ORIGINAL ARTICLE

Not in the mind of neurasthenic lazybones but in the cell nucleus: patients with chronic fatigue syndrome have increased production of nuclear factor kappa beta

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Abstract

There is now some evidence that chronic fatigue syndrome is accompanied by an activation of the inflammatory response system and by increased oxidative and nitrosative stress. Nuclear factor kappa beta (NFκβ) is the major upstream, intracellular mechanism which regulates inflammatory and oxidative stress mediators.

In order to examine the role of NFκβ in the pathophysiology of CFS, this study examines the production of NFκβ p50 in unstimulated, 10 ng/mL TNF-α (tumor necrosis factor alpha) and 50 ng/mL PMA (phorbolmyristate acetate) stimulated peripheral blood lymphocytes of 18 unmedicated patients with CFS and 18 age-sex matched controls.

The unstimulated ($F=19.4$, $df=1/34$, $p=0.0002$), TNF-α-($F=14.0$, $df=1/34$, $p=0.0009$) and PMA-($F=7.9$, $df=1/34$, $p=0.008$) stimulated production of NFκβ were significantly higher in CFS patients than in controls. There were significant and positive correlations between the production of NFκβ and the severity of illness as measured with the FibroFatigue scale and with symptoms, such as aches and pain, muscular tension, fatigue, irritability, sadness, and the subjective feeling of infection.

The results show that an intracellular inflammatory response in the white blood cells plays an important role in the pathophysiogy of CFS and that previous findings on increased oxidative stress and inflammation in CFS may be attributed to an increased production of NFκβ. The results suggest that the symptoms of CFS, such as fatigue, muscular tension, depressive symptoms and the feeling of infection reflect a genuine inflammatory response in those patients. It is suggested that CFS patients should be treated with antioxidants, which inhibit the production of NFκβ, such as curcumin, N-Acetyl-Cysteine, quercitin, silimarin, lipoic acid and omega-3 fatty acids.

INTRODUCTION

For decades, CFS patients were (and still are) dismissed as lazybones or hypochondriacs. Since 1994, the baffling illness has received recognition by the introduction of diagnostic criteria [1]. Nevertheless, many medical doctors and insurance companies still assert that CFS merely is a mental condition. Doctors who treat CFS patients as suffering from a biological disorder and scientists who deal with the psycho-neuro-immune pathophysiology of CFS are often considered quacks by some of their colleagues, insurance companies and anti-quack societies, which are sometimes officially supported by governments, e.g. the Dutch government, in order to eliminate the scientific view that CFS is an organic disorder. The latter obviously would mean that the national health care system is obliged to financially support those patients who now are considered hypochondriacs and thus are suspended from the national health care systems. In accordance, the mainstream, "evidence based" treatment for CFS is cognitive behavioural therapy, which means that patients with CFS are being treated as having a mental illness with "treatments" that do not treat any underlying pathophysiology.

There is, however, evidence that CFS is accompanied by severe immune disorders, such as activation of the inflammatory response system (IRS) and increased oxidative and nitrosative stress with a significant damage to membrane lipids and functional proteins [2–4].

Activation of the IRS is shown by the following: a) an increased expression of T cell activation markers, such as CD26 and CD38 [5]; b) alterations in the production of pro-inflammatory cytokines [6–8]; c) signs of peripheral inflammation, such as increased plasma concentrations of the alpha-2 globulin fraction obtained by electrophoresis and decreased serum zinc levels [9]; and d) decreased cellular immune responses, such as decreased mitogen–induced lymphocyte responses and mitogen-induced expression of CD69, an early activation marker [3,10,11].

Increased oxidative and nitrosative stress in CFS are shown by a) higher LDL thiobarbituric acid reactive substances (TBARS) [12]; b) increased isoprostane levels and oxidized low density lipoproteins (LDL) [13]; c) elevated protein carbonyl levels [14]; and d) increased IgM antibody levels directed against fatty acids (oleic acid), by-products of lipid peroxidation (MDA and azelaic acid), and anti-S-farnesyl-L-cysteine, and NO derivates, such as nitro-tyrosine, nitro-phenylalanine, nitro-arginine, nitro-tryptophan and nitro-cysteine [4]. These findings show that CFS is characterized by an IgM-mediated immune response directed against neoepitopes, which have become immunogenic through a) oxidative damage to lipid membrane components and by-products of lipid peroxidation; and b) modification of endogenous proteins by nitrosative stress (NO and peroxynitrite). Moreover, there are some reports showing that CFS is accompanied by a decreased antioxidant status in the blood, such as a) significantly lower serum levels of zinc, a strong antioxidant; and b) lowered plasma levels of dehydroepiendrosterone-sulfate, a hormone with strong antioxidant properties [9,15].

