Review

Oxidative stress and antioxidative parameters and metal ion content in patients with fibromyalgia syndrome: implications in the pathogenesis of disease

G. Fatima[1], S.K. Das[1], A.A. Mahdi[2]

[1]*Department of Rheumatology, and*
[2]*Department of Biochemistry, King George's Medical University, Lucknow, India.*

Ghizal Fatima, PhD
Siddharth Kumar Das, MD, PhD
Abbas Ali Mahdi, PhD

Please address correspondence to:
Dr Ghizal Fatima,
Department of Rheumatology,
King George's Medical University,
Lucknow 226003 (Uttar Pradesh), India.
E-mail: ghizalfatima8@gmail.com

Received on November 7, 2012; accepted in revised form on May 30, 2013.

Clin Exp Rheumatol 2013; 31 (Suppl. 79): S128-S133.

Key words: fibromyalgia syndrome, oxidative stress, antioxidative parameters, metal ions

Competing interests: none declared.

ABSTRACT

Fibromyalgia syndrome (FMS) is characterised by diffuse muscle pain, poor sleep and unrelenting fatigue. Individuals with FMS may also experience headaches, anxiety, depression, poor memory, numbness and tingling in the extremities, cold hands and feet, irritable bowel syndrome and lowered immune function. FMS is a common chronic pain syndrome of unknown etiology and limited treatment options. Previous studies have reported oxidative stress in FMS patients, but the results were inconsistent. Oxidative stress and nitric oxide is involved in FMS pathophysiology, however, it is still not clear whether oxidative stress abnormalities are the cause of FMS. There are several studies indicating oxidative stress in patients with FMS. Oxidant (Malondialdehyde) and antioxidant (Superoxide dismutase) balances were found to be changed in FMS patients. Furthermore, increased free radical levels may be responsible for the development of FMS and free radical-mediated oxidative stress including inflammatory cytokines may also play important roles in its pathogenesis. Moreover, oxidative stress is supposed to be increased in patients with FMS which is related to the severity of FMS symptoms. Therefore, it is important to understand whether the oxidative stress parameters are involved in FMS and what is the relationship between these and antioxidants in FMS patients. In this review we will elucidate the importance of oxidative stress and antioxidants and its possible relationship with FMS. Moreover, as metal toxicity is also reported to be involved in the pathogenesis of FMS, therefore we will also try to establish the role of toxic metals in the pathogenesis of FMS.

Introduction

Fibromyalgia syndrome (FMS) is a musculoskeletal pain disorder manifested by diffuse myalgia, localised areas of tenderness, fatigue, lowered pain thresholds, and nonrestorative sleep. FMS patients often develop an increased response to painful stimuli (hyperalgesia) and experience pain from normally nonnoxious stimuli (allodynia). Diagnostically, previous rheumatological criteria require the presence of 11 or more of 18 specified tender points on physical examination (1), however, the initial FMS criteria included tenderness on pressure (tender points) in at least 11 of 18 defined anatomic sites with the presence of widespread pain. Therefore, in the recent criteria it is clear that apart from the pain other seminal features of the disorder such as cognitive dysfunction, unrefreshing sleep, fatigue and mood disorders also play an important role in the diagnosis (2, 3). Despite being a common disorder which occurs more often in women than in men (4), with an estimated prevalence of 0.5% to 5.8% in the general population of North America and Europe (5, 6), its pathogenic mechanism remains elusive. Recently, oxidative stress has been proposed as a relevant event in the pathogenesis of this disorder (7, 8).

Most of the body's energy is produced by the enzymatically controlled reaction of oxygen with hydrogen in oxidative phosphorylation occurring within the mitochondria during oxidative metabolism. During this enzymatic reduction of oxygen to produce energy, free radicals are formed (9). In the normal condition there is a balance between Reactive Oxygen Species (ROS) and antioxidants within the cell, in the membranes and in the extracellular space. Endogenous free radical scavengers,

namely antioxidants, are overwhelmed by excessive production of ROS. The ROS attack the polyunsaturated fatty acids (PUFAs) in the membrane lipids, thereby leading to lipid peroxidation (LP) resulting in loss of fluidity of the membranes, changes in membrane potentials and eventually rupture leading to the release of cell and organelle contents (10).

