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Multiple Sclerosis and Its Relationship with Oxidative Stress, Glutathione Redox System, ATPase System, and Membrane Fluidity

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Abstract

Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS) with a focus on inflammation, demyelination, and damage to axons leading to neurological deficits. MS pathology is associated with excessive reactive oxygen species (ROS) and generation of reactive nitrogen species (RNS), causing oxidative/nitrosative stress. Deregulation of glutathione homeostasis and alterations in glutathione-dependent enzymes are implicated in MS. Reactive oxygen species enhance both monocyte adhesion and migration across brain endothelial cells. In addition, ROS can activate the expression of the nuclear transcription factor-kappa, which upregulates the expression of many genes involved in MS, such as tumor necrosis factor- α and nitric oxide synthase, among others, leading to mitochondrial dysfunction and energy deficits that result in mitochondrial and cellular calcium overload. Loss of mitochondrial membrane potential can increase the release of cytochrome c, one pathway that leads to neuronal apoptosis. Clinical studies suggest that omega-3 long-chain polyunsaturated fatty acids (PUFAs) including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have anti-inflammatory, antioxidant, and neuroprotective effects in MS and animal models of MS. Here, we review the relationship of oxidative stress, the

glutathione redox system, the ATPase system, and membrane fluidity with the development of MS. In addition, we describe the main findings of a clinical trial conducted with relapsing-remitting MS patients who received a diet supplemented with 4 g/day of fish oil or olive oil. The effects of PUFAs supplementation on the parameters indicated above are analyzed in this work.

Keywords: multiple sclerosis, oxidative stress, mitochondria, membrane fluidity, ATPase

1. Introduction

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS) with partially known etiology. It is the most common cause of neurological disability in young adults. Nutrition is commonly accepted as one of the possible environmental factors involved in the pathogenesis of MS. Omega-3 polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are fatty acids that possess several carbon-carbon double bonds. A diet supplemented with PUFAs has clinical and biochemical effects in patients with autoimmune diseases such as MS. Eicosapentaenoic acid and DHA are found in high proportions in fish oil, and these molecules may have anti-inflammatory, antithrombotic, antioxidant, immunomodulatory functions, and neuroprotective effects on the synaptogenesis and biogenesis of the neuronal membrane. Oxidative stress (OS) that is characterized by excessive production of reactive oxygen species and a reduction in antioxidant defense mechanisms have been implicated in the pathogenesis of MS. In consequence, a reduction in this phenomenon could be beneficial for MS patients [1]. In this work, we describe the relationship of several oxidative stress markers (glutathione redox system, mitochondrial ATPase activity, and membrane fluidity) with the development of MS. Furthermore, we describe the main findings of a clinical trial conducted with relapsing-remitting MS patients who received a diet supplemented with 4 g/day of fish oil or olive oil.

Pathologically, MS is characterized by perivenous infiltration of lymphocytes and macrophages in the brain parenchyma. There are four clinical manifestations of MS: relapsing-remitting, primary progressive, secondary progressive, and progressive-relapsing. The MS lesions are typically scattered, and the clinical picture can vary from a benign self-limiting disorder to severe and highly disabling disease. MS is a multifactorial disease involving genetic, immunological, and environmental factors that trigger the autoimmune process leading to the pathological changes of the disease. In this regard, it has been proposed that a viral infection in which self-antigens that generate molecular mimicry with myelin proteins cause a loss of tolerance against it, which results in the destruction of myelin mediated by activated T lymphocytes in white matter of the brain and sometimes extending into the gray matter, resulting in defects in the conduction of nerve impulses that leads to symptoms, depending on the affected site of the brain or spinal cord [1] (**Figure 1**).

According to the areas of myelin destruction, sensory or motor symptoms are affected (balance or vision disorders). The symptoms can change between an “outbreak” or relapse (emergence of new neurological symptoms or worsening of previous ones) and remission. Demyelinating lesions or “plaques” of different sizes and locations are spread throughout the CNS, and the onset of symptoms and response to treatment is unique to each patient [2].