Both activation of the IRS and the increased oxidative and nitrosative stress are probably intertwined phenomena in CFS [4]. Indeed, both phenomena may be caused by a same mechanism, such as an intracellular inflammation induced by increased production of nuclear factor kappa beta (NFκβ). NFκβ is the major upstream mechanism which regulates inflammatory and oxidative stress mediators [16]. Upon activation, NFκβ is translocated from the cytoplasma to the nucleus where it binds DNA promoter sequences and induces transcriptional activation of oxidative and nitrosative stress mediators, e.g. cyclo-oxygenase-2 (COX-2) and inducible NO synthase (iNOS), and inflammatory mediators, such as interleukin-1 (IL-1), IL-6, tumor necrosis factor-alpha (TNF-α) [16]. Therefore, we hypothesized that CFS could be accompanied by an increased production of NFκβ and that in CFS there are significant correlations between an increased NFκβ production and CFS symptoms pointing toward IRS activation, such as the subjective feeling of infection, pain (inflammatory pain) and depression, which is known to be related to inflammation [17].

The aims of the present study were to examine whether CFS is accompanied by an increased production of NFκβ and whether the increased NFκβ production is related to specific symptom profiles of CFS indicating IRS activation.

SUBJECTS AND METHODS

Subjects

Thirty-six subjects participated in the present study, 18 patients with CFS and 18 age-sex matched and unrelated controls (17 women and 1 man). Patients have been admitted to the M-Care4U Outpatient Clinics, Antwerp, Belgium. We made the diagnosis of CFS by means of the Centers for Disease Control and Prevention (CDC) criteria [1], i.e. the patient must have a severe chronic fatigue of six months or longer, while there is no other known medical condition which can explain the fatigue; and the patient must have four or more of the following symptoms: substantial impairment in short-term memory or concentration, sore throat, tender lymph nodes, muscle pain, multi-joint pain without swelling or redness, headache of a new type, pattern or severity, unrefreshing sleep, and post-exertional malaise lasting more than 24 hours. The FibroFatigue scale (Fibromyalgia and Chronic Fatigue Syndrome Rating Scale) [18] has been employed to rate the severity of illness. The total sum on the FibroFatigue scale was used to reflect severity of CFS and the individual symptoms were used to examine the symptom profiles of increased NFκβ production. The 12 symptoms are: pain, muscular tension, fatigue, concentration difficulties, failing memory, irritability, sadness, sleep disturbances, autonomic disturbances,

irritable bowel, headache, and subjective experience of infection.

We excluded subjects a) with a life-time diagnosis of psychiatric DSM-IV disorders, such as organic mental disorders, schizophrenia, bipolar disorder, depressive disorder, substance use disorders; b) who ever had been treated with anti-psychotic drugs, anticonvulsants or mood stabilizers; c) who had been taking antidepressants during the last year prior to the studies; d) with medical illnesses, e.g. essential hypertension, and arteriosclerosis, autoimmune disorders, diabetes type 1 or type 2, and inflammatory bowel disease; e) who had suffered acute inflammatory or allergic reactions the last 2 months prior to the studies; f) who had abnormal blood tests, such as alanine aminotransferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), calcium, creatinine, electrolytes, thyroid stimulating hormone (TSH), total protein, and iron or transferring saturation; and g) subjects with serological evidence for EBV or CMV infection. Patients and controls gave written informed consent after the study protocol was fully explained. The study has been approved by the local ethical committee.

Methods

PBMCs were obtained through histopaque-1077 density gradient centrifugation of peripheral blood samples of patients and controls. Isolated cells were resuspended in RPMI 1640 medium (Sigma R-0278) supplemented with 10% foetal calf serum and cultured overnight (18 hours). NFκβ p50 concentrations were determined on the cell lysates of unstimulated and 10 ng/mL TNF-α (tumor necrosis factor alpha) and 50 ng/mL PMA (phorbolmyristate acetate) stimulated mononuclear cell isolates. After harvesting the cells and washing with PBS/PIB (phosphate inhibitor buffer) cells were lysed with Nonidet P-40 and a nuclear cell extract was obtained after resuspension of the pellet in complete lysis buffer according to the protocol of the TransAM™ NFκβ p50 Transcription Factor Assay kit (Cat 41`096 & 41596; Active Motif, Carlsbad, California). All lysates were stored frozen at –80 °C until the measurements were performed simultaneously on the complete batch of samples. All results were measured quantitatively against a calibration curve provided by the manufacturer (TransAM™, Actif Motif, North America, CA) and expressed in ng/well. The analytical intra-assay coefficient of variation were <6.0%.

