REVIEW

Leaky gut in chronic fatigue syndrome: A review

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Abstract

There is now evidence that the pathophysiology of chronic fatigue syndrome (CFS) is related to inflammation and oxidative & nitrosative stress (IO&NS) with a) signs of immune activation and a suppression of ex vivo cellular immune responses; and b) damage to membrane lipids, functional proteins and DNA by O&NS. The above disorders are mediated by intracellular inflammation as indicated by an increased production of nuclear factor kappa Beta (NFκB), cyclo-oxygenase-2 (COX-2) and inducible NO synthase (iNOS). The above inflammatory reactions may by activated by a number of etiological factors, e.g. psychological stress, strenuous exercise, viral and bacterial infections.
The purpose of this paper is to review the evidence that an increased translocation of gram negative bacteria is another inflammatory pathway that is involved in CFS. The serum concentrations of IgM and IgA to lipopolysaccharide (LPS) of gram-negative enterobacteria, i.e. Hafnia Alvei; Pseudomonas Aeruginosa, Morganella Morganii, Pseudomonas Putida, Citrobacter Koseri, and Klebsielle Pneumoniae are significantly increased in patients with CFS. This suggests that in CFS there is an increased LPS translocation through a weakened tight junction barrier with subsequent gut-derived inflammation. This condition indicates an increased gut permeability or leaky gut. Treatment for 10-14 months with specific anti-inflammatory and -oxidative substances (NAIOSs), such as glutamine, N-acetyl cysteine and zinc, with or without immunoglobins intravenously (IVIg), significantly attenuates the initially increased IgA and IgM responses to LPS, showing that the gut-derived inflammation is attenuated and thus that the weakened tight junction barrier is partly restored. The attenuation of gut-derived inflammation predicts the clinical improvement 10-14 months after intake of NAIOSs. The above findings show that an increased translocation of gram negative bacteria with subsequent inflammation is a new pathway that contributes to the systemic IO&NS responses in CFS.

Introduction

Chronic Fatigue Syndrome (CFS) is a medical illness that is characterized by specific symptoms, such as fatigue, pain, infectious and neuropsychiatric symptoms (Fukuda *et al* 1994). Typical symptoms are substantial impairment in short–term memory or concentration; sore throat; tender cervical and axillary lymph nodes; muscle pain; multi–joint pain without selling or redness; headache of new type; unrefreshing sleep; post exertion malaise lasting more than 24 hours; and symptoms of irritable bowel syndrome (IBS). The symptoms must have persisted for at least six months (Fukuda *et al* 1994).

There is now evidence that CFS is an immune disorder characterized by an induction of inflammatory and oxidative and nitrosative stress (IO&NS) pathways.

1. Systemic inflammatory responses in CFS

A systemic inflammatory response is indicated by a number of findings. An increased production of pro-inflammatory cytokines, such as interleukin-1 (IL-1), IL-6 and tumor necrosis factor-α (TNFα). There is also an increased expression of T cell activation markers, such as CD26 and CD38, suggesting immune activation. Indicators of a peripheral inflammatory response are increased plasma concentrations of alpha-2 globulins, as obtained by electrophoresis, and decreased serum zinc levels (Aoki *et al* 1993; Barker *et al* 1994; Klimas *et al* 1990; Visser *et al* 1998; Patarca *et al* 1994; Linde *et al* 1992; Lloyd *et al* 1992; Gerrity *et al* 2004; Maes *et al* 2006a). Also, the suppression of ex vivo cellular immune responses, such as decreased mitogen–induced lymphocyte responses and mitogen-induced expression of CD69 indicates the presence of an immune response (Mihaylova *et al* 2007). It is also known that viral infections, e.g. with Ebstein Barr Virus may induce CFS. EBV viremia in CFS is accompanied by systemic inflammation, as indicated by an increased degradation of tryptophan (Bellmann-Weiler *et al* 2008). The catabolism of tryptophan is typically enhanced by pro-inflammatory cytokines, such as interferon-γ (IFNγ) and IFNα. Those cytokines can induce indoleamine 2,3-dioxygenase (IDO), the enzyme that converts tryptophan into tryptophan catabolites along the IDO pathway (TRYCATs) (Maes *et al* 2007a). In the former study, the authors (Bellmann-Weiler *et al* 2008) found that the serum concentrations of neopterin are positively correlated to signs of tryptophan degradation, suggesting that CFS patients due to EBV infection suffer from IFNγ-induced activation of cell-mediated immunity. These findings suggest that induction of IDO with an activation of cellular immunity and decreased plasma tryptophan are related to chronic fatigue following viral infections.

Also experimental induced inflammation is accompanied by fatigue. Thus, IFNα-based immunotherapy in HCV patients (hepatitis-C virus) has been shown to induce fatigue and "psychosomatic symptoms", including pain (Wichers *et al* 2005; Martin *et al* 2007; Bull *et al* 2008). These symptoms appear soon after starting the treatment. This indicates that fatigue, pain and psychosomatic symptoms are early signs of cytokine-induced inflammation. It is also known that IFNα-based immunotherapy can induce depression and even full blown major depression in a considerable number of HVC patients. However, the depressive symptoms occur some weeks after starting the treatment (Wichers *et al* 2005). Thus, it appears that fatigue/pain and depression are two different signs of inflammation: the former appearing some days after starting treatment, the latter appearing some weeks later. Also, the depressive reactions are predicted by the degree of fatigue one week after starting cytokine treatment (Wichers *et al* 2005). There is another argument showing that both symptom clusters have a different pathophysiology. Thus, the depressive symptoms respond well to serotonergic antidepressants, whereas the fatigue / pain symptoms do not (Martin *et al* 2007). Also translational research shows that, in rats, intraperitoneal injections of polyriboinosinic : polyribocytidylic acid (PIC), which mimics some of the effects of RNA viruses and which induces IFN, causes profound fatigue (Katafuchi *et al* 2005). The findings suggest that induction of inflammatory pathways may underpin fatigue.

