45]
Infections (reactivating and/or persistent);	[45-48]
Intestinal dysbiosis, inflammation and hyperpermeability, possibly associated with systemic inflammation;	[49-52]
Enhanced oxidative and nitrosative stress;	[56-59]
Mitochondrial dysfunction and damage to mitochondria;	[60-63]
Hypovolemia and/or low cardiac output and reduced blood and oxygen supply to muscles and brain, notably in an upright position and during exercise;	[11,64-66]
Reduced (maximum) oxygen uptake and anaerobic threshold;	[67-70]
Neurological abnormalities (including neuro-inflammation);	[71-74]
HPA axis dysfunction, including hypocortisolism and blunted HPA axis responses to response to adrenocorticotrophic hormone (ACTH);	[75-78]
Channelopathy (ion channel dysfunction);	[79-82]
(Long-lasting) abnormal responses to exertion.	[83-86]
	[87-90]
	[76,91-93]



**Figure 1** ME and CFS case definitions.

reflected by distinctive immunological aberrations<sup>[96,97]</sup> and other abnormalities<sup>[98,99]</sup>. Several pathogens have been reported to initiate ME/CFS, e.g. Epstein-Barr virus<sup>[100]</sup>, parvovirus B19<sup>[101]</sup>, and enteroviruses<sup>[48]</sup>. For example, 10-15% of individuals do not recover from infectious mononucleosis and fulfil the criteria for CFS<sup>[2]</sup> after six months<sup>[100,102]</sup>. The severity of the acute infection seems to predict the clinical outcome, rather than demographic, psychological, or microbiological factors<sup>[102,103]</sup>.

### Infections

Although contradicted by some studies, applying various methods various studies have found (multiple) infections or related antigens in patient subgroups (Table 3).

**Table 3** Pathogens/pathogen-related antigens observed in ME/CFS patient subgroups.

Pathogen and pathogen-associated antigens	References
<b>Viruses</b>	
Epstein-Barr Virus (EBV, HHV4)	[104-106]
Cytomegalovirus (CMV, HHV5)	[47,107,108]
Human Herpesvirus 6 (HHV6A and B)	[109,110]
Human Herpesvirus 7 (HHV7)	[106,111,112]
Enteroviruses	[113-115]
Parvovirus B19	[116-118]
Bornavirus	[119-121]
<b>Bacteria</b>	
Coxiella burnetii	[122-124]
Chlamydia pneumonia	[46,47,108]
Borrelia burgdorferi	[47,125,126]
Enterobacteria	[50,53]
<b>Other</b>	
Mycoplasma	[127-129]

### Intestinal dysbiosis and hyperpermeability

Some studies have observed intestinal dysbiosis<sup>[49,51]</sup>, intestinal inflammation and immune activation<sup>[52,130]</sup> and intestinal hyperpermeability, conceivably resulting into translocation of enterobacteria to the blood stream, thereby inducing systemic inflammation<sup>[50]</sup>. Inflammation and immune activation observed in ME/CFS have been associated with these gastro-intestinal abnormalities<sup>[53,54]</sup>. Bacteriotherapy (transcolonoscopic infusion of non-pathogenic enteric bacteria) showed long-term positive effects (15-20 year) in a substantial subgroup of ME/CFS patients with gastrointestinal complaints in a retrospective follow-up study<sup>[131]</sup>.

### Immunological abnormalities

Consistent findings in ME/CFS relate to immunological aberrations: (a) inflammation<sup>[41,132,133]</sup>; (b) (Th2-predominant) immune activation<sup>[48,134-136]</sup>; (c) immunosuppression, especially low NK cell activity (NKCA)<sup>[43,137,138]</sup>, blunted responses to mitogens<sup>[38,139,140]</sup>, and IgG deficiencies, most often IgG1 and IgG3<sup>[141,142]</sup>; and d) immune dysfunction, e.g. predominance of the humoral (Th2) immune response accompanied by suppression of the cellular (Th1) immune system, possibly due to altered glucocorticoid regulation of the immune response<sup>[143,144]</sup>, and dysregulation of the RNase-L pathway, likely due to cleavage of the native 83-kDa RNase L<sup>[145,146]</sup>.

### Increased oxidative and nitrosative stress

Elevated oxidative and nitrosative stress<sup>[36,88]</sup>, increased levels of superoxide (O<sub>2</sub><sup>-</sup>), nitric oxide (NO) and peroxynitrite (ONOO<sup>-</sup>), oxidative and nitrosative damage to DNA, proteins, lipids etc.<sup>[147-149]</sup>, and antioxidant depletion / increased antioxidant activity, e.g. vitamin A<sup>[150]</sup>, B<sup>[151]</sup>, C<sup>[56]</sup>, D<sup>[152]</sup>, E<sup>[56]</sup>, glutathione<sup>[153]</sup>, super oxide dismutase<sup>[62]</sup>

and zinc<sup>[154]</sup>, have been observed repetitively.

### Mitochondrial dysfunction and damage

Some studies have found structural mitochondrial damage, e.g. branching and fusion of mitochondrial cristae /mitochondrial degeneration<sup>[63]</sup>, substantially higher rates of deletion of common 4977 bp of mitochondrial DNA<sup>[155]</sup> and unusual patterns of mitochondrial DNA deletions in skeletal muscle<sup>[59]</sup>, while other studies implicate mitochondrial dysfunction<sup>[60,61,156]</sup>. Future research should provide clarity whether hypometabolism<sup>[157]</sup> and low oxygen uptake<sup>[75,78]</sup> and extraction<sup>[158]</sup> in ME/CFS is due to mitochondrial dysfunction and/or mitochondrial damage, circulatory deficits (see next paragraph) or other causes.