Statistics

Group mean differences were ascertained by means of analysis of variance (ANOVA) and covariance (ANCOVA). Differences between the unstimulated, PMA- and TNF-α-stimulated NFκβ production were examined by means or repeated measure (RM) design ANOVA. Relationships between variables were checked with Spearman's rank order correlation coefficients and canonical correlation analyses. The diagnostic performance of increased NFκβ was checked by means of ROC (receiver operating characteristics) analysis with computation of the area under the ROC curve, sensitivity, specificity and predictive value of a positive test result (PV+) and with kappa statistics [19]. The significance was set at α=0.05 (two tailed).

RESULTS

There were no significant differences in age (F=0.01, df=1/34, p=0.9) between normal controls (mean age ±SD=42.7 ± 9.0 years) and CFS patients (43.0 ± 9.2 years). There were no significant correlations between age and NFκβ (r=–0.12, non significant).

Figure 1 shows the scatter plot for the unstimulated, PMA- and TNF-α-stimulated production of NFκβ in CFS patients and in the controls. RM design ANOVA showed significantly higher production of NFκβ in CFS patients than in controls (F=28.3, df=1/102, p=0.00002), a significant effect of time (F=3.5, df=2/68, p=0.03) and no significant interaction between time × diagnostic groups (F=2.9, df=2/68, p=0.06). The Dunn test showed a significantly higher NFκβ production after stimulation with TNF-α (t=3.00, p=0.004) and PMA (t=2.28, p=0.02) than in the unstimulated condition. ANOVA showed that the unstimulated (F=19.4, df=1/34, p=0.0002), PMA- (F=7.9, df=1/34, p=0.008) and TNF-α-stimulated (F=14.0, df=1/34, p=0.0009) production of NFκβ were significantly higher in CFS patients than in controls. Covarying for age in an ANCOVA did not change this result.

ROC analysis performed on the NFκβ values showed that the area under the ROC curve (AUC) was highly significant for the unstimulated NFκβ production (AUC=86.6%) and the TNF-α-stimulated NFκβ production (AUC=81.9%), but less for the PMA-stimulated NFκβ production (70.01 %). Using a cut off value for the unstimulated NFκβ>0.245 ng/well, we found a significant discrimination of CFS patients from normal controls with a sensitivity of 66.7%, specificity=94.4% and a PV+=92.3% (6=0.61, t=4.63, p=0.0002, Y=0.71). Using a cut off value for the TNF-α-stimulated NFκβ production >0.4 ng/well, we found a significant discrimination of CFS patients from normal controls with a sensitivity of 66.7%, specificity=94.4 % and a PV +=92.3% (6=0.61, t=4.63, p=0.0002, Y=0.71). Spearman's rank order correlation analyses showed significant relationships between the total score on the FibroFatigue scale and the unstimulated (r=0.72, p=0.001) and TNF-α-stimulated (r=0.76, p=0.0004) NFκβ production. There was a trend towards a significant correlation between the FibroFatigue scale and the PMA-induced NFκβ production (r=0.35, p=0.16). The unstimulated production of NFκβ was significantly correlated to aches and pain (r=0.56, p=0.01), muscular tension (r=0.56, p=0.01), fatigue (r=0.78, p=0.0003), concentration difficulties (r=0.50, p=0.03) and the subjective experience of infection (r=0.84, p=0.0001). Spearman's rank order correlation analyses showed significant relationships between the TNF-α-stimulated NFκβ production and muscular

tension (r=0.56, p=0.01), fatigue (r=0.83, p=0.0001), irritability (r=0.61, p=0.005), and a subjective experience of infection (r=0.86, $p<10^{-4}$). Canonical correlation analyses with the 12 items of the FibroFatigue scale as dependent variables and the unstimulated and TNF-α-stimulated NFκβ production as explanatory variables showed that 32.1% of the variance in the symptoms was explained by the regression on the two NFκβ variables. Inspection of the loadings showed that aches and pain (0.63), muscular tension (0.72), fatigue (0.87), irritability (0.56), sadness (0.53), and the subjective feeling of infection (0.93) were related to the unstimulated (0.90) and TNF-α-stimulated (0.97) NFκβ production (shown are the significant loadings, i.e. those >0.50).

DISCUSSION

The major finding of this study is that the production of NFκβ is significantly increased in patients with CFS. Thus, these results give support to our a priori hypothesis that both IRS activation and oxidative or nitrosative stress in CFS (see Introduction) are interrelated phenomena, which are linked to a molecular, intracellular mechanism, i.e. an increased NFκβ production. Indeed, NFκβ is the major upstream mechanism which regulates inflammatory and oxidative stress mediators, such as pro-inflammatory cytokines, and COX-2 and iNOS production [16,20,21]. It is well established that the above pro-inflammatory cytokines are responsible for peripheral signs of IRS activation, such as lowered serum zinc and increases in the alpha-2 globulin fraction [9,17].