2. Induction of the O&NS pathways

Inflammatory stimuli are also accompanied by increased oxidative stress, that is an increased production of oxygen radicals with increased levels of peroxides (H_2O_2) and superoxide ($2O_2$-). Also, nitrosative stress may be increased with an increased production of nitric oxide (NO) and peroxynitrite ($ONOO^-$) by activated neutrophils and monocytes. Oxidation and nitration may cause chemical modifications of membrane fatty acids, functional proteins and DNA, which in turn may cause functional defects. Moreover, the damage by O&NS may cause the formation of neoepitopes which are strongly immunogenic, e.g. nitro-tyrosine (Ohmori & Kanayama 2005). There is now evidence that CFS is accompanied by an induction of various O&NS pathways.

The findings comprise: increased isoprostane levels; increased oxidized low density lipoproteins (LDL), increased protein carbonyl levels; and increased LDL thiobarbituric acid reactive substances (TBARS) (Kennedy *et al* 2005; Smirnova & Pall 2003; Vecchiet *et al* 2003). Translational research shows that in animal models of stress-induced chronic fatigue O&NS plays a key role (Singh *et al* 2002a; 2002b). Also, the anti-oxidative defences are decreased in CFS, as indicated by a) lower serum levels of zinc, a strong antioxidant; and b) lowered plasma levels of dehydroepiendrosterone-sulfate, a hormone with strong antioxidant properties (Maes *et al* 2005; Maes *et al* 2006a;).

There is also evidence for severe damage caused by O&NS in CFS. Thus, CFS is characterized by an increased IgM-mediated response to neoepitopes formed by damage to membrane fatty acids, e.g. oleic, palmitic, and myristic acid; to byproducts of lipid peroxidation, e.g. azelaic acid and malondialdehyde (MDA) (Maes *et al* 2006b); to functional intracellular fatty acids, such as phosphatidyl-inositol (Pi) (Maes *et al* 2007b); to NO derivates, such as nitro-tyrosine, nitro-phenylalanine, nitro-arginine, nitro-tryptophan and nitro-cysteine (Maes *et al* 2006b) and NO-bovine serum albumin (Maes *et al* 2008). Thus, these findings show that CFS is characterized by an IgM-mediated inflammatory response directed against fatty acids and proteins which are modified by oxygen radicals, NO and peroxynitrite. CFS is also characterized by an increased oxidative damage to the membrane of the red

blood cells (RBC) and to hemoglobin (Richards *et al* 2007). The latter may indicate that the RBC membrane of patients with CFS are damaged and that the peroxydized membrane bilayer has become more rigid (Richards *et al* 2007).

3. Intracellular inflammation

Recently, we detected that there is an intracellular inflammation in CFS. A major finding is that the production of nuclear factor kappa beta (NFκB), and the inducible enzymes cyclo-oxygenase-2 (COX-2) and inducible NO synthase (iNOS) are significantly increased in CFS (Maes *et al* 2007c; 2007d). These findings are also corroborated by gene expression studies which observed an increased NFκB gene expression in CFS (Kerr *et al* 2008). NFκB is the major intracellular mechanism that regulates and induces the IO&NS pathways (Brasier 2006). Once induced, NFκB will be translocated from the cytoplasma to the nucleus of the cell. In the nuclues NFκB binds with DNA promoter sequences to induce transcriptional activation of IO&NS pathways, such as COX-2, iNOS, and pro-inflammatory cytokines, such as IL-1β, IL-6, and TNFα (Brasier 2006). COX-2 is another key enzyme that is induced during inflammation. COX-2 is involved in the synthesis of prostaglandins and prostacyclins (Feng *et al* 1995). Upon stimulation, iNOS will generate nitric oxide (NO) by macrophages and neutrophils (Lipton 1996; Brown 1999). As explained above NO plays an important role in generating damage caused by O&NS. We found that - in CFS - the production of NFκB was significantly related to the production rates of both iNOS and COX-2 which shows that the increased production of NFκB drives the higher production rates of iNOS and COX-2 (Maes *et al* 2007c).

Therefore, we have argued that the peripheral signs of systemic IO&NS, as discussed above, should be attributed to intracellular inflammation. Indeed, NFκB may induce the production of the IO&NS pathways, such as that of the pro-inflammatory cytokines, which in turn can induce signs of systemic inflammation, such as decreased zinc and changes in acute phase proteins (Maes *et al* 2007c; 2007d). Moreover, the induction of COX-2 and iNOS is responsible for further induction of more IO&NS pathways, with causes an increased production of prostaglandins, NO, superoxide, peroxynitrite and eventually to the damage to proteins and fatty acids with the formation of neoepitopes which in turn will induce greater and more responses in the IO&NS pathways (Maes *et al* 2007c; 2007d).

4. Systemic and central inflammation and the symptoms of CFS

Systemic inflammation is often accompanied by central neuroinflammation. For example, challenge with LPS induces an activation of brain microglia with a chronically elevated production of pro-inflammatory mediators, such as TNFα (Qin *et al* 2007). The latter may remain elevated during 10 months (Qin *et al* 2007). Peripheral or central administration of LPS thus may cause brain neuroinflammation and an increased production of pro-inflammatory cytokines, such as IL-1β, IL-6 and TNFα, which in turn may induce specific symptoms, labeled as the sickness behavior syndrome (Qin *et al* 2007). As explained (Maes *et al* 1993; 2009), there is a strong similarity between the symptoms of sickness behavior, on the one hand, and those of depression (anorexia, weight loss, psychomotor retardation, anhedonia) and CFS (fatigue, pain, sleep disorders, soporific effects, cognitive disorders), on the other. In both disorders, there are highly significant correlations between sings of inflammation and the key symptoms of depression or CFS, which indicates that the symptomatology of both disorders is related to IO&NS. Thus, in depression, there is a strong correlation between serum haptoglobin levels (an acute phase protein) and the "psychosomatic" depressive symptoms, e.g. anorexia, weight loss, middle insomnia, psychomotor retardation, and loss of interest (Maes *et al* 1993; 2009). In CFS, we detected significant correlations between sings of IO&NS activation and specific symptoms: a) the serum IgM levels directed against fatty acids, MDA and azelaic acid are significantly related to aches and pain, muscular tension and fatigue (Maes *et al* 2006b); the serum IgM levels directed against Pi are significantly related to fatigue and depression (Maes *et al* 2007b); the IgM antibodies directed against the nitro-derivates of the amino-acids are significantly related to aches and pain, muscular tension and fatigue (Maes *et al* 2006b); and increased NFκB is significantly correlated to aches and pain, muscular tension, fatigue, irritability, sadness, and the subjective feeling of infection (Maes *et al* 2007c).