### Low blood volume, cardiac output and/or blood and oxygen supply

Several studies have established markedly reduced blood volume<sup>[64,65,75]</sup> and impaired cardiac function, indicated by decreased cardiac index/output and stroke volume<sup>[65,66,74]</sup>, when compared to healthy sedentary controls. Post-exertional malaise, flu-like symptoms and cognitive deficits seem to differentiate those with severe CFS from those with less severe CFS and to predict lower cardiac output<sup>[74]</sup>. In addition, some studies indicate low cardiac mass<sup>[66,159]</sup>. So, low cardiac output in ME/CFS could be due to reduced cardiac mass (a 'small heart')<sup>[160]</sup> and/or hypovolemia<sup>[65]</sup>. Low cardiac output and/or blood/oxygen supply to muscles<sup>[69]</sup> and brain<sup>[67]</sup> and/or mitochondrial abnormalities could explain the low exercise capacity/oxygen uptake<sup>[75,161]</sup> and elevated (ventricular) lactate levels<sup>[68]</sup>.

### Orthostatic abnormalities

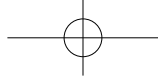
Orthostatic intolerance, (delayed) orthostatic hypotension and/or tachycardia (POTS), in patient subgroups has been implicated by various studies<sup>[73,162,163]</sup>. Orthostatic stress seems to induce cognitive deficits<sup>[24,164]</sup> and reduced angle-related mental task-activated cerebral blood flow velocity<sup>[164]</sup>. According to some authors<sup>[165]</sup>, POTS marks a distinct group of patients with distinct phenotypical features. Some studies have also observed abnormalities in (parasympathetic and sympathetic) heart rate variability at rest and on standing<sup>[166]</sup>.

### Neurological abnormalities

Over time various researchers have observed various neurological aberrations in ME/CFS patient subgroups<sup>[79,81]</sup>, e.g. diminished grey<sup>[82,167]</sup> and white matter<sup>[82,168]</sup>, reduced blood flow and hypoperfusion<sup>[169,170]</sup>, glucose hypometabolism in specific regions of the cerebrum and the brain stem<sup>[171]</sup>, an increased number of defects on SPECT scans, predominantly in the frontal and temporal lobes<sup>[172]</sup>, intracranial abnormalities on MRI and SPECT scans<sup>[173]</sup>, neuro-inflammation in various brain areas<sup>[174]</sup>, cerebrospinal fluid anomalies suggesting inflammation and immune activation<sup>[175,176]</sup>, and elevated ventricular lactate levels<sup>[68]</sup>.

### HPA axis dysfunction

HPA axis hypofunction in ME/CFS<sup>[83,177]</sup> can potentially manifest itself in (a) low basal levels and diurnal production levels (total production, variation during the day) of stress hormones, especially cortisol (hypocortisolism)<sup>[178,179]</sup>; (b) hyporesponsiveness of the HPA axis: blunted responses of the pituitary and the adrenal glands to provocation, e.g. reduced cortisol response to ACTH<sup>[85,180]</sup>; (c) diminished HPA axis responses to stress and exercise<sup>[181,182]</sup> and (d) enhanced sensitivity of the HPA axis to negative feedback to



cortisol<sup>[183,184]</sup> and increased sensitivity of the cellular immune system to the immunosuppressive effects of cortisol<sup>[143,144]</sup>.

**Abnormal responses to exercise**

Several studies have established (long-lasting) deviant responses to physical exertion in ME/CFS, when compared to sedentary controls, e.g. substantially lower values for oxygen uptake and workload at peak exercise and at the ventilatory or anaerobic threshold at a second exercise test 24 hours later<sup>[77,91,185]</sup>; a long-term increase of gene expression of metabolite-detecting receptors after sustained moderate exercise<sup>[311]</sup>; decreased prefrontal oxygenation during exercise and recovery<sup>[71]</sup>; (prolonged) severe oxidative stress<sup>[93]</sup> and (long-lasting) suppression of protective heat shock proteins<sup>[92]</sup> in response to exercise, especially when a history of high level physical activity and infection is present<sup>[150]</sup>; cognitive deficits induced by exercise<sup>[186]</sup>; and substantially higher increments of NO metabolites in relation to workload during exercise<sup>[187]</sup>. Post-exertional malaise in ME/CFS seems to be related to exercise-induced inflammation and immune activation<sup>[188,189]</sup>.

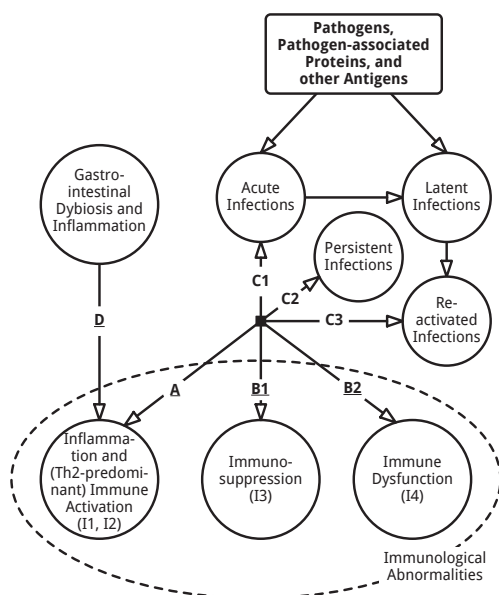
**PLAUSIBLE CAUSAL RELATIONSHIPS BETWEEN ABNORMALITIES**

**Figure 2, the 4I hypothesis: a key role for four types of immunological abnormalities**

Four immunological abnormalities underpin the 4I hypothesis for ME/CFS (Figure 2): inflammation (I1), (Th2-predominant) immune activation (I2), immunosuppression (I3) and immune dysfunction (I4).

**Potential immunological stimuli in ME/CFS**

The immune system seems to face three potential stimuli in ME/CFS: pathogens and associated antigens, due to acute, chronic and/or reactivated infections (Figure 2, A, B1 and B2), gastro-intestinal inflammation and hyperpermeability of the intestines, possibly resulting into translocation of enterobacteria to the blood stream (Figure 2, D), and auto-epitopes, due to oxidative and nitrosative damage to proteins, lipids etc. (Figure 3, K).