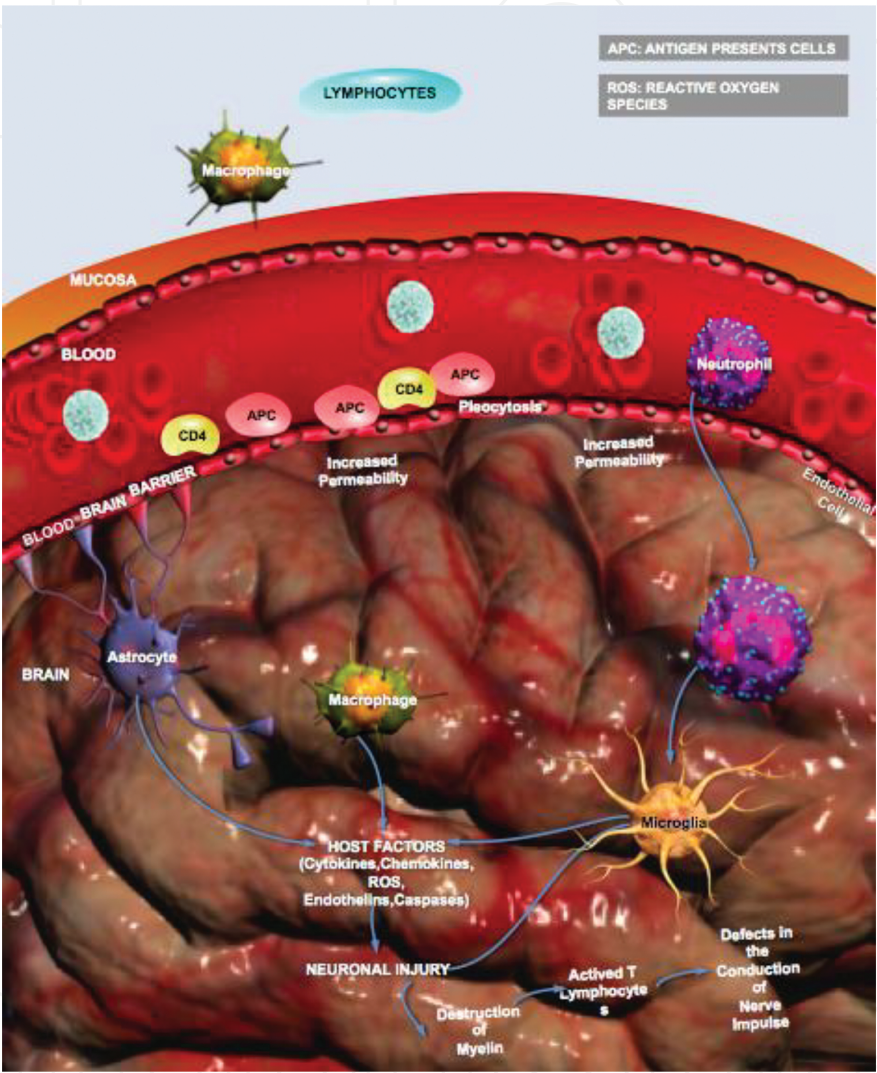


Figure 1. Immune-mediated destruction of myelin components in multiple sclerosis. Pathway Builder Online Tool was used to draw the figure [27].

2. Oxidative stress and multiple sclerosis

OS is a cellular state where the homeostasis of redox reactions is altered when the production of reactive oxygen (ROS) and reactive nitrogen species (RNS) exceed their elimination. These reactive species are generated, among other causes, by oxidative metabolism. Neurons of the CNS are very active in oxidative metabolism, as they are constantly exposed to low-to-

moderate levels of ROS, and these species are removed by antioxidants (melatonin, vitamin D, vitamin E, glutathione) and antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase, etc.). In chronic inflammatory diseases, such as MS, antioxidant defenses are overcome, which leads to oxidative stress [3].

Collectively, the ROS are reactive species derived from oxygen that include the superoxide anion ($O^{\bullet-}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($\bullet OH$). The RNS are reactive species derived from nitrogen and include nitric oxide ($NO\bullet$) and peroxynitrite ($ONOO^-$). The ROS and RNS are extremely unstable and reactive because they have an unpaired electron in their outer orbital. They take electrons from proteins, lipids, carbohydrates, and nucleic acids, causing damage to biological membranes, genetic material, and other macromolecules. The CNS is particularly vulnerable to oxidative damage since it has a very active mitochondrial metabolism, which leads to high levels of intracellular superoxide anions. Moreover, oligodendrocytes have low levels of antioxidant enzymes and a high concentration of iron. Unsaturated fatty acids are the most vulnerable to free radicals, and because myelin has a high lipid-to-protein ratio, it is a preferred target of ROS [4]. The ROS are generated by a number of cellular oxidative and metabolic processes including activity of the enzymes of the mitochondrial respiratory chain, xanthine oxidase, NADPH oxidase, monoamine oxidases, and metabolism of arachidonic acid (AA) mediated by the activity of lipoxygenases (LOX), and ROS are produced primarily by leakage of electrons in the mitochondrial respiratory chain [3].

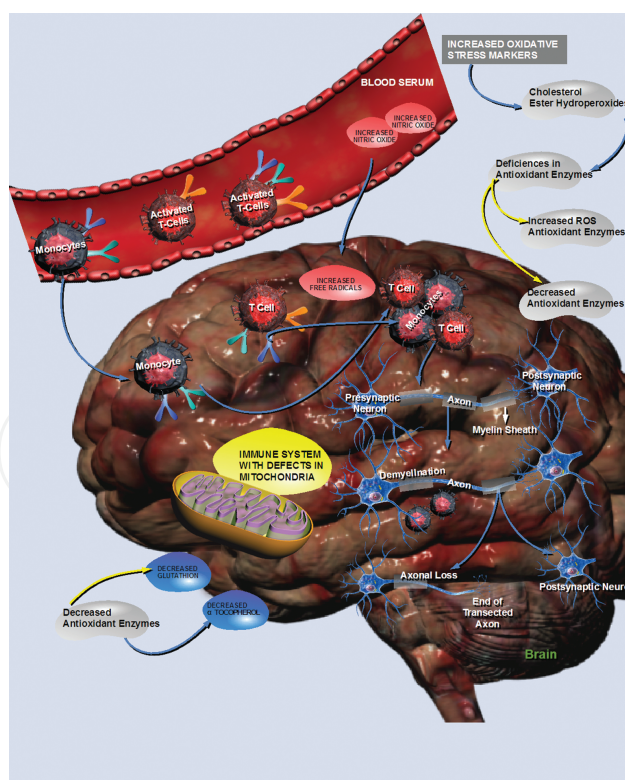


Figure 2. Oxidative stress levels are directly related to the progression of MS. Pathway Builder Online Tool was used to draw the figure [27].

Numerous studies in MS patients have shown an increase in the production of OS markers (such as cholesteryl ester hydroperoxides) and lower levels of uric acid (a ONOO⁻ scavenger). These changes are accompanied by significant deficiencies in antioxidant enzymes compared to healthy subjects. The increase in ROS coupled with decreased antioxidant capacity is not enough to entirely explain the pathogenesis of MS [4, 5]. Other reports suggest that the loss of myelin nerve sheath is possible because the immune system participates in combination with defects in the mitochondria, and these defects cause the generation of ROS and RNS. Macrophages and monocytes release mediators of OS that degrade the unsaturated fatty acids. The ROS have also been implicated as a mediator of demyelination of axonal damage in MS and experimental autoimmune encephalomyelitis (EAE) [6]. It is important to mention that in assessing platelets in MS patients, increased activity of free radicals with decreased levels of important antioxidants such as glutathione and alpha-tocopherol has been reported [7] (Figure 2).