COX-2 is responsible for multiple inflammatory actions in the tissues [20,22]. Upon stimulation iNOS can generate nitric oxide (NO) which can combine with superoxide anions (O^{2-}) to form peroxynitrite ($ONOO^{-}$), a free radical that can cause DNA fragmentation, lipid oxidation and tyrosine nitration [23–26]. Thus, the increased production of NFκβ in CFS may have caused the multivarious changes in the IRS and oxidative stress in CFS – which have been reviewed in the Introduction – through its effects on the pro-inflammatory cytokines, COX-2 and iNOS.

Another question is why CFS is accompanied by an increased production of NFκβ which – in turn – may have induced IRS activation and oxidative stress. As explained elsewhere [4], there are many factors which may trigger CFS, such as viral infections, bacterial LPS from infections and increased gut permeability (leaky gut), psychological stress and sustained strenuous exercise. Interestingly, all these factors are known to induce the production of NFκβ. In fact, NFκβ functions as a "smoke sensor" that detects the above threats and acts as a switch to turn inflammation on and off and, thus, could trigger CFS when the threats are severe or chronic and have caused chronic inflammation.

In the following we discuss the above factors, which are known to trigger CFS and may activate NFκβ as well. Firstly, NFκβ is activated by the presence of viral

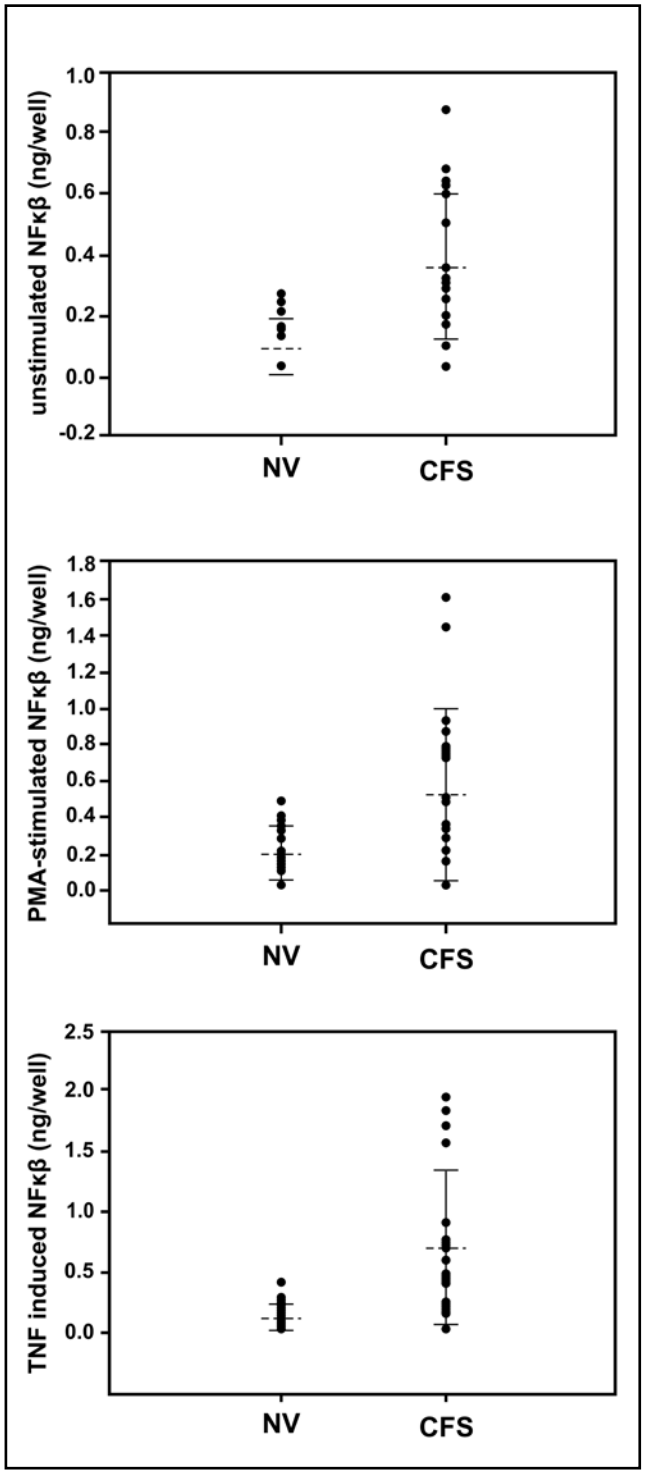

Figure 1. Scatter plot with mean (± 1 standard deviation) values of the NFκβ p50 concentrations in unstimulated, and tumor necrosis factor alpha (TNF-α) and phorbolmyristate acetate (PMA) stimulated mononuclear cell isolates of 18 patients with chronic fatigue syndrome (CFS) and 18 age-sex matched normal volunteers (NV).