**Figure 2** Four immunological abnormalities in ME/CFS underpinning the 4I hypothesis.

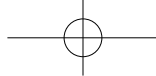
ME/CFS is often precipitated by infections, the severity of which seems to predict the clinical outcome<sup>[102,103]</sup>. Some studies implicate persistency of the pathogens<sup>[48]</sup> or antigens related to the pathogen<sup>[190]</sup> that instigated ME/CFS. Various infectious agents linked to ME/CFS are able to produce a persistent active infection, thereby establishing a constant incitement to the immune system<sup>[45]</sup>. In addition, some pathogens associated with ME/CFS, e.g. EBV<sup>[191]</sup> and HHV-6A/B<sup>[192]</sup>, are known to induce a life-long latent infection. Several studies suggest reactivation of these pathogens<sup>[111,193]</sup>, possible due to immunosuppression and immune dysfunction, e.g. a deficient EBV-specific B- and T-cell response<sup>[191]</sup>. The chronic or reactivated infection-hypothesis is contested<sup>[194]</sup>. However, as summarized, many studies have observed active infections in substantial patient subgroups. Infections would explain inflammation and immune activation in ME/CFS (Figure 2, A). It is also known that certain pathogens evade the immune system by immune modulation (Figure 2, B1 and B2), e.g. inhibiting the innate immune response, disrupting of T-cell function, and inducing a Th1->Th2 switch<sup>[195,196]</sup>.

Another possible immunological challenge relates to intestinal dysbiosis<sup>[49,51]</sup> and inflammation<sup>[130]</sup> and increased permeability of the intestinal barrier, thereby allowing enterobacteria to enter the blood stream, as indicated by elevated serum IgA levels against lipopolysaccharides (LPS) of gram-negative enterobacteria<sup>[50]</sup>. Intestinal dysbiosis and inflammation<sup>[54]</sup> and increased IgA responses to the LPS of commensal bacteria<sup>[53]</sup> have been associated with systemic inflammation and immune activation (Figure 2, D). It is known the gastro-intestinal tract can modulate the central nervous system by various blood-brain-barrier mediated mechanisms, e.g. through the secretion of NO and cytokines, thereby influencing behaviour<sup>[197]</sup>.

Finally, auto-epitopes, originating from oxidative and nitrosative damage to proteins and lipids (Figure 3, J), can induce autoimmune responses<sup>[198,199]</sup> (Figure 3, K). Although insufficiently explained, elevated levels of auto-antibodies have frequently been observed in ME/CFS, e.g. against serotonin<sup>[200,201]</sup>, gangliosides<sup>[200]</sup>, phospholipids<sup>[200]</sup>, including mitochondrial cardiolipins<sup>[202,203]</sup>, antinuclear antibodies<sup>[47,204]</sup>, muscarinic cholinergic receptor<sup>[204]</sup> and ssDNA<sup>[205]</sup>.

**A vicious cycle of oxidative and nitrosative stress induced by inflammation**

Oxidative and nitrosative stress play an important intermediate role in the 4I hypothesis. Inflammation will generate oxidative and nitrosative stress<sup>[206,207]</sup> (Figure 3, E), while oxidative and nitrosative stress can induce or amplify inflammation<sup>[208,209]</sup> (Figure 3, F). NO and reactive oxygen species (ROS) exert multiple immune-modulating effects<sup>[210,211]</sup>. ROS and reactive nitrogen species (RNS), including NO, can suppress NK<sup>[212,213]</sup> and T<sup>[214,215]</sup> cell cytotoxicity, which could contribute to immunosuppression (Figure 3, G). High levels of NO, produced by cytotoxic activated macrophages<sup>[216]</sup> may also play an important role in the shift from a Th1 to Th2 response<sup>[217,218]</sup> (Figure 3, H). While NO seems to suppress both Th1- and Th2-cell-mediated immunity at the early proliferation stage, high levels of NO appear to inhibit the Th1-cell differentiation of mature T helper (Th) cells<sup>[219]</sup>. Through several feedback loops oxidative and nitrosative stress can induce a self-perpetuating cycle of elevated oxidative and nitrosative stress, inflammation, elevated N-methyl-D-aspartate (NMDA) receptor activity, and ATP depletion, which is mediated by O<sub>2</sub><sup>-</sup>, NO and ONOO<sup>-</sup><sup>[220]</sup> (Figure 3, I).



Twisk FNM. 4I hypothesis for Myalgic Encephalomyelitis

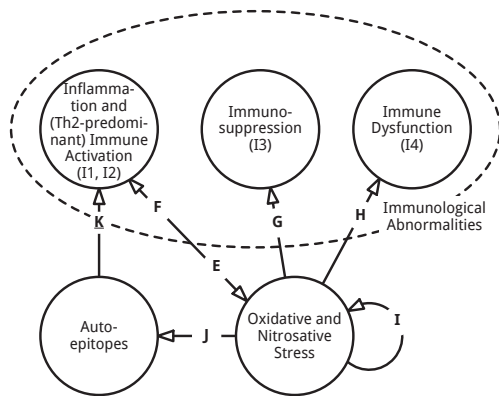


Figure 3 Immunological aberrations and oxidative and nitrosative stress in ME/CFS.