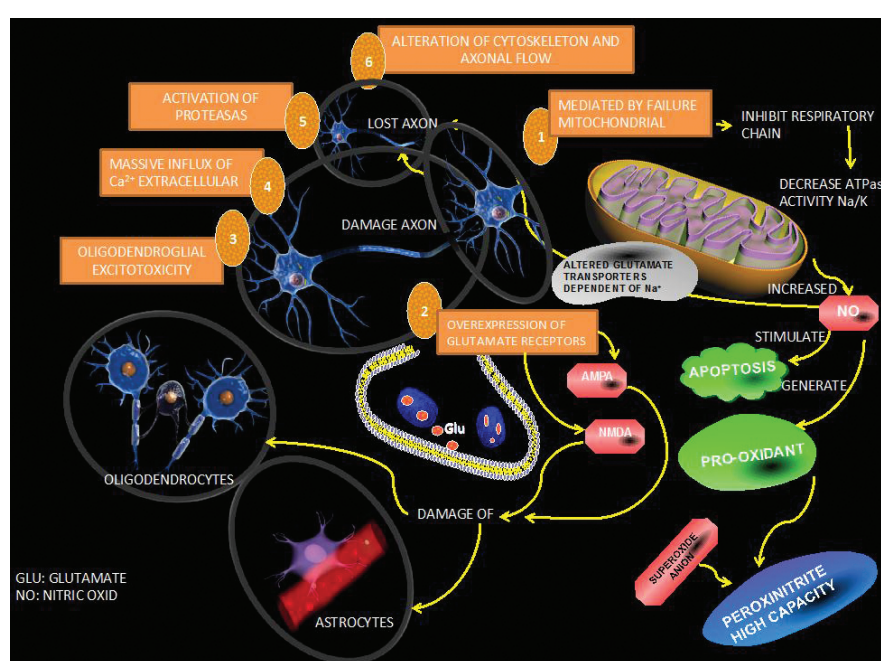


Figure 3. Damage to axons. Pathway Builder Online Tool was used to draw the figure [27].

The molecular mechanisms proposed to explain how ROS could specifically mediate brain damage are the following: (1) The lower levels of antioxidants can promote increased activity of lipoxygenase in CNS stimulating leukotriene production, thereby increasing the immunoinflammatory processes in the cerebral cortex; (2) the damage to myelin can be caused by activation of T cells that may be activated for the presence of free radicals produced by the synthesis route of AA. Then appear the markers of OS associated with reduced activity of superoxide dismutase and the increase in glutamine, followed by increases of •OH and the production of peroxides which ultimately has a negative impact on myelin. After that, the evident changes in mitochondrial activity and finally changes in membrane fluidity (particularly, mitochondrial membranes) appear [8].

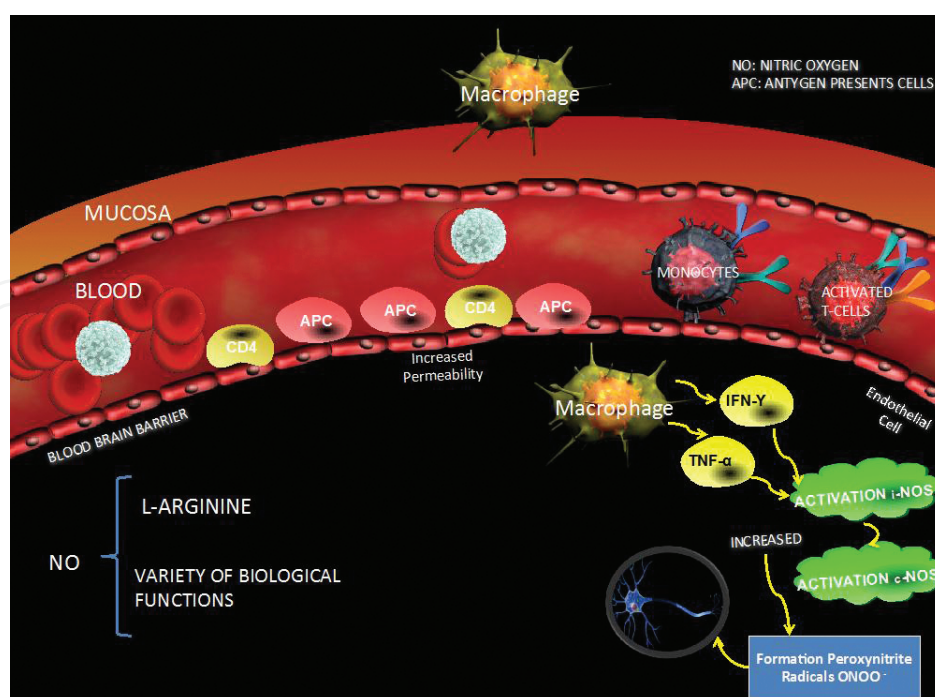


Figure 4. Effect of nitric oxide in inflammation. Pathway Builder Online Tool was used to draw the figure [27].

Paraclinical studies have shown an increased metabolism of the RNS in serum, lymphocytes, and cerebrospinal fluid of MS patients, which correlate to pathology studies. ONOO^- is also closely associated with acute inflammatory lesions [9]. Damage to axons is mediated by the following: (1) failure in mitochondrial energy metabolism due to inhibition of the respiratory chain by nitric oxide, which in turn causes a decrease in Na^+/K^+ ATPase activity and alters Na^+ -dependent glutamate transporters, (2) over-expression of glutamate receptors, (3) oligodendroglial excitotoxicity, (4) massive influx of extracellular Ca^{++} , (5) activation of proteases, and (6) impaired axonal transport. These mechanisms produce glutamate excitotoxicity and increased generation of nitric oxide leading to nitrosative stress. Nitric oxide is a highly toxic element that by itself blocks nerve conduction, especially in demyelinated axons, and stimulates apoptosis. When nitric oxide is combined with the superoxide anion, it generates a potent free radical, the pro-oxidant peroxynitrite. Glutamate in turn causes neurodegeneration through the AMPA and NMDA receptors in oligodendrocytes and astrocytes (**Figure 3**). It is possible to explain the role of mediators using an experimental model of autoimmune encephalitis: Protection against the experimental disease occurs after administration of a glutamate antagonist [10].