infections. Indeed, in a subset of patients with CFS, IgM serum antibodies to Epstein-Barr virus [27], cytomegalovirus [28], herpes VI virus [29] are present, suggesting that CFS may be due to a persistent enteroviral infection [30]. In the latter study, up to 31% of CFS patients had significant titres of enteroviral IgM. Secondly, also LPS produced during bacterial infections or acquired by the increased translocation of gram negative bacteria may induce NFκβ and thus trigger CFS. In this respect, it has been reported that a high prevalence of infections with gram-negative bacteria may be found among CFS patients [31]. We reported that CFS is accompanied by increased serum IgA and IgM levels against the LPS of gram negative enterobacteria, indicating an increased translocation of gram-negative enterobacteria in CFS. The latter is probably caused by an increased gut-intestinal permeability [32]. A third mechanism revolves around the effects of psychological stress. Previously, it was shown that psychological stress in humans may induce the production of pro-inflammatory cytokines [33,34] and may induce oxidative stress [35–38], thus supporting the hypothesis that also NFκβ is induced by psychological stress. Recently it has been proposed that activation of NFκβ evoked by psychosocial stress, may directly target various tissues and thus constitutes one link between external stress and the body [39]. Interestingly, chronic unpredictable stress in rats can increase LPS-induced NFκβ activation in the frontal cortex and the hippocampus [40]. Finally, sustained strenuous exercise, which is another established trigger factor for CFS, may generate oxidative and nitrosative stress [41,42]. Recently, the activation of NFκβ has been observed in muscles subjected to intense exercise, a phenomenon which may cause muscle inflammation, damage and protein breakdown [43].

Another major finding of this study is that the increased production of NFκβ is significantly correlated to the key symptoms of CFS, i.e., a) the subjective feeling of infection; b) affective symptoms, such as sadness and irritability; and c) aches and pain, muscular tension, and fatigue. a) Previously, we reported that the subjective experience of infection is related to another marker of immune activation in CFS, i.e. lowered serum zinc [9]. The correlations between the subjective feeling of infection and NFκβ production and lowered zinc show that this symptom reflects a genuine intracellular inflammatory response in CFS. b) There is now a vast literature that inflammation is often accompanied by affective symptoms and that administration of pro-inflammatory cytokines and LPS may induce fatigue and depression [17,44,45]. c) Previously, significant correlations were detected between signs of oxidative and nitrosative stress and inflammation and aches and pain, muscular tension and fatigue. For example, Vecchiet *et al.* [12] reported that increased oxidative stress and decreased antioxidant defences are related to the extent of fatigue in patients with CFS. Kennedy *et al.* [13] showed that joint pain and postexertional malaise correlated well with isoprostane levels. Maes *et al.* [4] found significant correlations between increased IgM levels against a phletora of neoepitopes directed against lipids and proteins and aches and pain, muscular symptoms and fatigue. Other results implicate a causal role for oxidative stress in those symptoms. For example, Jammes *et al.* [46] described that the response of CFS patients to incremental exercise associates an accentuated oxidative stress together with marked alterations of the muscle membrane excitability. Matuszczak *et al.* [47] report that administration of N-acetyl-cysteine (NAC), a strong antioxidant, delays muscle fatigue during repetitive handgrip exercise, supporting the hypothesis that oxidative stress is a causal factor in human muscle fatigue. It is well-known that COX-2 is implicated in the production of musceloskeletal pain and that COX-2 inhibitors may alleviate these symptoms [48]. COX-2-selective inhibition has also a beneficial effect on muscle fatigue resistance in elderly patients with inflammation of infectious origin [49].

In summary, our results show that an intracellular inflammatory response in the white blood cells with increased production of NFκβ, the master modulator for inflammation and oxidative stress, plays an important role in the pathophysiogy of CFS. Thus, previous findings reporting increased oxidative stress and activation of the IRS in CFS may be explained by an increased production of NFκβ. The results suggest that the symptoms of CFS, such as fatigue, muscular tension, depressive symptoms and the feeling of infection reflect a genuine intracellular inflammatory response in those patients. It is suggested that CFS patients should be treated with substances that inhibit the production of NFκβ, e.g. curcumin, NAC, quercitin, lipoic acid, and silimarin [50–55] and with omega-3 fatty acids, which are decreased in CFS [55] and inhibit the production of NFκβ [56].

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REFERENCES

1 Fukuda K, Straus SE, Hickie I, Sharpe MC, Dobbins JG, Komaroff A: The chronic fatigue syndrome: a comprehensive approach to its definition and study. International Chronic Fatigue Syndrome Study Group. Ann Intern Med. 1994; **121**(12): 953–959.

