

**Universidade de Lisboa
Faculdade de Farmácia**



Nutritional Status and Antioxidant Capacity in Multiple Sclerosis

Sara Vicente de Sousa Duarte

Monografia orientada pela Professora Doutora Cristina Maria
Leitão de Carvalho, Professora Associada

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**Trabalho Final de Mestrado Integrado em Ciências Farmacêuticas
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Resumo

A Esclerose Múltipla é uma doença neurológica que afecta o sistema nervoso central, onde linfócitos auto-reactivos promovem um processo de inflamação com proliferação de linfócitos T. Esses linfócitos activam também a microglia, que conduz a lesões na mielina, desacelerando o fluxo de impulsos eléctricos para determinada região do corpo, consoante o local onde ocorre a desmielinização. Por esta razão, os sintomas sentidos pelos doentes portadores de Esclerose Múltipla, são muito diferentes entre si, dificultando o processo de diagnóstico. À luz do conhecimento actual, a susceptibilidade genética, os factores ambientais – como a exposição ao fumo do tabaco, a baixa exposição à luz solar e, conseqüentemente, os níveis baixos de vitamina D – e a dieta serão determinantes para a etiologia.

A existência de uma maior incidência de Esclerose Múltipla em países industrializados, onde as dietas são mais calóricas, com um maior consumo de produtos processados, ricos em açúcar e, simultaneamente, menos nutritivas, pobres em vitaminas e minerais, tem suscitado interesse em diversos grupos de investigação. Recentemente, também se tem estudado como as opções alimentares afectam a flora intestinal e como esta alteração se relaciona com a inflamação observada nas fases iniciais da Esclerose Múltipla. Nesse sentido, a dieta do indivíduo terá um papel fundamental, podendo as escolhas alimentares mais saudáveis diminuir a inflamação.

Os doentes de Esclerose Múltipla apresentam valores inferiores de alguns nutrientes, comparativamente com indivíduos saudáveis, como os níveis de vitamina D, entre outras e alguns minerais essenciais. Quando a dieta é ajustada de forma a corrigir essas diferenças, os doentes mostram melhorias na evolução da doença. O mesmo acontece com a toma de suplementos vitamínicos, nomeadamente vitamina D. Atualmente, os medicamentos disponíveis apenas conseguem atrasar a progressão da doença. No entanto, apesar dos benefícios associados à alteração da dieta, o doente não é aconselhado pelo médico, que o segue, relativamente à importância dessas alterações. Os doentes de Esclerose Múltipla são indivíduos que se mostram bastante disponíveis em alterar os seus hábitos alimentares e estilo de vida, no entanto, muitas vezes, fazem-no sem aconselhamento ou recorrendo a medicinas alternativas.

Orientadora: Doutora Cristina Maria Leitão de Carvalho, Professora Associada

Palavras-chave: Esclerose Múltipla; Inflamação; Stress Oxidativo; Nutrição; Antioxidantes

*Resumo redigido sem o novo acordo ortográfico.

Abstract

Multiple Sclerosis is a neurological disease that affects the central nervous system, in which self-reactive lymphocytes promote an inflammatory process, leading to the proliferation of T lymphocytes. They also activate microglia, which leads to lesions in myelin, slowing the flow of electrical impulses to certain regions of the body, depending on the place where demyelination occurs. Hence, the symptoms experienced by patients with Multiple Sclerosis are very different, making the diagnosis process difficult. For what is currently known, genetic susceptibility, environmental factors – such as exposure to tobacco smoke, low exposure to sunlight and, consequently, low levels of vitamin D – and diet are determining factors for the etiology.

The higher incidence of Multiple Sclerosis in industrialized countries, where diets are more caloric, with a greater consumption of processed products, rich in sugar and, simultaneously, less nutritious, poor in vitamins and minerals, has raised interest in different investigation groups. Recently, it has also been studied how food choices affect the intestinal flora and how these changes may be related to the inflammatory status that is observed in the initial stages of Multiple Sclerosis. Taking that into account, the individual's diet will play a fundamental role, and healthier food choices could reduce inflammation.

Multiple Sclerosis patients have lower levels of some nutritional factors compared to healthy individuals, such as vitamin D levels, among other vitamins, and some essential minerals. When the diet is adjusted to correct these differences, patients show improvements managing the progression of the disease. The same happens with taking vitamin supplements, namely vitamin D. Despite those benefits, upon diagnosis, Multiple Sclerosis patients are not advised about the importance of changes in diet, even if the medications currently available only delay the progression of the disease. Multiple Sclerosis patients are very willing to change their eating habits and lifestyle. However, most of them do it without any clinical counseling or by selecting alternative medicines.

Keywords: Multiple Sclerosis; Inflammation; Oxidative Stress; Nutrition; Antioxidants

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A realização desta monografia foi um processo onde muitas pessoas contribuíram, até sem o saber.

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Foi na Faculdade de Farmácia da Universidade de Lisboa que eu própria, conheci o meu diagnóstico e foi aqui que, pela primeira vez, apesar das limitações e dificuldades sentidas há muito tempo, tive o apoio inesgotável de alguns Professores que fui conhecendo. Não posso deixar de referir, logo do início desta caminhada, com o diagnóstico recentemente conhecido, o apoio do Professor Doutor Paulo José Pinto Salústio. Eu tinha perdido a visão do meu olho direito poucos dias antes de realizar o seu exame e nunca esquecerei a sua ideia e cuidado que teve em imprimir o enunciado em folha A₃ para que eu conseguisse realizá-lo. Pequenos grandes gestos como este ajudaram-me a não desistir desta caminhada e contribuíram para o seu culminar.