**HPA axis hypofunction as a potential sequel of inflammation, immune activation and oxidative stress**

The HPA axis and the immune system are bidirectionally interconnected<sup>[221]</sup>. Some authors have suggested that the immunological abnormalities in ME/CFS, e.g. inflammation, are secondary to HPA axis hypofunction<sup>[177]</sup>, possibly due to a “stress crash”: a switch from HPA axis hyper- to hypofunction<sup>[26]</sup>. However looking at various observations this doesn't seem very likely. Hypocortisolism is only present in a minority of the patients<sup>[222]</sup>, HPA axis dysfunction is not present during the early stages of ME/CFS<sup>[223]</sup> and seems to develop gradually and to be more pronounced the longer ME/CFS exists<sup>[224]</sup>, whereas immune activation and inflammation are often already present at the onset of the illness<sup>[102]</sup>. In contrast, chronic inflammation and immune activation can induce “adrenal exhaustion” gradually through various pathways (figure 4, L1): (a) (synergistic) suppression of cortisol release and the cortisol response to adrenocorticotropic hormone (ACTH) by tumor necrosis factor alpha (TNF $\alpha$ )<sup>[225,226]</sup>, possibly mediated by NO<sup>[227]</sup>, and interleukin (IL)-1<sup>[228]</sup>, (b) HPA axis desensitizing<sup>[229]</sup>, resulting into a long-lasting state of LPS tolerance to a second exposure of LPS, affecting the response of plasma TNF $\alpha$  and HPA-hormones to LPS<sup>[230]</sup>, (c) reduced adrenal response to ACTH as a consequence of elevated levels of interleukin-10<sup>[231]</sup>, associated with Th2 and Treg immune responses. Adrenal responses can also be inhibited by oxidative<sup>[232,233]</sup> and nitrosative stress/NO<sup>[234,235]</sup> (Figure 4, L2). A typical endocrine abnormality in ME/CFS relates to increased sensitivity of the cellular immune system<sup>[143,144]</sup> and HPA axis<sup>[183,184]</sup> to glucocorticoids (Figure 4, M1 and M2), plausibly explaining the paradoxical combination of hypocortisolism and Th2 predominance in ME/CFS.

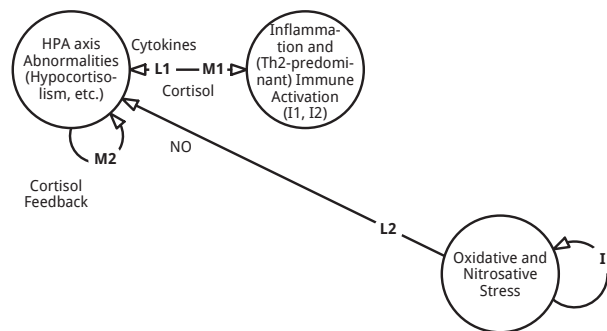


Figure 4 Inflammation, immune activation and oxidative and nitrosative stress and their potential contribution to HPA-axis dysfunction in ME/CFS.

**Mitochondrial dysfunction induced by oxidative and nitrosative stress**

In addition to causing damage to lipids, proteins and DNA, inducing neo-epitopes, oxidative and nitrosative stress can have various other negative effects (Figure 5).

ROS and RNS, including ONOO-, exert multiple effects on mitochondria, including mitochondrial dysfunction, damage, and apoptosis<sup>[236,237]</sup> (Figure 5, N). NO and RNS inhibit the respiratory chain, leading to elevated O<sub>2</sub> production, and, after reaction with NO, to increased ONOO- levels, further impeding mitochondrial respiration<sup>[238]</sup> (Figure 5, O).

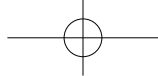
**Cardiovascular abnormalities due to oxidative and nitrosative stress**

NO plays an important role in the cardiovascular system and increased levels of NO can have various adverse cardiovascular effects<sup>[239]</sup> (Figure 5, P). NO is a potent vasodilator<sup>[240,241]</sup> and seems essential in autoregulation of blood flow, both in large arteries and at the microcirculatory level, thereby determining the distribution of flow among the various vascular networks<sup>[239]</sup>. Therefore elevated basal NO levels in ME/CFS could explain decreased peripheral resistance and low blood pressure (hypotension)<sup>[242]</sup>. Although possibly mediated by other pathways, these phenomena are induced in healthy subjects by exercise<sup>[243]</sup>. Exercise-induced NO, added to elevated basal levels, might partially account for post-exertional hypotension and delayed recovery in ME/CFS<sup>[242]</sup>. Furthermore, elevated NO levels could also be involved in reduced myocardial contractility, disrupted autonomic modulation of myocardial function and abnormalities in heart rate variability<sup>[239,244,245]</sup>. The cardiovascular aberrations and/or mitochondrial dysfunction/damage could plausibly explain reduced oxygen uptake<sup>[158]</sup> and oxygenation<sup>[71]</sup> during exercise in ME/CFS (Figure 5, Q).

**Neurological aberrations as potential consequences of inflammation, oxidative and nitrosative stress, cardiovascular abnormalities and reduced oxygen uptake**