2 Gerrity TR, Papanicolaou DA, Amsterdam JD, Bingham S, Grossman A, Hedrick T, Herberman RB, Krueger G, Levine S, Mohagheghpour N, Moore RC, Oleske J, Snell CR: CFIDS Association of America. Immunologic aspects of chronic fatigue syndrome. Report on a Research Symposium convened by The CFIDS Association of America and co-sponsored by the US Centers for Disease Control and Prevention and the National Institutes of Health. Neuroimmunomodulation 2004; **11**: 351–357.

3 Mihaylova I, Bosmans Em Maes M: Deceased expression of CD69 in chronic fatigue syndrome in relation to inflammatory markers: evidence for a severe disorder in the early activation of T lymphocytes and natural killer cells. Neuroendocrinol Lett 2007; **28**(4): 477–487.

4 Maes M, Mihaylova I, Leunis JC. Chronic fatigue syndrome is accompanied by an IgM-related immune response directed against neopitopes formed by oxidative or nitrosative damage to lipids and proteins. Neuroendocrinol Lett 2006; **27**: 615–621.
5 Klimas NG, Salvato FR, Morgan R, Fletcher MA: Immunologic abnormalities in chronic fatigue syndrome. J Clin Microbiol. 1990; **28**(6): 1403–1410.
6 Visser J, Blauw B, Hinloopen B, Brommer E, de Kloet ER, Kluft C, Nagelkerken L: CD4 T lymphocytes from patients with chronic fatigue syndrome have decreased interferon-gamma production and increased sensitivity to dexamethasone. J Infect Dis 1998; **177**(2): 451–454.
7 Patarca R, Klimas NG, Lugtendorf S, Antoni M, Fletcher MA: Dysregulated expression of tumor necrosis factor in chronic fatigue syndrome: interrelations with cellular sources and patterns of soluble immune mediator expression. Clin Infect Dis 1994; **18** Suppl 1: S147–53.
8 Linde A, Andersson B, Svenson SB, Ahrne H, Carlsson M, Forsberg P, Hugo H, Karstorp A, Lenkei R, Lindwall A, *et al*: Serum levels of lymphokines and soluble cellular receptors in primary Epstein-Barr virus infection and in patients with chronic fatigue syndrome. J Infect Dis 1992; **165**(6): 994–1000.
9 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.
10 Lloyd A, Hickie I, Hickie C, Dwyer J, Wakefield D: Cell-mediated immunity in patients with chronic fatigue syndrome, healthy control subjects and patients with major depression. Clin Exp Immunol 1992; **87**(1): 76–79.
11 Barker E, Fujimura SF, Fadem MB, Landay AL, Levy JA: Immunologic abnormalities associated with chronic fatigue syndrome. Clin Infect Dis 1994; **18** Suppl 1: S136–141.
12 Vecchiet J, Cipollone F, Falasca K, Mezzetti A, Pizzigallo E, Bucciarelli T, De Laurentis S, Affaitati G, De Cesare D, Giamberardino MA: Relationship between musculoskeletal symptoms and blood markers of oxidative stress in patients with chronic fatigue syndrome. Neurosci Lett 2003; **335**(3): 151–154.
13 Kennedy G, Spence VA, McLaren M, Hill A, Underwood C, Belch JJ: Oxidative stress levels are raised in chronic fatigue syndrome and are associated with clinical symptoms. Free Radic Biol Med. 2005; **39**(5): 584–549.
14 Smirnova IV, Pall ML: Elevated levels of protein carbonyls in sera of chronic fatigue syndrome patients. Mol Cell Biochem 2003; **248**(1–2): 93–95.
15 Maes M, Mihaylova I, De Ruyter M: Decreased dehydroepiandrosterone sulfate but normal insulin-like growth factor in Chronic Fatigue Syndrome (CFS): Relevance for the inflammatory response in CFS. Neuroendocrinol Lett 2005; **26**(5): 487–492.
16 Brasier AR: The NF-kappaB regulatory network. Cardiovasc Toxicol 2006; **6**: 111–130.
17 Maes, M: Major depression and activation of the inflammatory response system. Adv Exp Med Biol 1999; **461**: 25–46.
18 Zachrisson O, Regland B, Jahreskog M, Kron M, Gottfries CG: A rating scale for fibromyalgia and chronic fatigue syndrome (the FibroFatigue scale). J Psychosom Res 2002; **52**(6): 501–509.
19 Zweig MH, Campbell G: Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. Clin Chem 1993; **39**(4): 561–477.
20 Chen J, Zhao M, Rao R, Inoue H, Hao CM: C/EBP{beta} and its binding element are required for NF{kappa}B-induced COX2 expression following hypertonic stress. J Biol Chem 2005; **280**: 16354–16359.
21 Wong HR, Finder JD, Wasserloos K, Lowenstein CJ, Geller DA, Billiar TR, Pitt BR, Davies P: Transcriptional regulation of iNOS by IL-1 beta in cultured rat pulmonary artery smooth muscle cells. Am J Physiol 1996; **271**(1 Pt 1): L166–L171.
22 Khanapure SP, Garvey DS, Janero DR, Letts LG: Eicosanoids in inflammation: biosynthesis, pharmacology, and therapeutic frontiers. Curr Top Med Chem 2007; **7**(3): 311–340.