Recently, we have discussed that LPS administration and activation of the systemic IO&NS pathways are accompanied by increased levels of pro-inflammatory cytokines in the brain with activation of microglia and, thus, with neuroinflammation (Qin *et al* 2007). Previously we have discussed that the symptoms of CFS may be induced by a) intracellular inflammation with increased COX-2 and iNOS levels, which may induce pain, muscle pain, inflammatory malaise, neurocognitive disorders (Maes *et al* 2007c; 2007d); b) increased production of pro-inflammatory cytokines, which may induce fatigue, depression, and inflammatory malaise (Maes *et al* 2009); and c) damage caused by O&NS, which may induce fatigue, muscle pain, and muscle tension (Maes *et al* 2006b; Maes 2009). Administration of LPS may provoke comparable symptoms in animal models (Borowski *et al* 1998; Lacosta *et al* 1999). The above findings suggest that IO&NS may have induced the symptoms of CFS.

5. Trigger factors for intracellular inflammation and CFS as well

We have explained previously that CFS may be induced by a number of trigger factors, such as viral infections, psychological stress and sustained strenuous exercise (Maes 2009). Increased serum antibodies to Epstein-Barr virus (Lerner *et al* 2004), human cytomegalovirus (Beqaj *et al* 2008), herpes VI virus (Patnaik 1995), and human parvovirus B19 (Seishima *et al* 2008) are present in CFS. Enterovirus VP1, RNA and non-cytopathic viruses are significantly more detected in the stomach of patients with CFS (Chia & Chia 2008). Therefore, it has been suggested that CFS may be due to persistent viral infections (Dowsett *et al* 1990). These findings are corroborated by recent findings that treatment with valacyclovir for six months shows a significant efficacy in treating CFS by increasing energy levels (Lerner *et al* 2007). Also, psychological stress is known to induce the IO&NS pathways, such as the production of pro-inflammatory cytokines (Maes *et al* 1998); lipid peroxidation and oxidative/nitrosative DNA damage (Aleksandrovskii *et al* 1988; Pertsov *et al* 1995; Sivonova *et al* 2004; Irie *et al* 2001); and LPS-induced NFκB activation in the frontal cortex and the hippocampus (Munhoz *et al* 2006). Sustained strenuous exercise throughout childhood and early adult life can increase the risk to develop CFS and to generate IO&NS (Orhan *et al* 2004; Klapcinska *et al* 2005), including increased NFκB in muscles (Kramer & Goodyear 2007). Thus, the various factors which are known trigger factors of CFS also induce the IO&NS pathways including the production of NFκB. Recently, we detected a new pathway that may induce systemic inflammation and that is related to CFS, i.e. an increased translocation of LPS with consequent gut-induced inflammation.

6. Increased translocation of LPS from gram negative enterobacteria

We found that the prevalences and median values for the plasma IgA and IgM levels directed against LPS of various enterobacteria (Hafnia Alvei; Pseudomonas Aeruginosa, Morganella Morganii, Proteus Mirabilis, Pseudomonas Putida, Citrobacter Koseri, and Klebsielle Pneumoniae) are significantly greater in patients with CFS than in normal controls (Maes *et al* 2007e). This suggests that there is a systemic inflammation directed against LPS, which is induced by the presence of increased intestinal permeability (leaky gut) with enlarged spaces between the cells of the gut wall and a loss of the protective barrier. Such a phenomenon is known to cause an increased bacterial translocation and an increased translocation of LPS (endotoxin), which both cause increased LPS circulating levels in the peripheral blood and consequently an increased production of NFκB, which in turn can induce the IO&NS pathways (Maes *et al* 2007e). Phrased differently, the increased plasma IgA and IgM levels against the LPS of gram negative enterobacteria in CFS indicate the presence of an increased gut permeability and a mounted immune response directed against LPS of the enterobacteria.

Leaky gut is known to be a driver of systemic inflammation (Wischmeyer 2006). For example, in abdominal postoperative patients increased gut permeability is a cause of systemic inflammation, while an attenuation of leaky gut is accompanied by a reduced systemic inflammation (Quan *et al* 2004). The pathway which causes gut-derived inflammation is bacterial translocation or translocation of LPS from gram negative bacteria, whereby bacteria or LPS are increasingly translocated from the gut into the blood (Wischmeyer 2006; Quan *et al* 2004). Phrased differently, "increased intestinal permeability" allows normally poorly invasive enterobacteria or the LPS from those bacteria to exploit the enlarged spaces to cross the gut epithelium (Wischmeyer 2006). The increased translocation of LPS or bacteria causes infections or induces the IO&NS pathways in the peripheral blood and liver through a primary induction of NFκB, which eventually induces central neuroinflammation.

We also detected significant correlations between the increased IgA-mediated immune response directed against LPS and muscular tension, fatigue, concentration difficulties, failing memory, autonomic disturbances, the subjective experience of infection and symptoms of IBS (Wischmeyer 2006). Thus, the relationship between the increased plasma IgA levels directed against LPS and IBS reflects gut-induced inflammation and not "mental stress" or "something in the mind" as most psychiatrists would posit. In our clinical experience IBS may precede CFS, while in other patients IBS develops together with the fatigue and pain symptoms or develops later in the course of illness. This suggests that "gut-derived inflammation" caused by increased LPS translocation may be a primary cause of CFS or may be a secondary phenomenon which may further aggravate the existing IO&NS. In any case, these results show that gram negative enterobacteria may be one of the etiological factors which can trigger CFS (Wischmeyer 2006).

7. Causes of an increased gut permeability

The intestinal barrier may be compromised by various etiological factors which are also known to induce CFS or chronic fatigue due to an organic disorder or condition. These are amongst others: psychological stress (Meddings & Swain 2000; Cameron & Perdue 2005); sustained strenuous exercise (Davis *et al* 2005); surgery and trauma (Riddington *et al* 1996; Pape *et al* 1994), alcoholism (Bjarnason *et al* 1984), the use of non-steroid anti-inflammatory drugs (NSAIDs) (Bjarnason *et al* 1986), chemotherapeutic agents (Coltart *et al* 1988), prolonged use of antibiotics (Berg 1992; Viljoen *et al*

2003), radiation (Coltart *et al* 1988), AIDS/HIV (Lim *et al* 1993), autoimmune disorders (Sundqvist *et al* 1982) and inflammatory bowel disease (Shanahan 1994).