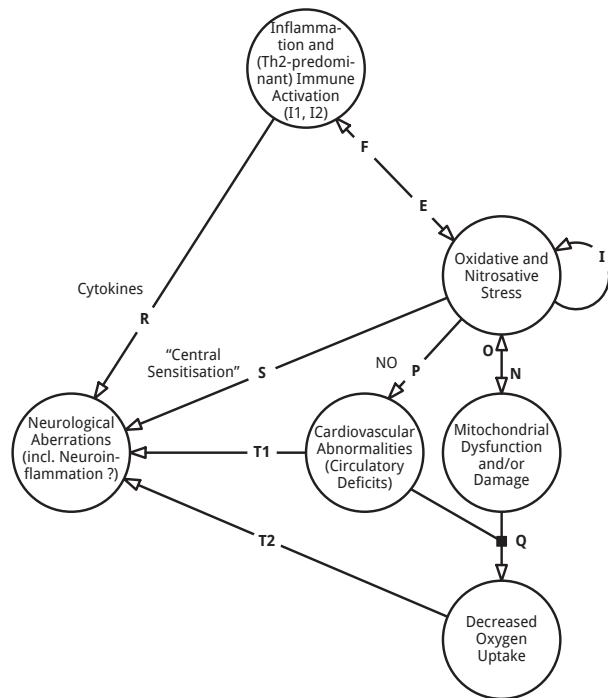
Various neurological abnormalities could be induced by the abnormalities in ME/CFS. First, systemic inflammation can induce neuroinflammation and “sickness behavior”<sup>[246]</sup> (Figure 5, R). Pro-inflammatory cytokines, induced by peripheral inflammation, can access the central nervous system through various pathways, and induce cytokines, amplify cytokine signals and release secondary messengers, e.g. NO, in the brain thereby influencing virtually every aspect of brain function<sup>[247]</sup>. Peripheral inflammation affects neuroendocrine function, NMDA receptor activity, neurotransmitter metabolism, and neurogenesis<sup>[248]</sup>. The behavioral effects of peripheral inflammation include depression, “fatigue”, psychomotor slowing, cognitive dysfunction and sleep disruption<sup>[247]</sup>. In addition to systemic inflammation-induced “sickness behaviour”, other mechanisms by which immunological aberrations affect the nervous system in ME/CFS have been suggested, e.g. an elevated release of cytokines by glial cells<sup>[249]</sup>, auto-immune pathways, involving vasoactive neuropeptides damaging the blood-brain barrier and blood-spinal barrier<sup>[250]</sup>, and infections of the nervous system by neurotropic viruses<sup>[45]</sup>. A recent study established evidence for brain glial activation in patients with chronic pain, a phenomenon repetitively observed in animal models<sup>[251]</sup>. Activation of microglia or astrocytes, related to neuro-inflammation, in widespread brain areas<sup>[174]</sup> could account for the chronic pain experienced by many patients with ME/CFS.

Second, increased levels of NO-stimulated glutamate release and



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hypersensitivity of NMDA receptors<sup>[252,253]</sup> could account for “central sensitisation”, manifesting itself in enhanced sensitivity of the central nervous system to various stimuli, e.g. sound, and hyperalgesia (Figure 5, S). Others have proposed an NO-independent O<sub>2</sub>-mediated pathway to induce hyperalgesia<sup>[254,255]</sup>. Third, cardiovascular abnormalities, resulting into reduced cerebral blood flow<sup>[70]</sup> (Figure 5, T1) and cerebral oxygenation<sup>[256]</sup> (Figure 5, T2), induced or intensified by exertion<sup>[71]</sup>, would account for reduced ATP synthesis in the central nervous system, as implicated by increased ventricular lactate levels<sup>[68]</sup>.



**Figure 5** The potential neurological, cardiovascular and metabolic effects of inflammation, immune activation and oxidative and nitrosative stress.

### The physiological effects of exercise and stress can explain post-exertional “malaise”

Exercise and psychological stress could amplify pre-existing immunological abnormalities, oxidative and nitrosative stress, and intestinal hyperpermeability (Figure 6). Since these anomalies can plausibly explain various typical symptoms of ME and CFS (next paragraph), the physiological effects of exercise and psychological stress would intensify abnormalities already present in rest. This could explain a (prolonged) aggravation of symptoms after a minor exertion: post-exertional “malaise”.

Exercise has well-known beneficial effects<sup>[257,258]</sup>. Strenuous exercise induces an increase in the pro-inflammatory cytokines TNF $\alpha$ , IL-1 $\beta$ , and IL-8 and the inflammatory cytokine IL-6, produced locally in the skeletal muscle in response to exercise<sup>[259,260]</sup>. This release is counterbalanced by the release of IL1 $\beta$  and TNF $\alpha$  inhibitors and IL-10<sup>[259]</sup>. In chronic inflammatory diseases, exercise could have adverse effects through a combination of exercise-induced stimulation of immune signals with leukocytes previously affected by other stress, inflammatory, or immune mediators<sup>[261]</sup>. Deviant effects of exercise on inflammation and immune activation in ME/CFS (Figure 6, X1) are illustrated by the observations that the severity of symptom flare after moderate exercise is directly linked to increased levels of IL-1 $\beta$ , IL-12, IL-6, IL-8, IL-10, and IL-13 8 hours post-exercise<sup>[188]</sup>, that travelling from home to the hospital is sufficient for significantly

elevated TGF- $\beta$  levels<sup>[262]</sup>, that exercise induces a sustained increase in plasma TNF- $\alpha$  in patients, not in controls<sup>[262]</sup>, and that moderate exercise induces a larger 48 hours post-exercise area under the curve for IL-10<sup>[15]</sup>. In addition, since acute<sup>[263]</sup> and chronic<sup>[264]</sup> stress can induce inflammation<sup>[264]</sup>, psychological stress could amplify pre-existing (low-grade) inflammation in ME/CFS.

Exercise can also have immunosuppressive effects. While moderate exercise seems to stimulate immunity, prolonged strenuous exercise seem to suppress immune function, e.g. NK cell activity and antibody synthesis<sup>[265,266]</sup>. Since the aerobic threshold seems (profoundly) decreased in ME/CFS, “low-level” anaerobic exercise can amplify immunosuppression, e.g. diminished NK cell cytotoxicity and antibody levels, observed in ME/CFS (Figure 6, X2).

In addition, eccentric exercise could induce and/or intensify immune dysfunction in ME/CFS (Figure 6, X3), e.g. inducing or amplifying a Th2-predominance<sup>[267,268]</sup>. Acute, subacute or chronic stress can suppress cellular (Th1) immunity and boost humoral (Th2) immunity, due to a differential effect of glucocorticoids and catecholamines on Th1/Th2 cells and type 1/type 2 cytokine synthesis<sup>[263]</sup>. In general, physiological and psychological stress may cause a selective suppression of Th1 functions and a shift towards a Th2 response, protecting the host from systemic ‘overshooting’ with pro-inflammatory cytokines<sup>[269]</sup>.