23 Fehr T, Schoedon G, Odermatt B, Holtschke T, Schneemann M, Bachmann MF, Mak TW, Horak I, Zinkernagel RM: Crucial role of interferon consensus sequence binding protein, but neither of interferon regulatory factor 1 nor of nitric oxide synthesis for protection against murine listeriosis. J Exp Med 1997; **185**(5): 921–931.
24 Murad F: Nitric oxide signaling: would you believe that a simple free radical could be a second messenger, autacoid, paracrine substance, neurotransmitter, and hormone? Recent Prog Horm Res 1998; **53**: 43–60.
25 Lipton SA: Neuronal protection and destruction by NO. Cell Death Differ 1999; **6**: 943–951.
26 Brown GC: Nitric oxide and mitochondrial respiration. Biochim. Biophys. Acta 1999; **1411**: 351–369.
27 Lerner AM, Beqaj SH, Deeter RG, Fitzgerald JT: IgM serum antibodies to Epstein-Barr virus are uniquely present in a subset of patients with the chronic fatigue syndrome. In Vivo 2004; **18**(2): 101–106.
28 Lerner MA, Beqaj SH, Deeter RG, Fitzgerald JT: IgM serum antibodies to human cytomegalovirus nonstructural gene products p52 and CM2(UL44 and UL57) are uniquely present in a subset of patients with chronic fatigue syndrome. In Vivo 2002; **16**(3): 153–159.
29 Patnaik M, Komaroff AL, Conley E, Ojo-Amaize EA, Peter JB: Prevalence of IgM antibodies to human herpesvirus 6 early antigen (p41/38) in patients with chronic fatigue syndrome. J Infect Dis 1995; **172**(5): 1364–1367.
30 Dowsett EG, Ramsay AM, McCartney RA, Bell EJ: 1990. Myalgic encephalomyelitis–a persistent enteroviral infection? Postgrad. Med. J 1990; **66**(777): 526–530.
31 Devanur LD, Kerr JR. Chronic fatigue syndrome. J Clin Virol. 2006; **37**(3): 139-50.
32 Maes M, Mihaylova I, Leunis JC: Increased serum IgA and IgM against LPS of enterobacteria in chronic fatigue syndrome (CFS): Indication for the involvement of gram-negative enterobacteria in the etiology of CFS and for the presence of an increased gut-intestinal permeability. J Affect Disord. 2007; **99**(1–3): 237–240.
33 Maes M, Song C, Lin A, De Jongh R, Van Gastel A, Kenis G, Bosmans E, De Meester I, Benoy I, Neels H, Demedts P, Janca A, Scharpe S, Smith RS: The effects of psychological stress on humans: increased production of pro-inflammatory cytokines and a Th1-like response in stress-induced anxiety. Cytokine 1998; **10**(4): 313–318.
34 Maes M, Song C, Lin A, DeJong R, Van Gastel A, Kenis G, Bosmans E, DeMeester I, Neels H, Janca A, Scharpe S, Smith S: Immune and clinical correlates of psychological stress-induced production of interferon-(and IL-10 in humans. In: Plotnikoff NP, Faith RE, Murgo AJ, Good RA, Eds: Cytokines, Stress and Immunity, 1998; 39–50.
35 Aleksandrovskii IuA, Poiurovskii MV, Neznamov GG, Seredeniia SB, Krasova EA: [Lipid peroxidation in emotional stress and neurotic disorders] Zh Nevropatol Psikhiatr Im S S Korsakova 1988; **88**(11): 95–101.
36 Pertsov SS, Balashova TS, Kubatieva AA, Sosnovskii AS, Pirogova GV, Abramov VM: [Lipid peroxidation and antioxidant enzymes in rat brain in acute emotional stress: effect of interleukin-1beta] Biull Eksp Biol Med 1995; **120**(9): 244–247.
37 Sosnovskii AS, Kozlov AV: [Increased lipid peroxidation in the rat hypothalamus after short-term emotional stress] Biull Eksp Biol Med 1992; **113**(5): 486–488.
38 Sivonova M, Zitnanova I, Hlincikova L, Skodacek I, Trebaticka J, Durackova Z: Oxidative stress in university students during examinations. Stress 2004; **7**(3): 183–188.
39 Bierhaus A, Wolf J, Andrassy M, Rohleder N, Humpert PM, Petrov D, Ferstl R, von Eynatten M, Wendt T, Rudofsky G, Joswig M, Morcos M, Schwaninger M, McEwen B, Kirschbaum C, Nawroth PP: A mechanism converting psychosocial stress into mononuclear cell activation. Proc Natl Acad Sci USA 2003; **100**(4): 1920–1925.
40 Munhoz CD, Lepsch LB, Kawamoto EM, Malta MB, Lima Lde S, Avellar MC, Sapolsky RM, Scavone C: Chronic unpredictable stress exacerbates lipopolysaccharide-induced activation of nuclear factor-kappaB in the frontal cortex and hippocampus via glucocorticoid secretion. J Neurosci 2006; **26**(14): 3813–3820.
41 Orhan H, van Holland B, Krab B, Moeken J, Vermeulen NP, Hollander P, Meerman JH: Evaluation of a multi-parameter biomarker set for oxidative damage in man: increased urinary excretion of lipid, protein and DNA oxidation products after one hour of exercise. Free Radic Res 2004; **38**(12): 1269–1279.