Under physiologic conditions, nitric oxide is produced from L-arginine by constitutive nitric oxide synthase (cNOS) and participates in a variety of important biological functions such as immunoregulation of inflammatory reactions, the downregulation of tumor necrosis factor (TNF)- α production, MHC II expression in macrophages, induction of apoptosis in CD4 cells, physiological regulation of the mitochondrial respiratory chain, inhibition of antigen presentation, and leukocyte adhesion and migration. However, during inflammatory reactions, exposure of macrophages to interferon (IFN)- γ and TNF- α results in the activation of the

inducible isoenzyme of NOS (iNOS), which increases up to 10 times the levels of nitric oxide. Nitric oxide facilitates the formation of peroxynitrite radicals. Only cells capable of generating a high flow of NO^\bullet have the potential for causing nitrosative stress. The role of nitric oxide in MS is therefore complex, and in fact, peroxynitrite is definitely more toxic than nitric oxide [9] (Figure 4).

3. Reactive oxygen species, cytokines, and axonal damage in multiple sclerosis

Mechanisms of axonal damage are the consequence of the presence of $\text{TNF-}\alpha$, matrix metalloproteinases (MMPs), ROS, antibodies, increased glutamate, and aspartate, and these molecules cause excitotoxicity in MS patients. Glutamate is increased in MS patients (active lesions) especially in white matter of normal appearance. Mature oligodendrocytes and astrocytes are highly sensitive to glutamate due to the expression of AMPA and NMDA receptors [9]. The myelin sheath can be damaged by cytokines, autoantibodies, ROS, proteolytic enzymes, and phagocytosis. Increased ROS by activated microglia (specialized macrophages of the CNS) during the immune response gives a state of increased lipid peroxidation, and the oligodendrocyte cell is the cell most susceptible to damage by ROS. Myelin degradation may be the result of lipid peroxidation mediated by peroxides, but the role of these specific toxic factors in the pathogenesis of MS remains partially elusive [9].

4. Glutathione system and multiple sclerosis

In a recent study, the oxidation of DNA in the nucleus of oligodendrocytes and oxidation of lipids in the myelin of oligodendrocytes and axons were observed. This oxidation was associated with the active process of demyelination and neurodegeneration. Active lesions in relapsing-remitting MS (RRMS) and progressive course patients were associated with inflammation, lipid peroxidation, and DNA oxidation [11]. Similarly, Ortiz et al. [12] observed an increase in serum lipid peroxides and nitrite/nitrate levels and the activity of glutathione peroxidase in patients with RRMS compared to healthy individuals.

Reduced ubiquinone and vitamin E levels, and reduced activity of the enzyme glutathione peroxidase in lymphocytes and granulocytes were reported (with a decrease in 51 and 78%, respectively), as well as a decrease in glutathione reductase activity in granulocytes (27%) and lymphocytes (8%) [13]. In contrast, in 2012, Tasset et al. [14] found an increase in activity of the glutathione reductase in patients with RRMS when compared to control subjects (1.3 ± 0.9 vs 0.3 ± 0.19 , $P < 0.01$), and an increased ratio of reduced glutathione to oxidized glutathione (GSH/GSSG) in these patients (28.2 ± 39.6 vs 4.0 ± 2.9 , $P < 0.01$). Similarly, an increase was found in the levels of oxidized glutathione and also increased concentrations of isoprostanes and malondialdehyde (MDA) in patients with MS [15, 16].

4.1. Glutathione deficiency and multiple sclerosis

There are several reports in the literature that relate the decrease or alteration of glutathione (GSH) metabolism with several neurodegenerative diseases. Biochemical analysis of postmortem brains has provided evidence for the generation of oxidative stress during the course of the disease since the total GSH content is reduced by 40–50% compared to controls. Also in several brain regions, we have found increased levels of lipid peroxidation [17]. The ratio GSH/GSSG (usually 10:1) is considered consistent with the concept of oxidative stress as an important part in the pathogenesis of MS. Moreover, low concentrations of GSH appear to be an important indicator of oxidative stress during the progression of MS. Although the decrease in GSH alone is not responsible for the degeneration of glial cells and neurons, reduced GSH could increase the susceptibility to other stressful factors and contribute to neuronal damage at glia and neuron cells. Glutathione has been reported to protect mitochondrial complex I activity against nitrosative stress, as S-nitrosoglutathione is formed. When this complex increases its content of nitrotyrosine and nitrosothiol groups in response to nitrosative stress, its activity is inhibited and therefore ATP production is diminished, which causes neuronal degeneration [10]. The role of glial cells in generating ROS in MS and the selective vulnerability of neurons is due to activated glial cells surrounding these neurons, as these glial cells are also directly involved in GSH levels. The engagement of the glutathione system in astroglial cells contributes to the reduction in its antioxidant defenses and so poor glial defense could contribute to existing neuronal damage (**Figure 5**) [10]. Furthermore, the specific activities of some enzymes that metabolize GSH are high, as in the case of glutathione peroxidase, glutathione reductase, and glutathione S-transferase. Other products of OS are also elevated, as in the case of 4-hydroxynonenal (4-HNE, a product of lipid peroxidation of polyunsaturated omega-6 fatty acids) [17].