Another mechanism by which exercise can amplify pre-existing abnormalities is intestinal hyperpermeability<sup>[270,271]</sup> (Figure 6, X4). Psychological and physiological stress can compromise the intestinal barrier function<sup>[272,273]</sup>. Intestinal hyperpermeability could result in translocation of enterobacteria to the blood stream, instigating inflammation in response to endotoxins.

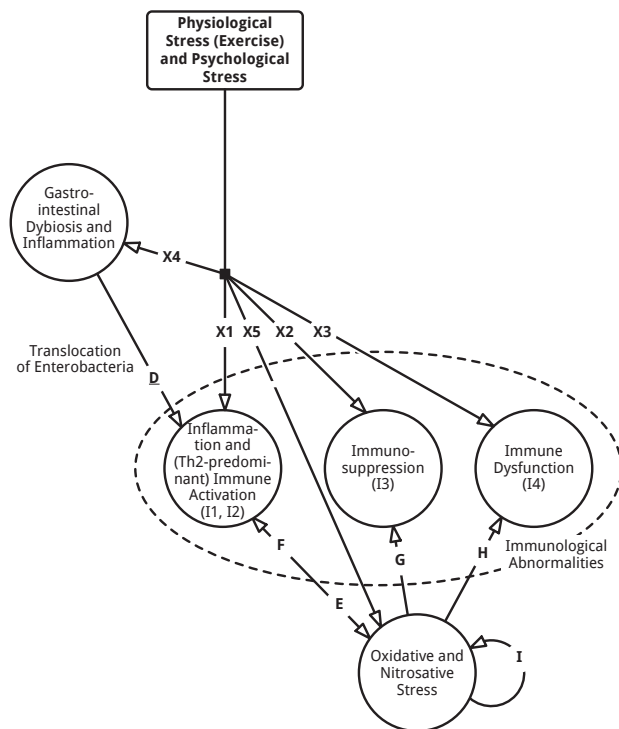
An additional pathway by which exercise can amplify pre-existing aberrations is oxidative<sup>[274,275]</sup> and nitrosative<sup>[274,276]</sup> stress as a result of exercise (Figure 6, X5). ME/CFS seems to be associated with a prolonged accentuated oxidative stress and reduced heat shock proteins responses to incremental exercise<sup>[93]</sup>. Several observations suggest an abnormal adaptive response to exercise in ME/CFS<sup>[92]</sup>, the severity of which seems to be related with premorbid physical activity and severe acute infections<sup>[150]</sup>. Through elevated glutamate release and subsequent NMDA receptor activation and induction of nuclear factor kappa-B (NF- $\kappa$ B), psychological stress enhances levels of NO and pro-oxidants in various brain areas<sup>[277,278]</sup>.

## CONCEIVABLE ETIOLOGY OF CHARACTERISTIC SYMPTOMS

The abnormalities observed in ME/CFS patients or substantial subgroups could explain the presence and variability of various characteristic symptoms (Figure 7 and Table 4). As explained in the previous paragraph the physiological effects of exercise in general and in ME/CFS in particular could account for the post-exertional “malaise”: exercise-induced intensification of symptoms<sup>[279]</sup>.

## DISCUSSION

The subjective and ambiguous criteria for CFS define a heterogeneous population of patients with chronic fatigue<sup>[32]</sup>. While “chronic fatigue” is obligatory for the diagnosis CFS<sup>[2]</sup>, easy muscle fatigability and cognitive impairment, but above all, post-exertional “malaise” are mandatory for the diagnosis ME, whether defined by the original criteria<sup>[304]</sup> or the recently proposed new<sup>[1]</sup> criteria. ME and CFS are two distinct, partially overlapping clinical entities<sup>[8]</sup>.



**Figure 6** The physiological effects of exercise and psychological stress on the core elements of the I4 hypothesis: inflammation and oxidative and nitrosative stress in ME/CFS.

Based upon observations, it is estimated that 30-60% of people fulfilling the criteria for CFS<sup>[2]</sup> meet the more strict criteria for ME. Fatigue is not obligatory for the diagnosis ME, and since the majority of the research in the last decades have used the CFS criteria for patient selection, it is unknown how many patients fulfilling the ME criteria don't meet the case definition for CFS. Despite the confusion created by the use of the CFS criteria, various studies have observed typical abnormalities in the ME/CFS patient group or significant ME/CFS patient subgroups.

More consistent findings relate to four types of immunological abnormalities in ME/CFS: inflammation (I1), (Th2-predominated) immune activation and counteractive immunoregulatory responses (I2), immunosuppression (I3), and immune dysfunction (I4). These immunological abnormalities and their direct and indirect sequels can account for various abnormalities observed in ME/CFS and several typical symptoms, including post-exertional "malaise" and "weakness". ME/CFS often has a sudden, "flu-like" onset. Whether the original infection persists and perpetuates the illness or is only a "hit-and-run" infection remains subject to debate. However, using different methods and samples, various infections have been observed in substantial ME/CFS patient subgroups. Immunosuppression (I3) and immune dysfunction (I4), either due to pathogens modulating and evading the immune system (as an effect) or enabling chronic/reactivating infections (as a cause) or both, seem to play a key role in the etiology. (Chronic) inflammation (I1) and immune activation (I2), both observed repetitively in ME/CFS, can induce and sustain a vicious circle of reactive oxygen and nitrogen species and peroxynitrite. Elevated oxidative/nitrosative stress has various detrimental effects: inflammation (I1), immunosuppression (I3), immune dysfunction (I4), the generation of auto-epitopes (due to oxidative and nitrosative damage to proteins, mitochondria etc.), mitochondrial dysfunction, cardiovascular deficits, et cetera-. Gastro-intestinal dysbiosis and inflammation and intestinal hyperpermeability, found by some studies, could result into translocation of enterobacteria into the blood stream, thereby inducing a third potential immunological stimulus in ME/CFS (i.e. LPS). HPA axis dysfunction, especially hypocortisolism and HPA axis hyporesponsiveness, can explain some immunological abnormalities, but seem to arise at a later stage of the disease. On the other hand, inflammation, immune activation and oxidative and nitrosative stress can induce hypocortisolism and a blunted adrenal response to ACTH through various pathways gradually. The endocrine and immunological anomalies in ME/CFS reflect a paradox: reduced adrenal output (cortisol) combined with suppression of the (cellular) immune system, (possibly) due to