Therefore, increased oxidative stress results from an imbalance between products of oxidation and antioxidant defences. These toxic molecules become highly reactive in their formation because of their altered number of unpaired valence electrons. There are several inflammatory clinical conditions associated with increased oxidative stress, data also suggest a relationship between oxidative stress and pain perception, furthermore, oxidative stress is also found increased in patients with chronic fatigue syndrome (CFS) (11-13). There is little information about oxidative stress in FMS. Moreover, there are several disorders associated with oxidative stress that is manifested by LP, protein oxidation and other markers. Studies have shown evidence that oxidative stress may have a role in the pathophysiology of FMS (14-16). Therefore, in this review, we will also try to elucidate the complex network of oxidative and antioxidative imbalance in women with FMS and try to establish the role of toxic metal ion content in these patients.

Consequences of oxidative stress

Oxidative stress, arising as a result of an imbalance between free radical production and antioxidant defences, is associated with damage to a wide range of molecular species including lipids, proteins, and nucleic acids. Lipoprotein particles or membranes characteristically undergo the process of LP, giving rise to a variety of products including short chain aldehydes such as malondialdehyde or 4-hydroxynonenal, alkanes, and alkenes, conjugated dienes, and a variety of hydroxides and hydroperoxides. Many of these products can be measured as markers of LP. Oxidative damage to proteins and nucleic acids similarly gives rise to a variety of specific damage products as a result of modifications of amino acids or nucleotides (17). Such oxidative damage might also lead to cellular dysfunction, and it might contribute to the pathophysiology of a wide variety of diseases including FMS.

Oxidative stress in FMS

LP and carbonylated proteins, the end products of membrane damage which are induced by ROS, are increased in the plasma of patients with FMS (18, 19). The role of free radical-mediated oxidative damage was investigated in the etiopathogenesis of FMS by Fassbender and Wegner (20), who suggested that muscle tender points in FMS result from local hypoxia. Another study showed abnormal oxygen pressure at the muscle surface above trigger points (21). Bengtsson *et al*. (22) investigated oxidative metabolism and found that adenosine diphosphate and phosphoryl creatine levels decreased and adenosine monophosphate and creatine levels increased in FMS patients. Furthermore, total antioxidant capacity or antioxidant enzymes such as superoxide dismutase (SOD) and Catalase are decreased in the plasma of patients with FMS (23).

Research has been directed to the plasma or serum of patients as a study model, with a need for cellular models, as this is the place where activation and control of the ROS-producing machinery occur. In this regard, hydrogen peroxide (H_2O_2), as one of the free oxygen radicals that results from the oxygen of the ROS, has been found increased in neutrophils of patients with FMS. Similarly, high levels of superoxide of mitochondrial origin (O_2^-) have been observed in the peripheral blood mononuclear cells of patients with FMS (24). In this model, patients had low levels of CoQ10, a vital element in the mitochondrial respiratory chain whose primary mission is the electron transport from complexes I and II to III, in addition to regulating the coupling of proteins, the pore transition and mitochondrial oxidation of fatty acids, an important antioxidant and membrane, so that a deficiency of the cell induces a drop in the activity of complex II + III, complex III, and complex IV, plus reduces the expression of mitochondrial proteins involved in oxidative phosphorylation, decreases mitochondrial membrane potential and increases the production of ROS (24). But, from a physiological point of view, what relationship exists between oxidative stress and the symptoms of FMS? It is known that ROS are involved in the etiology of one of the major symptoms of FMS, and that is pain.

The superoxide radical plays an important role in the development of pain on one side by peripheral and central nervous system sensitisation and thus induces an alteration of nociception, and on the other hand contributes to it through the activation of several cytokines such as tumour necrosis factor- alpha (TNF-α), interleukin-1 (IL-1), and IL-6 (25). The role of cytokines in FMS has been widely discussed, although not as an etiologic mechanism, but as a factor in the worsening of symptoms (25, 26). Although the mechanisms by which oxidative stress can alter muscle sensitivity are still unknown, it is possible that oxidative damage interferes with the muscles by reducing local nociceptors, which causes a decrease in the pain threshold (27). On the other hand, LP has been associated with a typical symptom of FMS: fatigue. High levels of LP, as well as an interesting correlation with this symptom, have been demonstrated in CFS, a disease with a high rate of comorbidity with FMS (24).