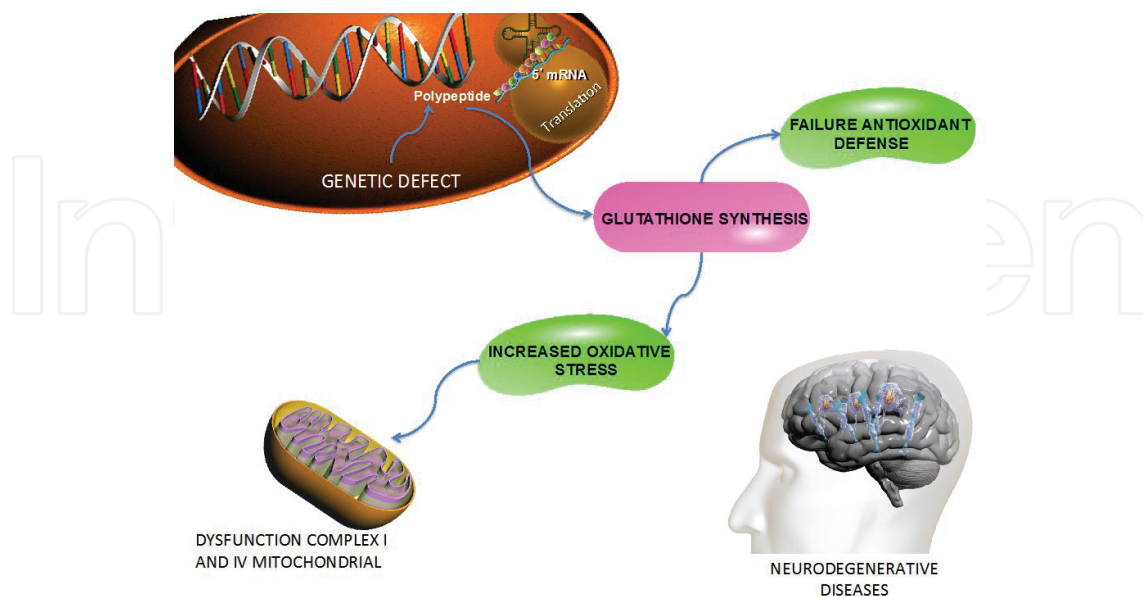


Figure 5. Genetic defect in glutathione synthesis and neurodegenerative diseases. Pathway Builder Online Tool was used to draw the figure [27].

A new proposal is that a genetic defect of glutathione synthesis may be the initial event in the failure of the antioxidant defenses. In neurodegenerative diseases, a decreased GSH level is accompanied by dysfunction of the mitochondrial complex I and complex IV and promotes oxidative stress [18]. We found a significant decrease in GSH levels in the cerebrospinal fluid of patients with this disease, and, in addition, proton magnetic resonance studies have shown a 50% decrease in GSH levels in the frontal cortex of patients with MS (**Figure 5**).

5. Mitochondria

Mitochondria are granular and filamentous organelles found in the cytoplasm of all eukaryotic cells and are the main site of adenosine triphosphate (ATP) synthesis by the processes of oxidative phosphorylation. These organelles vary in size and shape depending on the source and metabolic status, but are often ellipsoids of about 5 microns in diameter and 1 micron long. A typical eukaryotic cell contains more than 2,000 mitochondria, which takes up about one-fifth of the cell volume, an amount that is needed to meet the energy demands of the cell. Its main function is the mitochondrial respiration process in which the reducing power produced in the oxidation reactions enters the electron transport chain and energy is captured in the form of adenosine triphosphate (ATP). Mammalian tissues containing more mitochondria are the heart and brain [19]. The mitochondrion is formed by two membranes: the outer membrane and the inner membrane, which is highly folded, and the inner matrix is gel (approximately 50% water) [20].

The outer mitochondrial membrane contains porin, a pore-forming protein that allows diffusion of up to 10 kD molecules). The inner membrane contains approximately 75% protein and 25% lipids by weight, and it is much richer in outer membrane proteins. The inner membrane is permeable only to carbon dioxide (CO_2), oxygen (O_2), and water (H_2O). The passage of metabolites such as ATP, adenosine diphosphate (ADP), pyruvate, calcium ions (Ca^{2+}), and phosphate (PO_4) is regulated by controlling the transport proteins. This controlled permeability allows the generation of ionic gradients and results in the compartmentalization of metabolic functions between the cytoplasm and mitochondria. The inner membrane components of the respiratory chain are responsible for the synthesis of ATP (ATP synthase F_0F_1) [22], where the enzyme complex is housed. The inner membrane is arranged in ridges, giving it a large surface area: A single mitochondrion may have more than 10,000 sets of electron transfer systems (respiratory chain) and ATP synthase molecules distributed throughout the membrane's internal surface [21]. The inner membrane is, from the functional point of view, the most important because it contains the components of the respiratory chain and proteins necessary for the synthesis of ATP [21]. The mitochondrial matrix is the space delimited by the inner membrane and contains the pyruvate dehydrogenase complex and the enzymes of the tricarboxylic acid cycle (TCA), the fatty acid oxidation, and the oxidation of amino acids [21] (**Figure 6**).