But also activation of the systemic IO&NS pathways induces disruptions of gut permeability. a) Pro-inflammatory cytokines, such as IL-1β, IL-6, TNFα, and IFNγ are known to induce leaky gut by causing a loss of the gut tight junction barrier (Clark *et al* 2005; Chavez *et al* 1999; Yang *et al* 2003; Al-Sadi & Ma 2007). This phenomenon entails an enlarging of the spaces between the epithelial cells surrounding the gut wall, causing an increased permeability of the gut (Clark *et al* 2005; Chavez *et al* 1999; Yang *et al* 2003; Al-Sadi & Ma 2007). NFκB is essential in the loosening of the intestinal epithelial tight junction barrier. Thus, the IL-1β-induced leaky gut is mediated by NFκB activation (Al-Sadi & Ma 2007). Also, the TNFα-induced opening of the tight junction barrier is mediated by NFκB p50/p65 binding (Ye *et al* 2006; Ma *et al* 2005). Oxygen free radicals also cause a disruption of the tight junctions and thus of the intestinal barrier (Sun *et al* 2002). It is interesting to note that a depletion in certain natural anti-inflammatory and anti-oxidative substances (NAIOSs), e.g. glutamine, increases the risk to develop bacterial translocation (Wu *et al* 2004).

In conclusion, leaky gut may activate IO&NS pathways through increased bacterial and LPS translocation but the activation of the IO&NS pathways may also cause an opening of the tight junction barrier. Thus, leaky gut may be a primary causative factor in CFS or may be a secondary factor which further aggravates an existing activation of the IO&NS pathways, thus inducing a vicious circle between IO&NS activation and weakening of the tight junction barrier.

8. Treatment of leaky gut and CFS

There is now evidence that NAIOSs, such as glutamine, NAC, and zinc, have significant effects in treating leaky gut (Wu *et al* 2004; Olanders *et al* 2003; Sturniolo *et al* 2001; Chen *et al* 2003). Glutamine administration a) may repair the openings of the tight junction barrier and may decrease bacterial LPS translocation (Wu *et al* 2004); b) attenuates gut injuries and therefore may decrease gut-derived inflammation (Wischmeyer 2006); c) increases transmucosal resistance and decreases the mannitol flux through the epithelium and the prevalence of systemic infections (Foitzik *et al* 1997); d) reduces gut damage caused by NSAIDs and the consequent bacterial translocation in the rat (Ann *et al* 2004); e) reduces gut permeability, serum LPS concentrations and sings of systemic IO&NS (Quan *et al* 2004); f) improves gut permeability and decreases plasma LPS concentrations in injured patients (Zhou *et al* 2003); and g) significantly inhibits TNFα-induced bacterial translocation in caco-2 cells (Clark *et al* 2003). The abovementioned results show that glutamine is not only essential for the preservation of the functional tight junction barrier to microorganisms, but also that glutamine administration reduces leaky gut, the loosening of the tight junction barrier; bacterial LPS translocation, and gut-derived inflammation (Clark *et al* 2003; Buchman 1999). Zinc administration improves damaged rat intestines and stimulates gut repair and results in improved barrier integrity (Tran *et al* 1999; 2003; Di Leo *et al* 2001). Also, NAC administration tightens leaky gut and ameliorates gut-derived inflammation (Sun *et al* 2002).

Therefore, we have examined the effects of the above NAIOSs which had been taking during 12-14 months on the initially increased IgA and IgM responses to translocated LPS in patients with CFS (Maes *et al* 2007f; Maes & Leunis 2008). We found that the intake of those NAIOSs attenuates the increased IgM and IgA responses directed to LPS. This shows that these NAIOSs reduce gut-derived inflammation. By inference it may be deduced that these NAIOSs restore the loosened tight junction barrier. We found that the attenuation of the IgM responses directed to LPS was more pronounced than those in the IgA responses. We have explained this phenomenon because serum IgA responses indicate the more chronic pathogenic conditions (Maes & Leunis 2008).

Moreover, we found that the normalization of the IgA and IgM responses directed to LPS may in part predict the clinical outcome in CFS. Other predictors for a good outcome are: a younger age at onset of the CFS, the younger age of the patient and a shorter duration of the CFS (< 5 years). Thus, the attenuation of the translocation of LPS and consequently of the gut-derived inflammation predicts a better clinical outcome. These results support the view that restoration of the weakened tight junction barrier with reduced translocation of LPS by treatment with NAIOSs is accompanied by an improvement in some patients or with a total clinical remission in others. Thus, although improvement of gut-derived inflammation by NAIOSs is accompanied by a clinical improvement it is certainly not always accompanied by clinical remission. The above and the fact that the longer duration of illness is another risk factor for a worse clinical response indicates that other pathophysiogical factors are involved. These may constitute: damage by O&NS to membrane fatty acids, functional proteins and DNA and autoimmune responses, which frequently occur in CFS.

In our published case report (Maes *et al* 2007f), the patient was treated with NAIOSs together with immunoglobins intravenously (IVIg). IVIg is used since it is effective in reducing IO&NS reactions: this treatment reduces NFκB production; pro-inflammatory cytokine production; T-cell activation; and autoimmune reactions (since it contains antiidiotypic antibodies against human autoantibodies) (Garcia *et al* 2007; Skansen-Saphir *et al* 1998; Menezes *et al* 1997; Wu *et al* 2006; Makata *et al* 2006; Rossi & Kazatchkine 1989). Interestingly, IVIg also decreases bacterial translocation (Herek *et al* 2000). Thus, CFS patients with very severe leaky

gut together with severe IO&NS activation and autoimmune reactions can best be treated with the abovementioned NAIOSs together with IVIg.