Nitric oxide (NO) is a highly diffusible and labile, gaseous messenger molecule, involved in various biological functions such as vasodilatation or vascular regulation, modulation of nociception, immune function, neurotransmission and excitation–contraction coupling (28, 29). NO also acts as a metabolic regulator during exercise (28). The production of NO from L-arginine is catalysed by the dioxygenase, nitric oxide synthase (NOS), which has three isoforms: neuronal (nNOS), inducible (iNOS) and endothelial (eNOS). Recently, NO has been shown to modulate levels of ROS in a variety of cells (30). Larson *et al*. (31) suggested the possible role of NO in pain modulation in FMS. Interestingly, significant correlations are observed between the levels of

antioxidants in both plasma and serum with respect to the score on a visual analogue pain scale, and the degree of morning stiffness by patients (19). On the other hand, LP in serum has demonstrated a high degree of correlation with the level of depression presented by patients with FMS (18), which shows the relationship between the balance of oxidants/antioxidants and symptoms of FMS. Signs of oxidative stress in FMS include a high level of oxidative damage to DNA, as seen in biopsy samples of patients with FMS.

Mitochondrial abnormalities in FMS support a mitochondrial defect as a contributor. As the number of mitochondria was significantly lower in patients with FMS than in controls and also seemed to be morphologically altered. The ultrastructural changes in patients with FMS are characterised by abnormalities in muscle tissue that cause increased DNA fragmentation and changes in the number and size of mitochondria. These cellular changes are not signs of apoptosis. But, persistent focal contractions in muscle may contribute to ultrastructural tissue abnormalities as well as to the induction of nociceptive transmission from muscle to the central nervous system (32). Moreover, since mitochondria supply energy to the cell through oxidative phosphorylation, the lower level of ATP that results from a low mitochondrial activity may explain the low exercise capacity and fatigue reported in patients with FMS (33). These results suggest that ROS production in mitochondria may be involved in oxidative stress, and CoQ10 deficiency and mitochondrial dysfunction could be also involved in the pathophysiology of FMS. These results confirm the oxidative stress background of FMS, probably due to a defect in the antioxidant system (SOD, CoQ10) and a high production of ROS. Finding the origin of oxidative stress could help us to understand the pathophysiology of FMS, and to offer new therapeutic strategies for this disease.

Antioxidant defence systems

An antioxidant can be defined as: "any substance that, when present in low concentrations compared to that of an oxidisable substrate, significantly delays or inhibits the oxidation of that substrate" (34). The physiological role of antioxidants, as this definition suggests, is to prevent damage to cellular components arising as a consequence of chemical reactions involving free radicals. In recent years, a substantial body of evidence has been developed supporting a key role for free radicals in many fundamental cellular reactions and suggesting that oxidative stress might be important in the pathophysiology of common diseases including atherosclerosis, chronic renal failure, and diabetes mellitus. Because radicals have the capacity to react in an indiscriminate manner leading to damage to almost any cellular component, an extensive range of antioxidant defences, both endogenous and exogenous, are present to protect cellular components from free radical induced damage.

Antioxidant in FMS:

ROS, such as superoxide radical anion, hydroxyl radical and hydrogen peroxide, are produced in metabolic and physiological processes and harmful oxidative reactions may occur in organisms. The oxidative effects of ROS are controlled by exogenous antioxidants such as vitamins E and C, and also by endogenous antioxidants such as scavenger enzymes (SOD and glutathione peroxidase (GPx), bilirubin and uric acid (35). When the production of damaging free radicals exceeds the capacity of the body's antioxidant defences to detoxify them, a condition known as oxidative stress occurs, such that its increase in oxidant and decrease in antioxidant cannot be prevented, and the oxidative/antioxidative balance shifts towards the oxidative status.

Cellular injury can be caused by oxidative stress which has been implicated in over a hundred disorders, including FMS (36). Inherent to its function, skeletal muscle is continuously exposed to fluctuations in its redox environment as, during exercise, ROS production by the mitochondrial respiratory chain increases. Therefore, the capability of skeletal muscle to respond to oxidative stress may not be surprising. *In vitro* experiments have demonstrated that skeletal myocytes adapt to oxidative stress by upregulation of antioxidant enzymes such as copper, zinc, SOD, catalase, and GPx (37). This suggests that under some conditions (such as aging, poor sleep, or microtrauma) antioxidant capacity may be impaired, as it may result in more oxidative stress in skeletal muscle. Potential triggers of oxidative stress in the muscle compartment include hypoxia and local sources of reactive oxygen and nitrogen species; skeletal muscle trophical state, contractility and fatigability may be affected by oxidative stress, resulting in skeletal muscle dysfunction (38).