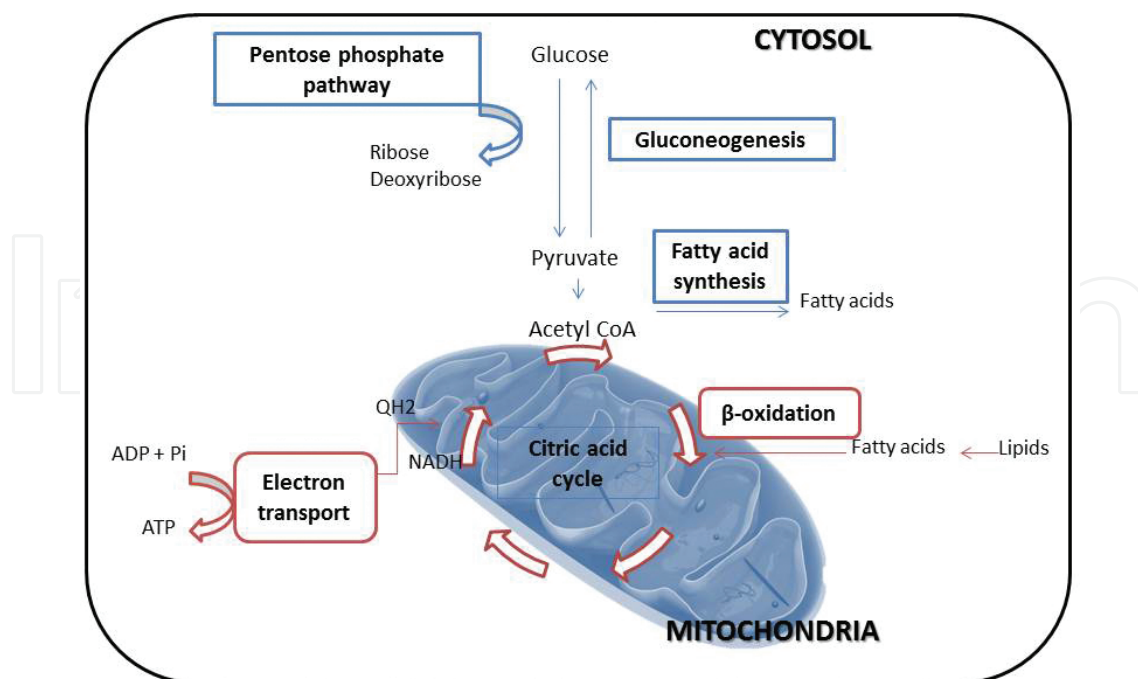


Figure 6. Main mitochondrial metabolic pathways. An important component of metabolic regulation is specialization. Mitochondria have a role in biosynthesis, catabolism, and energy metabolism, including citric acid cycle, oxidative phosphorylation, and fatty acid breakdown pathways.

The chemical energy required for cellular activities such as biosynthesis, transportation of ions and molecules, and mechanical work comes from ATP. Mitochondria generate more than 90% of the energy needed for the proper functioning of tissues that are highly dependent on aerobic metabolism, such as the brain and heart. This subcellular organelle provides the energy necessary for the production of ATP [22]. Depending on cell type and metabolic state, mitochondria consume approximately 90–95% of the oxygen consumed by the cell. The energy of this process, in which electrons are transferred from the substrates of the TCA to oxygen, is coupled to vectorial transport of H^+ from the mitochondrial matrix space [22].

The electron carriers, reduced nicotinamide dinucleotide adenine (NADH) and reduced flavin dinucleotide adenine ($FADH_2$), originating mainly in the TCA cycle, confer the energy that electrons carry. This energy is released gradually along the respiratory chain in the mitochondrial inner membrane. In this membrane, an exchange of electrons between the enzymatic complexes is given by NADH or $FADH_2$ [20].

The complexes are as follows: I (NADH-ubiquinone reductase), II (succinate-ubiquinone reductase), III (ubiquinol-cytochrome c reductase), IV (cytochrome oxidase), and V (ATP synthase complex F_0F_1) [20]. Electron transport is carried out by complexes I, III, and IV that produce a flow of electrons accompanied by a movement of protons from the mitochondrial matrix to the intermembrane space (space between the inner and outer mitochondrial membrane). This produces a difference in proton concentration and a difference in charge across the membrane [20]. The proton-motive force generated thereby drives protons through the F_0F_1 -ATP synthase, allowing condensation of a phosphate group to ADP, with the formation

of ATP [23]. Meanwhile, the complex F_0F_1 -ATP synthase is an enzyme located in the inner membrane of the mitochondria, responsible for ATP synthesis from ADP and a phosphate group (P_i), and the energy is supplied by a flow of protons (H^+). The difference between the terms ATPase and ATP synthase is that the enzyme has a dual function: It breaks down ATP to ADP and P_i (activated ATPase), and it also allows for catalyzing P_i binding of ADP using the proton gradient for ATP synthesis (ATP synthase activity). As complex V has both functions, we can name it indiscriminately when speaking in general terms of the enzyme [23]. This enzyme is constituted by two components: a soluble portion (F_1), located in aqueous medium, and another portion (F_0), which is lipid soluble. The F_0 part is inserted into the lipid bilayer and is sensitive to the antibiotic oligomycin (Figure 7).

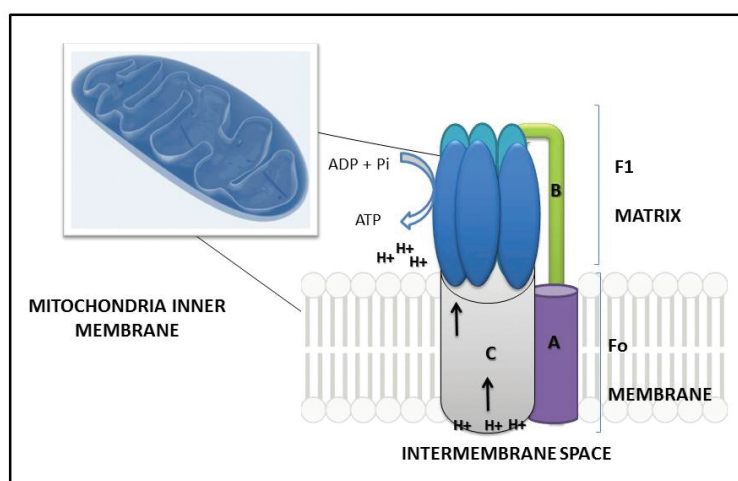


Figure 7. ATP synthase complex structure. The ATP synthase complex plays a central role in energy transduction in living cells that uses energy released by the movement of protons down a transmembrane electrochemical gradient to drive the synthesis of ATP. This enzyme is located in the inner membrane of mitochondria and is constituted by two parts: a soluble portion (F_1) in aqueous medium and another portion (F_0) lipid soluble. The F_0 part is inserted into the lipid bilayer.