In conclusion, gut-derived inflammation due to increased LPS translocation is a novel pathway in CFS. Leaky gut can be a primary cause of CFS or may develop during the course of CFS because the activated IO&NS pathways with increased cytokine and NFκB production induce a loosening of the tight junction barrier. The latter can be treated by specific NAIOSs with or without IVIg which in turn may have a clinical efficacy in patients with CFS who suffer from leaky gut. However, this treatment is rather expensive and takes 10-14 months. Therefore, leaky gut is a new drug target to develop novel drugs useful in treating leaky gut and CFS.

REFERENCES

1 Aleksandrovskii JA, Poiurovskii MV, Neznamov GG, Seredeniia SB, Krasova EA (1988). Lipid peroxidation in emotional stress and neurotic disorders. *Zh Nevropatol Psikhiatr Im S S Korsakova*. **88**(11): 95–101.

2 Al-Sadi RM & Ma TY (2007). IL-1beta causes an increase in intestinal epithelial tight junction permeability. *J Immunol*. **178**(7): 4641–4649.

3 Ann JY, Kim SJ, Han SP, Kim JW, Kim HJ, Do JH *et al* (2004). Effect of glutamine on the non-steroidal anti-inflammatory drug-induced bacterial translocation. *Korean J Gastroenterol*. **44**(5): 252–258.

4 Aoki T, Miyakoshi H, Usuda Y, Herberman RB (1993). Low NK syndrome and its relationship to chronic fatigue syndrome. *Clin Immunol Immunopathol*. **69**: 253–265.

5 Barker E, Fujimura SF, Fadem MB, Landay AL, Levy JA (1994). Immunologic abnormalities associated with chronic fatigue syndrome. *Clin Infect Dis*. **18**(Suppl.1): S136–141.

6 Bellmann-Weiler R, Schroecksnadel K, Holzer C, Larcher C, Fuchs D, Weiss G (2008). IFN-gamma mediated pathways in patients with fatigue and chronic active Epstein Barr virus-infection. *J Affect Disord*. **108**(1–2): 171–176.

7 Beqaj SH, Lerner AM, Fitzgerald JT (2008). Immunoassay with cytomegalovirus early antigens from gene products p52 and CM2 (UL44 and UL57) detects active infection in patients with chronic fatigue syndrome. *J Clin Pathol*. **61**(5): 623–626.

8 Berg RD (1992). Bacterial translocation from the gastrointestinal tract. *J Med*. **23**: 217–244.

9 Bjarnason I, Peters TJ, Wise RJ (1984). The leaky gut of alcoholism: possible route of entry for toxic compounds. *Lancet*. **1**(8370): 179–82.

10 Bjarnason I, Williams P, Smethurst P, Peters TJ, Levi AJ (1986). Effect of non-steroidal anti-inflammatory drugs and prostaglandins on the permeability of the human small intestine. *Gut*. **27**(11): 1292–1297.

11 Borowski T, Kokkinidis L, Merali Z, Anisman H (1998). Lipopolysaccharide, central in vivo biogenic amine variations, and anhedonia. *Neuroreport*. **9**(17): 3797–3802.

12 Brasier AR (2006). The NF-kappaB regulatory network. *Cardiovasc Toxicol*. **6**: 111–130.

13 Brown GC (1999). Nitric oxide and mitochondrial respiration. *Biochim Biophys Acta*. **1411**: 351–369.

14 Buchman AL (1999). Glutamine for the gut: mystical properties or an ordinary amino acid? *Curr Gastroenterol Rep*. **1**(5): 417–423.

15 Bull SJ, Huezo-Diaz P, Binder EB, Cubells JF, Ranjith G, Maddock C, *et al* (2008). Functional polymorphisms in the interleukin-6 and serotonin transporter genes, and depression and fatigue induced by interferon-alpha and ribavirin treatment. *Mol Psychiatry*. May 6, doi:10.1038/mp.2008.48.

16 Cameron HL, Perdue MH (2005). Stress impairs murine intestinal barrier function: improvement by glucagon-like peptide-2. *J Pharmacol Exp Ther*. **314**(1):214–220.

17 Chavez AM, Menconi MJ, Hodin RA, Fink MP (1999). Cytokine-induced intestinal epithelial hyperpermeability: role of nitric oxide. *Crit Care Med*. **27**(10): 2246–2251.

18 Chen P, Soares AM, Lima AA, Gamble MV, Schorling JB, Conway M *et al* (2003). Association of vitamin A and zinc status with altered intestinal permeability: analyses of cohort data from northeastern Brazil. *J Health Popul Nutr*. **21**(4): 309–315.

19 Chia JK & Chia AY (2008). Chronic fatigue syndrome is associated with chronic enterovirus infection of the stomach. *J Clin Pathol*. **61**(1): 43–48.

20 Clark EC, Patel SD, Chadwick PR, Warhurst G, Curry A, Carlson GL (2003). Glutamine deprivation facilitates tumour necrosis factor induced bacterial translocation in Caco-2 cells by depletion of enterocyte fuel substrate. *Gut*. **52**(2): 224–230.

21 Clark E, Hoare C, Tanianis-Hughes J, Carlson GL, Warhurst G (2005). Interferon gamma induces translocation of commensal Escherichia coli across gut epithelial cells via a lipid raft-mediated process. *Gastroenterology*. **128**(5):1258–1267.

22 Coltart RS, Howard GC, Wraight EP, Bleehen NM (1988). The effect of hyperthermia and radiation on small bowel permeability using 51 Cr EDTA and 14C mannitol in man. *Int J Hyperthemia*. **4**: 467–477.

23 Davis MS, Willard MD, Williamson KK, Steiner JM, Williams DA (2005). Sustained strenuous exercise increases intestinal permeability in racing Alaskan sled dogs. *J Vet Intern Med.* **19**(1): 34–39.

24 Di Leo V, D'Incà R, Barollo M, Tropea A, Fries W, Mazzon E *et al* (2001). Effect of zinc supplementation on trace elements and intestinal metallothionein concentrations in experimental colitis in the rat. *Dig Liver Dis*. **33**(2): 135–139.

25 Dowsett EG, Ramsay AM, McCartney RA, Bell EJ (1990). Myalgic encephalomyelitis – a persistent enteroviral infection? *Postgrad Med J*. **66**(777): 526–530.