Although the etiology of FMS remains unknown, recent data suggest that the oxidant/antioxidant balance may play a role in its development (39). It is suggested that oxidant/antioxidant imbalance is related to the disease process, and the increase in free radical levels may be responsible for the development of FMS. It has been proposed that FMS is caused by dysoxygenosis resulting from persistent and incremental oxidative stress on enzyme systems involved with cellular oxygen utilisation (40). Bramwell *et al*., (41) suggested that ascorbic acid treatment may be provided, to improve on clinical findings and quality of life in FMS patients. It has been suggested that the modified 'Myers cocktail', which consists of magnesium, calcium, B vitamins, and vitamin C has been found to be effective against acute asthma attacks, migraines, CFS, FMS, acute muscle spasm, upper respiratory tract infections, chronic sinusitis, seasonal allergic rhinitis, cardiovascular disease, and other disorders (42).

Antioxidants in FMS treatment:

To focus on clinical point of view in FMS which still is largely uncertain and is one of the main problems of this disease: lack of effective treatments. This leads the specialists to treat the symptoms of the disease rather than its causes, sometimes leading to a worsening of the disorders side-effects, and in many cases, these drugs induce an increase in oxidative stress. CoQ_{10} has been shown in invitro experiments with peripheral blood mononuclear cells of patients with FMS, either through its

antioxidant role or by offsetting the deficit significantly, to reduce ROS levels and to induce a mitochondrial degradation pathway known as autophagic mitophagia (24). This result could provide insights into the beneficial effect obtained in patients after administration of CoQ_{10} along with Gingko biloba shown by a pilot study in which there was a significant improvement in quality of life of patients (43). Fatigue, one of the most typical symptoms of FMS, has been reduced by treatment with CoQ_{10} in both animal and human physical fatigue models after exercise (44, 45). It should also be noted that CoQ_{10} has been shown to reduce muscle pain induced by statins in patients (46), and animal models have proven an anti-inflammatory and antinociceptive effect (47), and CoQ_{10} has recently been observed to regulate the expression of certain pro-inflammatory cytokine genes such as TNF-α (48), whose role has already been described in FMS (49).

Antioxidant therapies have proven effective in many pathological processes in which oxidative stress plays an important role. CoQ_{10}, Vitamin E or alphatocopherol, vitamin C or ascorbic acid, melatonin, SOD, vitamin A or retinol, glutathione, N-acetylcysteine, etc., are some of the antioxidants used in randomised trials of patients with a variety of diseases. However, in the case of FMS, there are still no double blind and placebo controlled trials in which the possible mechanisms demonstrate the benefits of these therapies in general and of CoQ_{10} in particular. The complexity of this disease makes it difficult to assess effectiveness of a single treatment, thus requiring a multidisciplinary therapeutic approach in which the use of antioxidants would acquire a role as co-treatment. Although oxidative stress in the FMS is an accepted fact, its role in the disease from a physiological point of view is not yet clear, and the mechanism by which high levels of free radicals, low levels of antioxidants or both processes simultaneously can have effects on the worsening of symptoms is still unknown. Therefore, further studies are necessary in this regard, as well as the design of controlled trials on the therapeutic effect of antioxidants.

Oxidative stress parameter like LP levels have been implicated in the severity of the clinical symptoms in FMS and it has been suggested that antioxidant therapy could be beneficial in FMS (25). In addition, prevention of LP has been demonstrated to be neuroprotective in a variety of neuropathological paradigms (50). It is known that LP, as a consequence of oxidative stress, indirectly reflects intracellular ROS generation, and ROS are known to be implicated in the etiology of pain, one of the most prominent symptoms in FMS, by inducing peripheral and central hyperalgesia (51). Study on the effect of dietary glutamate on FMS and irritable bowel symptoms suggest that dietary glutamate may be contributing to FMS symptoms (52). All these data support the idea that antioxidant therapy may be beneficial in FMS patient. Nevertheless, although oxidative stress is accepted to be involved in the pathophysiology of FMS, and the mitochondrial dysfunction could be involved in this disease, more studies are necessary to elucidate the origin of this oxidative disorder and its role in the etiology of FMS. In conclusion, the hypothesis that mitochondrial dysfunction is the origin of oxidative stress in FMS patients, could help to understand the complex pathophysiology of this disorder and may lead to development of new therapeutic strategies for prevention and treatment of this disease.

Toxic metals and fibromyalgia syndrome

Lead (Pb) and aluminum (Al) metals are non-essential and toxic, magnesium (Mg) and zinc (Zn) are essential metals and the toxicity occurs when they are deficient, causing cognitive dysfunction and interfere with energy metabolism resulting in fatigue, paresthesia and weakness, all hallmarks of FMS. Heavy metals toxicity caused by increasing levels of pollution and use of chemicals in industry is a growing threat to health and development. Distribution of the metal in different target organs varies with route, dose, and duration of exposure (53). A number of FMS patients are unknowingly suffering from heavy metal poisoning *i.e.* Al, Pb and arsenic (Ar). Metal Al is present in our food, water supply, and soil; most people suffer from some degree of Al toxicity. After years of accumulated exposure and storage of it in body tissues, It is clear that Al causes poisoning to the nervous system with a range of symptoms that are similar to the FMS like disturbed sleep, nervousness, emotional instability, memory loss, headaches, and impaired intellect.