**Table 4 A** plausible explanation for characteristic symptoms of ME/CFS by abnormalities.

Symptom *	Abnormalities	References
1a "Fatigue" (loss of energy, weakness)	Decreased oxygen uptake, resulting into ATP depletion	[280,281]
1b	Elevated oxidative and nitrosative stress, resulting into contractile dysfunction	[275,276]
2a Muscle fatigability and post-exertional muscle weakness and pain	Decreased oxygen uptake, resulting into ATP depletion and increased lactate synthesis, prolongedly aggravated as a consequence of muscle exertion	[282,283]
3a Widespread (muscle and joint) pain	Inflammation, inducing "sickness behavior", including inflammatory pain, and has a negative impact on nociception	[284,285]
2b, 3b Widespread (muscle and joint) pain and post-exertional muscle pain	Neurological abnormalities / central sensitisation, due to elevated levels of NF-kB, NO, ROS, ONOO-, and NMDA receptor activity	[286-288]
2c, 3c Widespread (muscle and joint) pain and post-exertional muscle weakness and pain	Neuro-inflammation, implicated by activation of microglia or astrocytes	[251,289]
4 Orthostatic intolerance	Cardiovascular abnormalities / circulatory deficits and autonomic dysfunction, due to increased levels of NO	[245,290]
5 Depression	Neurological abnormalities / "sickness behaviour"	[291,292]
6 Headaches	Neurological abnormalities, as part of "sickness behaviour" and/or result of low blood and/or oxygen supply, possibly resulting into increased lactate levels	[293-295]
7 Hypersensitivity to light, sound and odours	Neurological abnormalities / Central sensitisation due to neuro-inflammation, induced by peripheral inflammation, and/or increased levels of NO	[286,296,297]
8 Cognitive deficits (brain fog")	Neurological abnormalities / reduced blood flow to and/or oxygenation of the brain	[70,298]
9 Flu-like feeling	Neurological abnormalities / "sickness behaviour"	[291,299]
10 Sleep disturbances	Neurological abnormalities / "sickness behaviour"	[300,301]
11 Stress intolerance	HPA axis abnormalities, including defective stress responses	[27,181]
12 Abdominal pain / "irritable bowel"	Intestinal dysbiosis and inflammation	[302,303]
13 Post-exertional "malaise": an exercise-/stress-induced increase of symptoms	An increase in abnormalities mentioned above	[279]

\* The numbers refer to the cause-and-effect relationships between abnormalities and symptoms in figure 7.