26 Feng L, Xia Y, Garcia GE, Hwang D, Wilson CB (1995). Involvement of reactive oxygen intermediates in cyclooxygenase-2 expression induced by interleukin-1, tumor necrosis factor-alpha, and lipopolysaccharide. *J Clin Invest*. **95**(4): 1669–1675.

27 Foitzik T, Stufler M, Hotz HG, Klinnert J, Wagner J, Warshaw AL *et al* (1997). Glutamine stabilizes intestinal permeability and reduces pancreatic infection in acute experimental pancreatitis. *J Gastrointest Surg*. **1**(1): 40–46; discussion 46–7.

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

29 Garcia JM, Espanol T, Gurbindo MD, Casas CC (2007). Update on the treatment of primary immunodeficiencies. *Allergol Immunopathol (Madr)*. **35**(5): 184–192.

30 Gerrity TR, Papanicolaou DA, Amsterdam JD, Bingham S, Grossman A, Hedrick T *et al* (2004). 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*. **11**: 351–357.

31 Herek O, Ozturkk H, Ozyurt M, Albay A, Cetinkursun S (2000). Effects of treatment with immunoglobulin on bacterial translocation in burn wound infection. *Ann Burns Fire Disasters*. **XIII**(1): 1–7.

32 Irie M, Asami S, Nagata S, Miyata M, Kasai H (2001). Relationships between perceived workload, stress and oxidative DNA damage. *Int Arch Occup Environ Health*. **74**(2): 153–157.

33 Katafuchi T, Kondo T, Take S, Yoshimura M (2005). Enhanced expression of brain interferon-alpha and serotonin transporter in immunologically induced fatigue in rats. *Eur J Neurosci*. **22**(11): 2817–2826.

34 Kennedy G, Spence VA, McLaren M, Hill A, Underwood C, Belch JJ (2005). Oxidative stress levels are raised in chronic fatigue syndrome and are associated with clinical symptoms. *Free Radical Biol Med*. **39**(5): 584–589.

35 Kerr JR, Petty R, Burke B, Gough J, Fear D, Sinclair LI *et al* (2008). Gene expression subtypes in patients with chronic fatigue syndrome/myalgic encephalomyelitis. *J Infect Dis*. **197**(8): 1171–1184.

36 Klapcinska B, Kempa K, Sobczak A, Sadowska-Krepa E, Jagsz S, Szoltysek I (2005). Evaluation of autoantibodies against oxidized LDL (oLAB) and blood antioxidant status in professional soccer players. *Int J Sports Med*. **26**(1): 71–78.

37 Klimas N, Salvato F, Morgan R, Fletcher MA (1990). Immunologic abnormalities in chronic fatigue syndrome. *J Clin Microbiol*. **28**: 1403–1410.

38 Kramer HF & Goodyear LJ (2007). Exercise, MAPK, and NF-kappaB signaling in skeletal muscle. *J Appl Physiol*. **103**(1): 388-395.

39 Lacosta S, Merali Z, Anisman H (1999). Behavioral and neurochemical consequences of lipopolysaccharide in mice: anxiogenic-like effects. *Brain Res*. **818**(2): 291–303.

40 Lerner AM, Beqaj SH, Deeter RG, Fitzgerald JT (2004). IgM serum antibodies to Epstein-Barr virus are uniquely present in a subset of patients with the chronic fatigue syndrome. *In Vivo*. **18**(2): 101–106.

41 Lerner AM, Beqaj SH, Deeter RG, Fitzgerald JT (2007). Valacyclovir treatment in Epstein-Barr virus subset chronic fatigue syndrome: thirty-six months follow-up. *In Vivo*. **21**(5): 707–713.

42 Lim SG, Menzies IS, Lee CA, Johnson MA, Pounder RE (1993). Intestinal permeability and function in patients infected with human immunodeficiency virus. A comparison with coeliac disease. *Scand J Gastroenterol*. **28**(7): 573–580.

43 Linde A, Anderson B, Svenson SB, Ahrne H, Carlsson M, Forsberg P *et al* (1992). Serum level of lymphokines and soluble cellular receptors in primar EBV infection and in patients with chronic fatigue syndrome. *J Infect Dis*. **165**: 994 –1000.

44 Lipton SA (1996). Neuronal protection and destruction by NO. *Cell Death Differ*. **6**: 943–951.

45 Lloyd A, Hickie I, Hickie C, Dwyer J, Wakefield D (1992). Cell-mediated immunity in patients with chronic fatigue syndrome, healthy controls and patients with major depression. *Clin Exp Immunol*. **87**: 76–79.

46 Ma TY, Boivin MA, Ye D, Pedram A, Said HM (2005). Mechanism of TNF-alpha modulation of Caco-2 intestinal epithelial tight junction barrier: role of myosin light-chain kinase protein expression. *Am J Physiol Gastrointest Liver Physiol*. **288**(3): G422–430.

47 Maes M, Meltzer HY, Scharpé S, Cooreman W, Uyttenbroeck W, Suy E, Vandervorst C *et al* (1993). Psychomotor retardation, anorexia, weight loss, sleep disturbances, and loss of energy: psychopathological correlates of hyperhaptoglobinemia during major depression. *Psychiatry Res*. **47**(3): 229–241.

48 Maes M, Song C, Lin A, De Jongh R, Van Gastel A, Kenis G *et al* (1998). The effects of psychological stress on humans: increased production of pro-inflammatory cytokines and a Th1-like response in stress-induced anxiety. *Cytokine*. **10**(4): 313–318.

49 Maes M, Mihaylova I, De Ruyter M (2005). Decreased dehydroepiandrosterone sulfate but normal insulin-like growth factor in chronic fatigue syndrome (CFS): relevance for the inflammatory response in CFS. *Neuro Endocrinol Lett*. **26**(5): 487–492.

50 Maes M, Mihaylova I, De Ruyter M (2006a). Lower serum zinc in Chronic Fatigue Syndrome (CFS): relationships to immune dysfunctions and relevance for the oxidative stress status in CFS. *J Affect Disord*. **90**(2–3): 141–147.

51 Maes M, Mihaylova I, Leunis JC (2006b). Chronic fatigue syndrome is accompanied by an IgM-related immune response directed against neopitopes formed by oxidative or nitrosative damage to lipids and proteins. *Neuro Endocrinol Lett*. **27**(5): 615–621.