Toxic metals trigger the production of free radicals leading to oxidative stress and depletion of the body's master antioxidant as well as influencing the metabolism of metallothioneins (small metal binding proteins high in sulphur) (54). Toxic metals are also known to stimulate the production of inflammatory messengers known as cytokines in the immune system causing immense pain (55). A study by Pernambuco *et al*. demonstrated increased levels of IL-17A in FMS patients. And also reported positive correlation in the levels of IL-17A and of other cytokines. Therefore, strengthening the hypothesis of the involvement of inflammatory mechanisms in the development of FMS (56). However, study on trace element pattern in patients with FMS could not demonstrate abnormal levels of trace elements in blood or urine of FMS patients and, thus, does not support the hypothesis that trace element abnormalities play any significant role in the development of FMS (57). Muscle pain has been associated with Mg and selenium (Se) deficiency. Therefore, Mg and Se status were investigated in FMS where Mg abnormalities were found associated with impairment of thiamin metabolism and antioxidant status (plasma malondialdehyde) and serum Se levels were reported to be unchanged in patients with FMS (58).

Bazzichi *et al*. (59) investigated the role of intracellular levels of the high energy adenosine triphosphate nucleotide (ATP) and essential divalent cations, calcium and Mg, in platelets of patients affected by primary FMS and reported significant lower ATP levels inside platelets of patients and higher calcium concentrations together with significant increased Mg levels in platelets of primary FMS patients than in

controls. Therefore, this study suggests that disturbances in the homeostasis of platelet ATP metabolism-signaling and calcium-magnesium flows might have a relevance in the pathogenesis of FMS. Furthermore, the relationship between serum antioxidant vitamins, Mg levels, and clinical parameters in patients with primary FMS was evaluated by Sakarya *et al*., and this study reported no significant differences in the levels of vitamins A, C, and E and Mg between control subjects and patients with FMS. In addition, no statistically significant correlations were found between serum vitamins A, C, and E, and Mg and number of Tender Point Counts (TPC), Fibromyalgia Impact Questionnaire (FIQ), in patients with FMS. According to the results of this study, it was asserted that other complex mechanism may play an important role in the pathophysiology of FMS without plasma antioxidant vitamins and Mg levels (60).

Heavy metal toxicity is a growing threat to health and development. Research has also suggested that serum Mg and Zn levels may play an important role in the pathophysiology of FMS (61). These effects and more are known to be important in FMS. Thus, the symptoms of FMS alone is suggestive of metal toxicity, symptoms such as fatigue, poor memory and concentration, and "brain fog", could all be caused or exacerbated by toxic metals like Al and Pb. An increased susceptibility to allergies and HPA-axis dysfunction has long been thought to be central to CFS and FMS. Metal toxicity (Al, Pb etc) is characterised by diffuse muscle pain, poor sleep, chronic malaise, brain fog, dizziness, headaches, anxiety and numbness which are same as that of symptoms of FMS. Our bodies need some trace amounts (Al, mercury (Hg), Pb, copper (Cu), Ar and others), but at high levels these metal toxins can be quite serious, even fatal. They are found in the air, soil, drinking water, fuel, medicines, cosmetics etc. they produce many like symptoms of FMS. The immune and nervous systems are closely linked and it has been suggested that exposure to toxic metals could upset the delicate balance between them and lead to diseases such as CFS and FMS where neuroimmune dysfunction is present (62). Through increasing oxidative stress toxic metals can damage mitochondrial DNA and cause mitochondrial dysfunction which inevitably leads to fatigue and other symptoms associated with FMS (63). Toxic metals not only increase oxidative stress but also deplete glutathione, and the amino acid cysteine vital for its production, thus lowering the body's defences against free radicals.