On the other hand, pathophysiological features exhibited the association between mitochondrial dysfunction, decreased activity of complex I and complex IV of the electron transport chain, and the glutathione system in MS [23].

6. Mitochondria and multiple sclerosis

In acute phases of the disease, axonal degeneration correlates with the severity of inflammation. This type of injury has been used in an experimental model of autoimmune encephalomyelitis (EAE), where acute mitochondrial damage within axons is detected and later suffers from focal damage as a preliminary pathological step of axonal damage [15].

Complex IV of the mitochondrial electron transport chain has a binding site for O_2 (The final acceptor in the respiratory chain) and catalyzes the reduction in O_2 to H_2O . Interestingly, nitric oxide inhibits mitochondrial respiration by reacting with either the reduced or the oxidized

binuclear site of cytochrome c oxidase, leading to ATP depletion. In cases of excessive nitric oxide production, complete inhibition of cytochrome c oxidase has been shown to contribute to pathology.

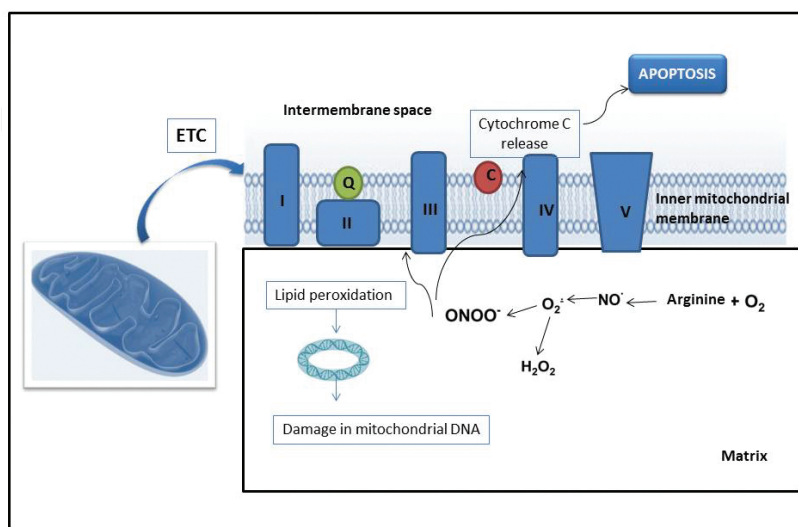


Figure 8. Association of mitochondrial electron transport chain with oxidative stress. The mitochondrial electron transport chain (ETC), which is composed of four multiprotein complexes named complex I–IV, has been recognized as one of the major cellular generators of free radicals. Leakage of electrons directly from the intermediate electron carriers generates reactive oxygen species that leads to membrane lipid peroxidation, mitochondrial DNA damage, and the release of cytochrome C to the cytosol triggering apoptosis.

At the same time, interrupting the electron transport chain by binding of NO to complex IV increases electron release, thus facilitating the formation of reactive oxygen species, firstly superoxide anion and subsequently H₂O₂ and OH[•]. Peroxynitrite has a direct effect on mitochondria leading to lipid peroxidation of membrane lipids and thus damaging the complexes of the respiratory chain and mitochondrial DNA. Opening of permeability transition pores and release of cytochrome C from mitochondria initiate apoptosis (**Figure 8**).

At the stage of acute inflammation, a set of mechanisms that alter mitochondrial function is produced. The energy deficit causes structural and functional damage to macromolecules by increased ROS that ultimately leads to severe axonal damage. In these events, the mitochondria has an important role; therefore, if we know what the mechanisms involved in glial and neuronal alterations are, we must be able to identify the elements that can be used as effector elements and design drugs to control and reduced harm during the stage of relapse [14].

Many demyelinated axons survive during a relapse, and these can become chronically demyelinated axons, in which case axonal mitochondria develop compensatory mechanisms to cope with the lack of myelin. There are reports in which inactive lesions from chronic demyelinated axons of patients with MS are observed. In such reports, they have found an increase in the activity of mitochondrial complex IV and increased synphilin anchoring protein [19]. However, axons progressively degenerate in chronic lesions of MS patients. In the absence of myelin, redistribution of Na⁺ occurs to maintain the transmission of nerve impulses that

increases energy demand, and this produces a situation of “virtual hypoxia.” At the end, the demand exceeds the capacity of axonal mitochondria to produce enough ATP, which causes an increase in the concentration of Ca^{2+} in the axon. Ca^{2+} pumping and extended levels of intramitochondrial calcium leads to opening pores, rupture of the outer mitochondrial membrane, and release of cytochrome C, finally leading to apoptosis (**Figure 8**).

One of the questions we have not answered is: Why are mitochondria helpless and overwhelmed by the energy demand and how does this happen? Are the axons unable to maintain stable mitochondrial activity in demyelination?. This reflects the inability of the cell to carry and generate mitochondria. Dutta et al. [23] have shown decreased gene expression of 26 nuclear-encoded subunits of the oxidative phosphorylation chain in non-demyelinated motor cortex from MS patients, which coincided with a significant reduction in activity of NADH dehydrogenase and ubiquinol-cytochrome c reductase. In the progressive phase of MS, it is postulated that chronically demyelinated axons are unable to maintain mitochondrial function, and thus, a deficit of ATP synthesis coupled with oxidative stress results in irreversible axonal damage.

7. Effect of fish oil (Omega3) and olive oil on membrane fluidity, ATPase activity in relapsing-remitting multiple sclerosis.