52 Maes M, Mihaylova I, Ruyter MD, Kubera M, Bosmans E (2007a). The immune effects of TRYCATs (tryptophan catabolites along the IDO pathway): relevance for depression – and other conditions characterized by tryptophan depletion induced by inflammation. *Neuro Endocrinol Lett*. **28**(6): 826–831.

53 Maes M, Mihaylova I, Leunis JC (2007b). Increased serum IgM antibodies directed against phosphatidyl inositol (Pi) in chronic fatigue syndrome (CFS) and major depression: evidence that an IgM-mediated immune response against Pi is one factor underpinning the comorbidity between both CFS and depression. *Neuro Endocrinol Lett*. **28**(6): 861–867.

54 Maes M, Mihaylova I, Bosmans E (2007c). 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. *Neuro Endocrinol Lett*. **28**(4): 456–462.

55 Maes M, Mihaylova I, Kubera M, Bosmans E (2007d). 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*. **28**(4): 463–469.

56 Maes M, Mihaylova I, Leunis JC (2007e). 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*. **99**(1–3): 237–240.

57 Maes M, Coucke F, Leunis JC (2007f). Normalization of the increased translocation of endotoxin from gram negative enterobacteria (leaky gut) is accompanied by a remission of chronic fatigue syndrome. *Neuro Endocrinol Lett*. **28**(6): 739–744.

58 Maes M & Leunis JC (2008). Normalization of leaky gut in chronic fatigue syndrome (CFS) is accompanied by a clinical remission in CFS: effects of age, duration of illness and increased IgA responses against translocated LPS of gram-negative bacteria. *Neuro Endocrinol Lett*. **29**(6):902–910.

59 Maes M, Mihaylova I, Kubera M, Leunis JC (2008). An IgM-mediated immune response directed against nitro-bovine serum albumin (nitro-BSA) in chronic fatigue syndrome (CFS) and major depression: evidence that nitrosative stress is another factor underpinning the comorbidity between major depression and CFS. *Neuro Endocrinol Lett*. **29**(3): 313–9.

60 Maes M (2009). Inflammatory and oxidative & nitrosative stress (IO&NS) pathways underpinning chronic fatigue, somatization and psychosomatic symptoms. *Curr Opinion Psychiatry*. **22**(1): 75–83.

61 Maes M, Yirmyia R, Noraberg J, Brene S, Hibbeln J, Perini G *et al* (2009). The inflammatory & neurodegenerative (I&ND) hypothesis of depression: leads for future research and new drug developments in depression. *Metab Brain Dis*. **24**(1): 27–53.

62 Makata H, Ichiyama T, Uchi R, Takekawa T, Matsubara T, Furukawa S (2006). Anti-inflammatory effect of intravenous immunoglobulin in comparison with dexamethasone in vitro: implication for treatment of Kawasaki disease. *Naunyn Schmiedebergs Arch Pharmacol*. **373**(5): 325–332.

63 Martin KA, Krahn LE, Balan V, Rosati MJ (2007). Modafinil's use in combating interferon-induced fatigue. *Dig Dis Sci*. **52**(4): 893–896.

64 Meddings JB, Swain MG (2000). Environmental stress-induced gastrointestinal permeability is mediated by endogenous glucocorticoids in the rat. *Gastroenterology*. **119**(4): 1019–1028.

65 Menezes MC, Benard G, Sato MN, Hong MA, Duarte AJ (1997). In vitro inhibitory activity of tumor necrosis factor alpha and interleukin-2 of human immunoglobulin preparations. Int *Arch Allergy Immunol*. **114**(4): 323–328.

66 Mihaylova I, DeRuyter M, Rummens JL, Bosmans E, Maes M (2007). 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. *Neuro Endocrinol Lett*. 2007; **28**(4): 477–83.

67 Munhoz CD, Lepsch LB, Kawamoto EM, Malta MB, Lima Lde S, Avellar MC *et al* (2006). Chronic unpredictable stress exacerbates lipopolysaccharide-induced activation of nuclear factor-kappaB in the frontal cortex and hippocampus via glucocorticoid secretion. *J Neurosci*. **26**(14): 3813–3820.

68 Ohmori H & Kanayama N (2005). Immunogenicity of an inflammation-associated product, tyrosine nitrated self-proteins. *Autoimmun Rev*. **4**(4): 224–9.

69 Olanders K, Borjesson A, Sun ZW, Andersson R (2003). Protective effects of N-acetyl-L-cysteine and platelet activating factor inhibition are not linked to intercellular adhesion molecule-1 expression after intestinal ischemia and reperfusion injury in rats. *Scand J Gastroenterol*. **38**(6): 618–625.

70 Orhan H, van Holland B, Krab B, Moeken J, Vermeulen NP, Hollander P *et al* (2004). 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*. **38**(12): 1269–1279.

71 Pape HC, Dwenger A, Regel G, Auf'm'Kolck M, Gollub F, Wisner D *et al* (1994). Increased gut permeability after multiple trauma. *Br J Surg*. **81**(6): 850–852.

72 Patarca R, Lugtendorf S, Antony M, Klimas NG, Fletcher MA (1994). Dysregulated expression of tumor necrosis factor in the chronic fatigue immune dysfunction syndrome: Interrelations with cellular sorces and patterns of soluble immune mediator expression. *Clin Infect Diseases*. **18**: 147–153.

73 Patnaik M, Komaroff AL, Conley E, Ojo-Amaize EA, Peter JB (1995): Prevalence of IgM antibodies to human herpesvirus 6 early antigen (p41/38) in patients with chronic fatigue syndrome. *J Infect Dis*. **172**(5): 1364–1367.

74 Pertsov SS, Balashova TS, Kubatieva AA, Sosnovskii AS, Pirogova GV, Abramov VM (1995). [Lipid peroxidation and antioxidant enzymes in rat brain in acute emotional stress: effect of interleukin-1beta]. *Biull Eksp Biol Med*. **120**(9): 244–247.

75 Qin L, Wu X, Block ML, Liu Y, Breese GR, Hong JS *et al* (2007). Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration. *Glia*. **55**(5): 453–462.

76 Quan ZF, Yang C, Li N, Li JS (2004). Effect of glutamine on change in early postoperative intestinal permeability and its relation to systemic inflammatory response. *World J Gastroenterol*. **10**(13): 1992–1994.