Conclusions

Free radicals have been implicated in the etiology of large number of major diseases including FMS. They can adversely alter many crucial biological molecules leading to loss of form and function. Such undesirable changes in the body can lead to diseased conditions. Antioxidants can protect against the damage induced by free radicals acting at various levels. Dietary and other components of plants form major sources of antioxidants. The relationship between free radicals, antioxidants and functioning of various organs and organ systems is highly complex and the discovery of 'redox signaling' is a milestone in this crucial relationship. The toxicity of metals like Al, Pb and Ar is mediated through free radicals generation in the cells that alters the oxidative stress parameters and may lead to FMS. Thus, a clear understanding on the oxidative stress, antioxidant parameters and disturbed metal ion level in patients with FMS may provide useful information to augment the understanding of pathophysiology and may also help in routine diagnosis of the patients. However, complete understanding of the biochemical events occurring at a cellular level to influence oxidative damage is required to guide future therapeutic advances. Furthermore, supplementation of the regular treatment with antioxidants, such as vitamins C and E may also be considered in these patients.

References

1. WOLFE F, SMYTHE HA, YUNUS MB *et al*.: The American College of Rheumatology Criteria for the classification of fibromyalgia. *Arthritis and Rheum* 1990; 33: 160-72.
2. WOLFE F, CLAUW DJ, FITZCHARLES MA *et al*.: The American College of Rheumatology preliminary diagnostic criteria for fibromyalgia and measurement of symptom severity. *Arthritis Care Res* (Hoboken) 2010; 62: 600-10.
3. SALAFFI F, SARZI-PUTTINI P: Old and new criteria for the classification and diagnosis of fibromyalgia: comparison and evaluation. *Clin Exp Rheumatol* 2012; 30: 3-9.
4. BARTELS EM, DREYER L, JACOBSEN S, JESPERSEN A: Fibromyalgia, diagnosis and prevalence. Are gender differences explainable? *Ugeskr Laeger* 2009; 171: 3588-92.
5. BRANCO JC, BANNWARTH B, FAILDE I *et al*.: Prevalence of fibromyalgia: a survey in five European countries. *Semin Arthritis Rheum* 2010; 39: 448-53.
6. LAWRENCE RC, FELSON DT, HELMICK CG *et al*.: Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. *Arthritis Rheum* 2008; 58: 26-35.
7. OZGOCMEN S, OZYURT H, SOGUT S *et al*.: Current concepts in the pathophysiology of fibromyalgia: the potential role of oxidative stress and nitric oxide. *Rheumatol Int* 2006; 26: 585-97.
8. PIECZENIK SR, NEUSTADT J: Mitochondrial dysfunction and molecular pathways of disease. *Exp Mol Pathol* 2007; 83: 84-92.
9. VALKO M, LEIBFRITZ D, MONCOL J *et al*.: Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem* 2007; 39: 44-84.
10. ESTERBAUER H, SCHAUR RJ, ZOLLNER H: Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radic Biol* 1991; 11: 81-128.
11. WANG ZQ, PORRECA F, CUZZOCREA S *et al*.: A newly identified role for superoxide in inflammatory pain. *J Pharmacol Exp Ther* 2004; 309: 869-78.
12. VECCHIET J, CIPOLLONE F, FALASCA K *et al*.: Relationship between musculoskeletal symptoms and blood markers of oxidative stress in patients with chronic fatigue syndrome. *Neurosci Lett* 2003; 335: 151-4.
13. KEENOY B, MOORKENS G, VERTOMMEN J, DE L I: Antioxidant status and lipoprotein peroxidation in chronic fatigue syndrome. *Life Sci* 2001; 68: 2037-49.
14. CORDERO MD: Oxidative stress in fibromyalgia: pathophysiology and clinical implications. (in Spanish) *Reumatol Clin* 2011; 7: 281-3.
15. CORDERO MD, DÍAZ-PARRADO E, CARRIÓN AM *et al*.: Is inflammation a mitochondrial dysfunction-dependent event in fibromyalgia? *Antioxid Redox Signal* 2013; 18: 800-7.
16. NEYAL M, YIMENICIOGLU F, AYDENIZ A *et al*.: Plasma nitrite levels, total antioxidant status, total oxidant status, and oxidative stress index in patients with tension-type headache and fibromyalgia. *Clin Neurol Neurosurg* 2012; 12: 453-2.
17. DE ZWART LL, MEERMAN JN, COMMANDEUR JM *et al*.: Biomarkers of free radical damage applications in experimental animals and in humans. *Free Radic Biol Med* 1999; 26: 202-26.
18. OZGOCMEN S, OZYURT H, SOGUT S *et al*.: Antioxidant status, lipid peroxidation and nitric oxide in fibromyalgia: etiologic and