42 Klapcinska B, Kempa K, Sobczak A, Sadowska-Krepa E, Jagsz S, Szoltysek I: Evaluation of autoantibodies against oxidized LDL (oLAB) and blood antioxidant status in professional soccer players. Int J Sports Med 2005; **26**(1): 71–78.
43 Bar-Shai M, Carmeli E, Reznick AZ: The role of NF-kappaB in protein breakdown in immobilization, aging, and exercise: from basic processes to promotion of health. Ann N Y Acad Sci 2005; **1057**: 431–447.
44 Wichers MC, Koek GH, Robaeys G, Praamstra AJ, Maes M: Early increase in vegetative symptoms predicts IFN-alpha-induced cognitive-depressive changes. Psychol Med 2005; **35**(3): 433–441.
45 Wichers M, Maes M: The psychoneuroimmuno-pathophysiology of cytokine-induced depression in humans. Int. J. Neuropsychopharmacol. 2002; **5**(4): 375–388.
46 Jammes Y, Steinberg JG, Mambrini O, Bregeon 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.
47 Matuszczak Y, Farid M, Jones J, Lansdowne S, Smith MA, Taylor AA, Reid MB: Effects of N-acetylcysteine on glutathione oxidation and fatigue during handgrip exercise. Muscle Nerve 2005; **32**(5): 633–638.
48 Luan Y, Xu W: 2006. The function of the selective inhibitors of cyclooxygenase 2. Mini Rev Med Chem 2006; **6**(12): 1375–1381.
49 Mets T, Bautmans I, Njemini R, Lambert M, Demanet C: The influence of celecoxib on muscle fatigue resistance and mobility in elderly patients with inflammation. Am. J. Geriatr. Pharmacother. 2004; **2**(4): 230–238.
50 Shao DZ, Lee JJ, Huang WT, Liao JF, Lin MT: Inhibition of nuclear factor-kappa B prevents staphylococcal enterotoxin A-induced fever. Mol Cell Biochem 2004; **262**(1–2): 177–185.
51 McCarty MF: Potential utility of natural polyphenols for reversing fat-induced insulin resistance. Med Hypotheses 2005; **64**(3): 628–635.
52 Csiszar A, Smith K, Labinskyy N, Orosz Z, Rivera A, Ungvari Z: Resveratrol attenuates TNF-alpha-induced activation of coronary arterial endothelial cells: role of NF-kappaB inhibition. Am J Physiol Heart Circ Physiol 2006; **291**(4): H1694–1699.
53 Kim BH, Cho SM, Reddy AM, Kim YS, Min KR, Kim Y: Down-regulatory effect of quercitrin gallate on nuclear factor-kappa B-dependent inducible nitric oxide synthase expression in lipopolysaccharide-stimulated macrophages RAW 264.7. Biochem Pharmacol 2005; **69**(11): 1577–1583.
54 Mackenzie GG, Zago MP, Erlejman AG, Aimo L, Keen CL, Oteiza PI: alpha-Lipoic acid and N-acetyl cysteine prevent zinc deficiency-induced activation of NF-kappaB and AP-1 transcription factors in human neuroblastoma IMR-32 cells. Free Radic Res 2006; **40**(1): 75–84.
55 Maes M, Mihaylova I, Leunis JC. 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. Neuroendocrinol Lett 2005; **26**(6): 745–751.
56 Zhao Y, Joshi-Barve S, Barve S, Chen LH. Eicosapentaenoic acid prevents LPS-induced TNF-alpha expression by preventing NF-kappaB activation. J Am Coll Nutr 2004; **23**(1): 71–78.