The mechanism of action for omega-3 PUFAs is suggested to be attributed to immunomodulation and antioxidant action [24]. For instance, omega-3 PUFAs decrease the production of inflammatory mediators (eicosanoids, cytokines, and ROS) and the expression of adhesion molecules. They both act directly by replacing AA as an eicosanoid substrate and inhibiting AA metabolism and indirectly by altering the expression of inflammatory genes through effects on transcription factor activation. Omega-3 PUFAs also give rise to anti-inflammatory mediators (resolvins and protectins) [25]. Effects of resolvins and protectins include reducing neutrophil trafficking, cytokine, and ROS regulation and lowering the magnitude of the inflammatory response [26].

Previously, we developed a twelve-month randomized double-blind controlled clinical trial in 50 patients with relapsing-remitting MS. Patients received an oral dose of 4 g/day of fish oil (containing a total of 800 mg of EPA and 1600 mg of DHA) or olive oil. Fasting blood samples were collected at baseline and after 6 and 12 months of the trial, in order to evaluate the effect of consumption of omega-3 PUFAs on some markers of oxidative stress at the peripheral level. The initial findings of this work were the decrease in serum levels of $\text{TNF}\alpha$, $\text{IL-1}\beta$, IL-6 , and nitric oxide metabolites compared with the placebo group [27].

On the other hand, after 12 months of intervention, supplementation with omega-3 PUFAs significantly enhanced the quantities of serum omega-3 highly unsaturated fatty acids compared with baseline values. Additionally, the levels of medium-chain monounsaturated fatty acids were significantly decreased. The olive oil supplementation induced minor decreases in EPA and DHA levels after 12 months of intervention. There were significant increases in both EPA and DHA in the group given fish oil supplementation compared to the

control group receiving olive oil. These increases were associated with a concomitant decrease in AA. Consequently, the omega-3 fatty acid index in the fish oil group increased significantly, and the ratios of n-6/n-3 and AA/EPA were decreased [28].

No differences in glutathione reductase activity and content of reduced glutathione, oxidized glutathione, and oxidized/reduced glutathione ratio were seen after 12 months of supplementation with omega-3 PUFAs. However, a trend in favor of omega-3 PUFAs supplementation was observed in GSSG levels and glutathione reductase activity at 12 months of intervention between the study groups [28].

A steady decrease in mitochondrial ATPase activity in platelets was observed in the groups given omega-3 fatty acid and the control group receiving olive oil. Membrane fluidity of platelets was significantly reduced in MS patients. Interestingly, a significant increase in platelet membrane fluidity was observed in the groups receiving omega-3 fatty acid and the control group receiving olive oil. As well, the fluidity of erythrocyte membranes was unchanged for both treatments (Unpublished results).

Epidemiological and experimental studies suggest an increased incidence of MS in populations with a high intake of saturated fats mainly from animal sources. Therefore, by consuming a diet high in fatty acids, without an appropriate number of unsaturates, a shift is produced in the integrity and functionality of the membrane [29]. An optimal balance in the consumption of fatty acids includes 35% polyunsaturated fatty acids and 65% saturated fatty acids, and the appropriate proportion of PUFA to maintain membrane balance is 50% omega-3 with 50% omega-6. The above ratio was a factor that inactivated the CD4 autoreactive cells in the CNS, a phenomenon that prevents the production of proinflammatory cytokines and free radicals [30].

Membrane fluidity depends on the temperature, the ratio of saturated/PUFA fatty acids, the presence of "lipid rafts," and the proportion of cholesterol present at the membrane [31]. Previous studies in patients with rheumatoid arthritis had increased cell membrane rigidity compared to membranes from those receiving immunomodulatory treatment. Our results showed diminished platelet membrane fluidity in MS patients and that proper membrane fluidity is restored with treatment of omega3 PUFAs. The increase in platelet membrane fluidity is directly related to the incorporation of PUFA's. Furthermore, the increase in membrane fluidity is accompanied with a significant decrease in mitochondrial ATPase activity. This ensures that the activity of ATP synthesis in mitochondria remains elevated.

8. Conclusions

The inflammatory process seen in MS is due to an excess production of pro-inflammatory cytokines, which leads to increased secretion of ROS. Oxidative stress plays a preponderant, key role in the pathogenesis of MS. Reactive oxygen species generated by macrophages have been implicated as mediators of demyelination and axonal damage in EAE and MS. The main findings of a clinical trial conducted with relapsing-remitting MS patients who received a diet supplemented with 4 g/day of fish oil or olive oil are the following:

1. Fish oil supplementation resulted in a high increase in proportions of EPA and DHA, leading to a decrease in AA concentrations as well as the AA/EPA ratio. These changes in fatty acids are indicative of a reduction in the production of inflammatory eicosanoids from AA and an increase in anti-inflammatory mediators such as resolvins and protectins.
2. No differences in glutathione reductase activity, content of reduced and oxidized glutathione, and GSH/GSSG ratio were seen after 12 months of supplementation. However, fish oil supplementation resulted in a smaller increase in GR compared with the control group. In addition, there was a significant change in glutathione reductase activity within subjects in the fish oil group after 6 months of treatment, while no significant differences within subjects were observed in the control group, suggesting a possible effect of fish oil on antioxidant defense mechanisms of the cell. Although glutathione reductase activity was not significantly different between the groups, fish oil supplementation resulted in a smaller increase in GR compared with the control group, suggesting a possible antioxidant effect of fish oil supplementation.
3. Membrane fluidity of platelets was significantly reduced in MS patients. That membrane property steadily increased in the groups given omega-3 fatty acid and the control group receiving olive oil. The increases in membrane fluidity of platelets were associated with a decrease in mitochondrial ATPase. As well, the fluidity of erythrocyte membranes was unchanged for both treatments (unpublished results).

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