therapeutic concerns. *Rheumatol Int* 2006; 26: 598-603.
19. ALTINDAG O, CELIK H: Total antioxidant capacity and the severity of the pain in patients with fibromyalgia. *Redox Rep* 2006; 11: 131-5.
20. FASSBENDER HG, WEGNER K: Morphology and pathogenesis of soft tissue rheumatism. *Z Rheumaforsch* 1973; 32: 355-74.
21. LUND N, BENGTSSON A, THORBORG P: Muscle tissue oxygen pressure in primary fibromyalgia. *Scand J Rheumatol* 1986; 15: 165-73.
22. BENGTSSON A, HENRIKSSON KG, LARSSON J: Reduced high energy phosphate levels in the painful muscles of patients with primary fibromyalgia. *Arthritis Rheum* 1986; 15: 1-6.
23. SENDUR OF, TURAN Y, TASTABAN E, YENISEY C, SERTER M: Serum antioxidants and nitric oxide levels in fibromyalgia: a controlled study. *Rheumatol Int* 2009; 29: 629-33.
24. CORDERO MD, DEMIGUEL M, MORENO FERNÁNDEZ AM *et al*.: Mitochondrial dysfunction and mitophagy activation in blood mononuclear cells of fibromyalgia patients: implications in the pathogenesis of the disease. *Arthritis Res Ther* 2010; 12: R17.
25. CORDERO MD, MIGUEL M, MORENO-FERNÁNDEZ AM: Mitochondrial dysfunction in fibromyalgia and its implication in the pathogenesis of disease. *Med Clin* (Barc). 2011; 136: 252-6.
26. MENZIES V, LYON DE: Integrated review of the association of cytokines with fibromyalgia and fibromyalgia core symptoms. *Biol Res Nurs* 2010; 11: 387-94.
27. FULLE S, MECOCCI P, FANO G *et al*.: Specific oxidative alterations in vastus lateralis muscle of patients with the diagnosis of chronic fatigue syndrome. *Free Radic Biol Med* 2000; 29: 1252-9.
28. KIGWELL BA: Nitric oxide-mediated metabolic regulation during exercise: effect of training in health and cardiovascular disease. *FASEB J* 2000; 14: 1685-96.
29. AKYOL O, ZOROGLU SS, ARMUTCU F *et al*.: Nitric oxide as a physiopathological factor in neuropsychiatric disorders. *In Vivo* 2004; 18: 377-90.
30. CEPINSKAS G, RUI T, KVIETYS PR: Interaction between reactive oxygen metabolites and nitric oxide in oxidant tolerance. *Free Radic Biol Med* 2002; 33: 433-40.
31. LARSON AA, GIOVENGO SL, RUSSELL IJ *et al*.: Changes in the concentrations of amino acids in the cerebrospinal fluid that correlate with pain in patients with fibromyalgia: implications for nitric oxide pathways. *Pain* 2000; 87: 201-11.
32. SPROTT H, SALEMI S, GAY RE *et al*.: Increased DNA fragmentation and ultrastructural changes in fibromyalgic muscle fibres. *Ann Rheum Dis* 2004; 63: 245-51.
33. YUNUS MB, KALYAN-RAMAN UP, KALYAN-RAMAN K *et al*.: Pathologic changes in muscle in primary fibromyalgia syndrome. *Am J Med* 1986; 81: 38-42.
34. HALLIWELL B, GUTTERIDGE JC: The definition and measurement of antioxidants in biological systems. *Free Radic Biol Med* 1995; 18: 125-6.
35. EREL O: A novel automated method to measure total antioxidant response against potent free radical reactions. *Clin Biochem* 2004; 37: 112-9.
36. HALLIWELL B: Free radicals, antioxidants and human disease: curiosity, cause or consequence. *Lancet* 1994; 344: 721-4.
37. FRANCO AA, ODOM RS, RANDO TA: Regulation of antioxidant enzyme gene expression in response to oxidative stress and during differentiation of mouse skeletal muscle. *Free Radic Biol Med* 1999; 27: 1122-32.
38. LUND N, BENGTSSON A, THORBORG P: Muscle tissue oxygen pressure in primary fibromyalgia. *Scand J Rheumatol* 1986; 15: 165-73.
39. SCHEIBMEIR HD, CHRISTENSEN K, WHITAKER SH, JEGAETHESAN J, CLANCY R, PIERCE JD: A review of free radicals and antioxidants for critical care nurses. *Intensive Crit Care Nurs* 2005; 21: 24-8.
40. ALI M: The cause of fibromyalgia: the respiratory-to-fermentative shift (the DysOx State) in ATP production. *J Integr Med* 2003; 8: 135-40.