77 Richards RS, Wang L, Jelinek H (2007). Erythrocyte oxidative damage in chronic fatigue syndrome. *Arch Med Res*. **38**(1): 94–98.

78 Riddington DW, Venkatesh B, Boivin CM, Bonser RS, Elliott TS, Marshall T *et al* (1996). Intestinal permeability, gastric intramucosal pH, and systemic endotoxemia in patients undergoing cardiopulmonary bypass. *JAMA*. **275**(13): 1007–1012.

79 Rossi F & Kazatchkine MD (1989). Antiidiotypes against autoantibodies in pooled normal human polyspecific Ig. *J Immunol*. **143**(12): 4104–4109.

80 Seishima M, Mizutani Y, Shibuya Y, Arakawa C (2008). Chronic fatigue syndrome after human parvovirus B19 infection without persistent viremia. *Dermatology*. **216**(4): 341–346.

81 Shanahan F (1994). Current concepts of the pathogenesis of inflammatory bowel disease. *Ir J Med Sci*. **163**: 544–549.

82 Singh A, Garg V, Gupta S, Kulkarni SK (2002a). Role of antioxidants in chronic fatigue syndrome in mice. *Ind J Exp Biol*. **40**(11): 1240–1244.

83 Singh A, Naidu PS, Gupta S, Kulkarni SK (2002b). Effect of natural and synthetic antioxidants in a mouse model of chronic fatigue syndrome. *J Med Food*. **5**(4): 211–220.

84 Sivonova M, Zitnanova I, Hlincikova L, Skodacek I, Trebaticka J, Durackova Z (2004). Oxidative stress in university students during examinations. *Stress*. **7**(3): 183–188.

85 Skansen-Saphir U, Andersson J, Bjork L, Ekberg C, Fehniger TE, Henter JI *et al* (1998). Down-regulation of lymphokine synthesis by intravenous gammaglobulin is dependent upon accessory cells. *Scand J Immunol*. **47**(3): 229–235.

86 Smirnova IV & Pall ML (2003). Elevated levels of protein carbonyls in sera of chronic fatigue syndrome patients. *Mol Cell Biochemistry*. **248**(1–2): 93–95.

87 Sturniolo GC, Di Leo V, Ferronato A, D'Odorico A, D'Inca R (2001). Zinc supplementation tightens "leaky gut" in Crohn's disease. *Inflamm Bowel Dis*. **7**(2): 94–98.

88 Sun Z, Lasson A, Olanders K, Deng X, Andersson R (2002). Gut barrier permeability, reticuloendothelial system function and protease inhibitor levels following intestinal ischaemia and reperfusion--effects of pretreatment with N-acetyl-L-cysteine and indomethacin. *Dig Liver Dis*. **34**(8): 560–569.

89 Sundqvist T, Lindstrom F, Magnusson KE, Sköldstam L, Stjernström I, Tagesson C (1982). Influence of fasting on intestinal permeability and disease activity in patients with rheumatoid arthritis. *Scand J Rheumatol*. **11**(1): 33–38.

90 Tran CD, Butler RN, Howarth GS, Philcox JC, Rofe AM, Coyle P (1999). Regional distribution and localization of zinc and metallothionein in the intestine of rats fed diets differing in zinc content. *Scand J Gastroenterol*. **34**(7): 689–695.

91 Tran CD, Howarth GS, Coyle P, Philcox JC, Rofe AM, Butler RN (2003). Dietary supplementation with zinc and a growth factor extract derived from bovine cheese whey improves methotrexate-damaged rat intestine. *Am J Clin Nutr*. **77**(5): 1296–1303.

92 Vecchiet J, Cipollone F, Falasca K, Mezzetti A, Pizzigallo E, Bucciarelli T *et al* (2003). Relationship between musculoskeletal symptoms and blood markers of oxidative stress in patients with chronic fatigue syndrome. *Neurosci Lett*. **335**(3): 151–154.

93 Viljoen M, Panzer A, Willemse N (2003). Gastro intestinal hyperpermeability: a review. *East Afr Med J*. **80**(6): 324–330.

94 Visser J, Blauw B, Hinloopen B, Broomer E, de Kloet ER, Kluft C, Nagelkerken L (1998). CD4 lymphocytes from patients with chronic fatigue syndrome have decreased interferon-gamma production and increased sensitivity to dexamethasone. *J Infect Dis*. **177**: 451–454.

95 Wichers MC, Koek GH, Robaeys G, Praamstra AJ, Maes M (2005). Early increase in vegetative symptoms predicts IFN-alpha-induced cognitive-depressive changes. *Psychol Med*. **35**(3): 433–441.

96 Wischmeyer PE (2006). Glutamine: role in gut protection in critical illness. *Curr Opin Clin Nutr Metab Care*. **9**(5): 607–612.

97 Wu GH, Wang H, Zhang YW, Wu ZH, Wu ZG (2004). Glutamine supplemented parenteral nutrition prevents intestinal ischemia- reperfusion injury in rats. *World J Gastroenterol*. **10**(17): 2592–2594.

98 Wu KH, Wu WM, Lu MY, Chiang BL (2006). Inhibitory effect of pooled human immunoglobulin on cytokine production in peripheral blood mononuclear cells. *Pediatr Allergy Immunol*. **17**(1): 60–68.

99 Yang R, Han X, Uchiyama T, Watkins SK, Yaguchi A, Delude RL *et al* (2003). IL-6 is essential for development of gut barrier dysfunction after hemorrhagic shock and resuscitation in mice. *Am J Physiol Gastrointest Liver Physiol*. **285**(3): G621–629.

100 Ye D, Ma I, Ma TY (2006). Molecular mechanism of tumor necrosis factor-alpha modulation of intestinal epithelial tight junction barrier. *Am J Physiol Gastrointest Liver Physiol*. **290**(3): G496–504.

101 Zhou YP, Jiang ZM, Sun YH, Wang XR, Ma EL, Wilmore D (2003). The effect of supplemental enteral glutamine on plasma levels, gut function, and outcome in severe burns: a randomized, double-blind, controlled clinical trial. *J Parenter Enter Nutr*. **27**(4): 241–245.