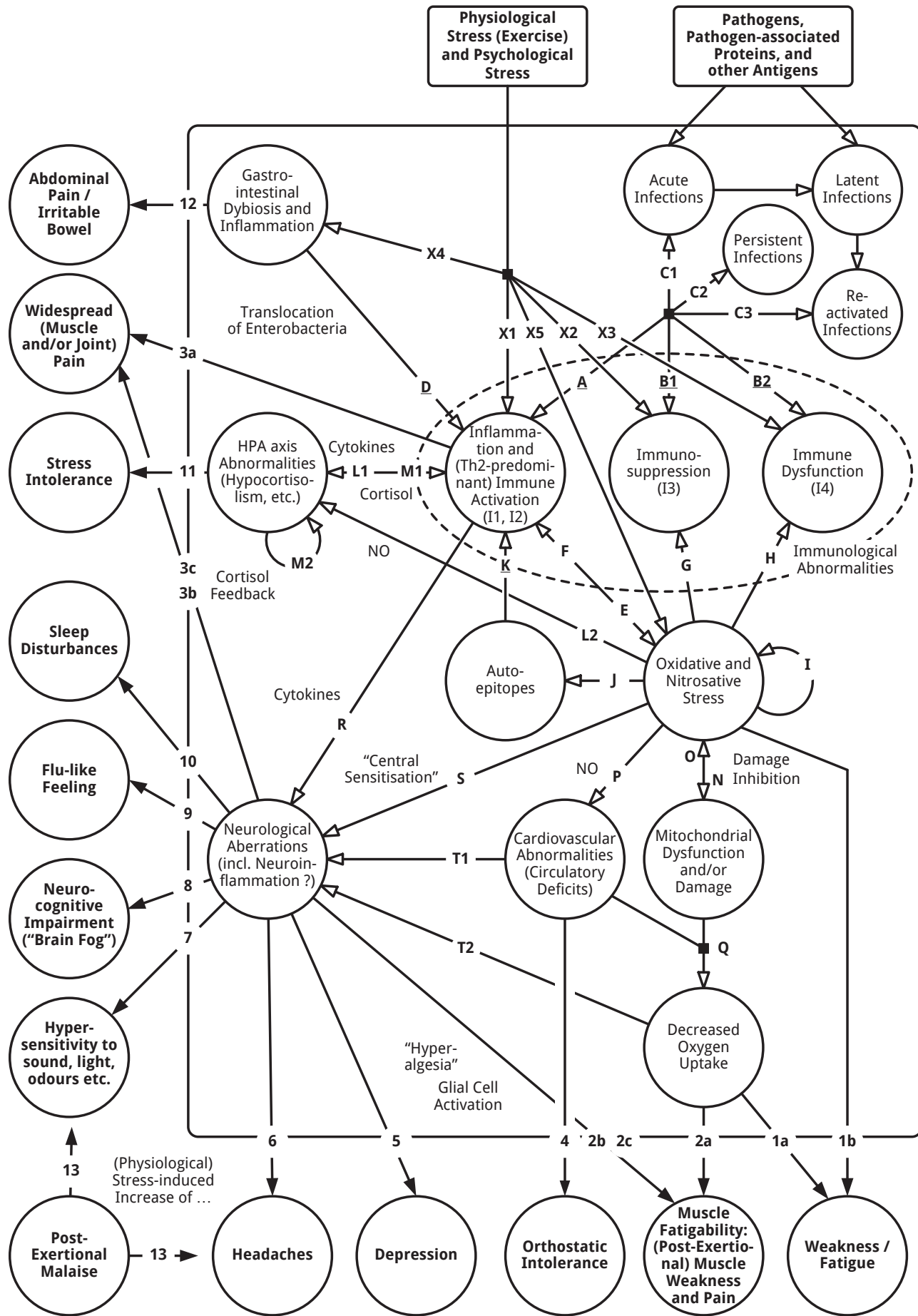


Figure 7 The abnormalities observed in ME/CFS could account for characteristic symptoms.



enhanced glucocorticoid sensitivity of the Th1 arm of the immune system. Finally, inflammation/immune activation, cardiovascular impairment and low oxygenation/oxygen uptake could account for various neurocognitive and neuropsychological abnormalities found in ME/CFS.

Other authors have proposed alternative explanatory models for ME/CFS. The ONOO-model, with a key role for the self-perpetuating vicious circle of elevated oxidative and nitrosative stress, resulting into peroxynitrite (ONOO-), proposed by Pall *et al*<sup>[305]</sup> is incorporated within the 4I explanatory model. The 4I explanatory model is also in line with the NO-induced central sensitisation-model of Meeus *et al*<sup>[306]</sup>. The 4I model has commonalities with the neuro-immunological (NI) model for ME/CFS, put forward by Morris and Maes<sup>[307]</sup>. However, there also some relevant differences. In essence, the NI model is a linear model in which a non-persistent infection induces a vicious circle of oxidative and nitrosative stress and inflammation, neo-epitopes (induced by oxidative and nitrosative damage to proteins) and autoimmunity. The 4I hypothesis embodies key roles for reactivating and chronic infections, immune dysfunction and Th2-dominated immune activation (next to inflammation), HPA axis dysfunction, e.g. hypocortisolism and blunted adrenal responses, enhanced sensitivity of the HPA axis and the (cellular) immune system to the suppressive effects of cortisol, and circulatory deficits<sup>[3]</sup>. In contrast with the hypothesis that maladaptive stress responses, either due to a stress crash<sup>[26]</sup> or “allostatic overload”<sup>[308]</sup>, are causing the immunological abnormalities seen in ME/CFS, the 4I hypothesis is based upon the premise that the immunological aberrations and oxidative/nitrosative stress can induce and sustain the endocrine abnormalities and defective stress responses through various pathways. The 4I hypotheses is consistent with the “alternate homeostatic state”-hypothesis of Craddock *et al*<sup>[309]</sup>, although, the 4I incorporates an opposing cause-and-effect-relationship between the immunological and endocrine abnormalities in ME/CFS.

## LIMITATIONS

While the abnormalities incorporated in the 4I model have been observed in ME/CFS patients or major patient subgroups repetitively, the cause-and-effect relationships between these abnormalities have often been established in general, non-ME/CFS-specific circumstances. However, the causal relations underpinning the 4I hypothesis, although speculative, collectively offer a plausible explanation for various abnormalities frequently observed in ME/CFS and characteristic symptoms.

Due to the heterogeneity of the CFS<sup>[2]</sup> patient group, the duration of the illness of the patients investigated, and the essential role of exertion-induced abnormalities, none of the abnormalities will be present in all CFS patients at all times and it is unlikely that here will ever be one abnormality present in all CFS<sup>[2]</sup> patients. In order to establish which abnormalities and causal relationships are applicable to specific patients, it is also essential to make a distinction between patients with post-exertional “malaise“, cognitive deficits and other characteristic symptoms of ME<sup>[1]</sup>, and CFS<sup>[2]</sup> patients without these typical symptoms and to investigate well-defined biological and symptomatic ME and CFS patient subgroups in more detail.

The 4I hypothesis can be tested by investigating the abnormalities in biological and clinical subgroups defined by biomarkers and objective measures of the clinical status<sup>[3]</sup>, by applying correlational analyses on the abnormalities present in patient subgroups, and by testing interventions aimed at reversing specific aberrations in patients, e.g. infections, immunological aberrations and oxidative/nitrosative stress.

## CONCLUSIONS

Immunologic abnormalities frequently observed in ME/CFS, inflammation (I1), (Th2-biased) immune activation (I2), immunosuppression (I3) and immune dysfunction (I4) seem to play a crucial role in the etiology and pathophysiology of ME/CFS. These immunological aberrations, combined with three potential immunological stimuli, can explain various other abnormalities observed in ME/CFS and underpin the 4I explanatory model for ME/CFS, which accounts for several characteristic symptoms.

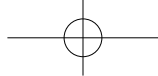
Future research should confirm various abnormalities and explore the aberrations in more detail in specific clinical and biological subgroups. Correlational analysis of co-existing abnormalities in patient subgroups and the outcomes of interventions aimed at specific abnormalities could be used to accept, refine or reject the cause-and-effect relationships incorporated in the 4I explanatory model for ME/CFS.

## CONFLICT OF INTERESTS

The Author has no conflicts of interest to declare.

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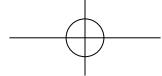
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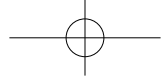
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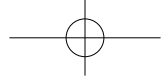
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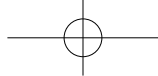
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