41. BRAMWELL B, FERGUSON S, SCARLETT N, MACINTOSH A: The use of ascorbigen in the treatment of fibromyalgia patients: a preliminary trial. *Altern Med Rev* 2000; 5: 455-62.
42. GABY ALAN R: Intravenous nutrient therapy: the "Myers' cocktail". ("Myers' Cocktail"). *Altern Med Rev* 2002; 7: 389-403.
43. LISTER RE: An open, pilot study to evaluate the potential benefits of coenzyme Q10 combined with Ginkgo biloba extract in fibromyalgia syndrome. *J Int Med Res* 2002; 30: 195-9.
44. FU X, JI R, DAM J: Antifatigue effect of coenzyme Q10 in mice. *J Med Food* 2010; 13: 211-5.
45. MIZUNO K, TANAKA M, NOZAKI S *et al*.: Antifatigue effects of coenzyme Q10 during physical fatigue. *Nutrition* 2008; 24: 293-9.
46. CASO G, KELLY P, MCNURLAN MA, LAWSON WE: Effect of coenzyme q10 on myopathic symptoms in patients treated with statins. *Am J Cardiol* 2007; 99: 1409-12.
47. JUNG HJ, PARK EH, LIM CJ: Evaluation of anti-angiogenic, anti-inflammatory and antinociceptive activity of coenzyme Q(10) in experimental animals. *J Pharm Pharmacol* 2009; 61: 1391-5.
48. SCHMELZER C, LINDNER I, RIMBACH G *et al*.: Functions of coenzyme Q10 in inflammation and gene expression. *Biofactors* 2008; 32: 179-83.
49. MENZIES V, LYON DE: Integrated review of the association of cytokines with fibromyalgia and fibromyalgia core symptoms. *Biol Res Nurs* 2010;11: 387-94.
50. KELLER JN, MATTSON MP: Roles of lipid peroxidation in modulation of cellular signaling pathways, cell dysfunction, and death in the nervous system. *Rev Neurosci* 1998; 9: 105-16.
51. WANG ZQ, PORRECA F, CUZZOCREA S *et al*.: A newly identified role for superoxide in inflammatory pain. *J Pharmacol Exp Ther* 2004; 309: 869-78.
52. HOLTON KF, TAREN DL, THOMSON CA, BENNETT RM, JONES KD: The effect of dietary glutamate on fibromyalgia and irritable bowel symptoms. *Clin Exp Rheumatol* 2012; 30 (Suppl. 74): 10-7.
53. DING W, ZHU Q: Metabolism of aluminum in rats. *J Chem Res Toxicol* 1997; 31: 338-41.
54. ANDREWS GK: Regulation of metallothionein gene expression by oxidative stress and metal ions. *Biochemical Pharmacology* 2000; 59: 95-104.
55. FOURNIÉ GJ, MAS M, CAUTAIN B *et al*.: Induction of autoimmunity through bystander effects: Lessons from immunological disorders induced by heavy metals. *J Autoimmun* 2001; 16: 319-26.
56. PERNAMBUCO AP, SCHETINO LP, ALVIM CC *et al*.: Increased levels of IL-17A in patients with fibromyalgia. *Clin Exp Rheumatol* 2013 Sep 10. Epub ahead of print.
57. ROSBORG I, HYLLÉN E, LIDBECK J, NIHLGÅRD B, GERHARDSSON L: Trace element pattern in patients with fibromyalgia. *Sci Total Environ* 2007; 385: 20-7.
58. EISINGER J, PLANTAMURA A, MARIE PA, AYAVOU T: Selenium and magnesium status in fibromyalgia. *Magnes Res* 1994; 7: 285-258.
59. BAZZICHI L, GIANNACCINI G, BETTI L *et al*.: ATP, calcium and magnesium levels in platelets of patients with primary fibromyalgia. *Clin Biochem* 2008; 41: 1084-90.
60. SAKARYA ST, AKYOL Y, BEDIR A, CANTURK F: The relationship between serum antioxidant vitamins, magnesium levels, and clinical parameters in patients with primary fibromyalgia syndrome. *Clin Rheumatol* 2011; 30: 1039-43.
61. SENDUR F, TASTABAN E, TURAN Y *et al*.: The relationship between serum trace element levels and clinical parameters in patients with fibromyalgia. *Rheumatol Int* 2008; 28: 1117-21.
62. LAWRENCE DA, KIM D: Central / peripheral nervous system and immune responses. *Toxicology* 2000; 142: 189-201.
63. PIECZENIK SR, NEUSTADT J: Mitochondrial dysfunction and molecular pathways of disease. *Exp Mol Pathol* 2007; 83: 84-92.