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“In December 1822, I ... was obliged to have my letters read to me,
and their answers written as my eyes were so attacked that
when fixed upon minute objects
indistinctness of vision was the consequence”

Augustus d'Este (1794 – 1848)

Abreviaturas

AP-1 – Activator Protein

APC – Antigen Presenting Cell

ARE – Antioxidant Response Elements

BBB – Blood - Brain Barrier

BF – Burkina Faso

BMI – Body Mass Index

BTN – Bovine Milk Protein Butyrophilin

CAT – Catalase

CIS – Clinically Isolated Syndrome

CNS – Central Nervous System

CSF – Cerebrospinal Fluid

DC – Dendritic Cell

EAE – Experimental Autoimmune Encephalomyelitis

EBV – Epstein-Barr Virus

EDSS – Extended Disability Status Score

EU – Europe

FA – Fatty Acids

FNB – Food and Nutrition Board

GCL – γ -Glutamylcysteine Ligase

GPx – Glutathione Peroxidase

GR – Glutathione Reductase

GSH – Glutathione (reduced form)

GSSG – Glutathione Disulfide (oxidized form)

HFCS – High-Fructose Corn Syrup

HOCl – Hypochlorous Acid

IFN- β – Interferon-beta

IFN- γ – Interferon-gamma

IM – Infectious Mononucleosis

Maf – Musculoaponeurotic Fibrosarcoma

sMaf – small Musculoaponeurotic Fibrosarcoma

MBP – Myelin Basic Protein

MFGM – Milk Fat Globule Membrane

MMP – Metalloproteinase

MOG – Myelin Oligodendrocyte Glycoprotein

MPO – Myeloperoxidase

MRI – Magnetic Resonance Imaging

MS – Multiple Sclerosis

NADPH – Nicotinamide Adenine Dinucleotide Phosphate

NF- κ B – Nuclear Transcription Factor- κ B

NO – Nitric Oxide

NO \cdot – Nitric Oxide Radical

NO $_2\cdot$ – Nitric Dioxide Radical

iNOS – Nitric Oxide Synthase

NOX – Nicotinamide Adenine Dinucleotide Phosphate Oxidase

Nrf2 – Nuclear Factor Erythroid 2-Related Factor2

$^1\text{O}_2$ – Singlet Oxygen

O $_2\cdot^-$ – Superoxide Anion Radical

O₃ – Ozone

O.E.C.D. – Organization for Economic Cooperation and Development

OH• – Hydroxyl Radical

OL – Oligodendrocytes

ONOO⁻ – Peroxynitrite

OS – Oxidative Stress

PA – Pernicious Anemia

PPMS – Primary-Progressive Multiple Sclerosis

PRMS – Progressive-Relapsing Multiple Sclerosis

PUFA – Polyunsaturated Fatty Acids

RCT – Randomized Clinical Trial

RNS – Reactive Nitrogen Species

RO• – Alkoxy Radical

ROO• – Peroxyl Radical

ROS – Reactive Oxygen Species

RRMS – Relapsing-Remitting Multiple Sclerosis

SCF – Scientific Committee on Food of the European Commission

SFA – Saturated Fatty Acid

SOD – Superoxide Dismutase

SPMS – Secondary-Progressive Multiple Sclerosis

TNF-α – Tumor Necrosis Factor-Alpha

Trx – Thioredoxin

UL – Upper Intake Level

UV – Ultraviolet

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1. Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS), the most important non-traumatic cause of neurological disability in young women, typically striking between 20 and 40 years of age (Farinotti et al., 2007). MS affects approximately 2.3 million people worldwide and is most prevalent in North America (140 cases per 100,000) and Europe (108 cases per 100,000) (Doshi and Chataway, 2016).

MS affects both brain and spinal cord and is caused by damage to the fatty myelin sheath, the protective covering that surrounds nerve cells, disrupting the normal flow of electrical impulses through the nerves (Armon-Omer et al., 2019). It is widely accepted that this neurological disease has an immune-mediated basis, however this theory has been questioned (van Rensburg et al., 2021).

MS causes a heterogeneous array of symptoms and signs because of differential involvement of motor, sensory, visual, and autonomic systems. Optic neuritis (inflammation of the optic nerve), Uhthoff's phenomenon (transient fluctuation or worsening of MS symptoms with a rise in body temperature) and Lhermitte's phenomenon (an abnormal electric shock-like sensation down the spine or limbs on neck flexion) are characteristic of MS (Doshi and Chataway, 2016). Some of the common symptoms and signs of MS include fatigue, numbness and weakness in the limbs, vision problems, bladder and bowel dysfunction, problems with balance and coordination and cognitive difficulties. The unpredictable range of symptoms can be explained by the different regions of the brain where lesions take place (Hauser and Cree, 2020).

MS relapses (exacerbation or flare-up) occur because of focal areas of demyelination evolving over 24 hours and persisting for days or weeks before generally improving (Doshi and Chataway, 2016). During MS relapses there is a sudden worsening or appearance of new MS symptoms. The formation of sclerotic plaques (Fig. 1), of which MS gets its name, represents the end stage of a process involving inflammation, demyelination and remyelination, oligodendrocyte depletion, and astrogliosis as well as neuronal and axonal degeneration (Compston and Coles, 2008).

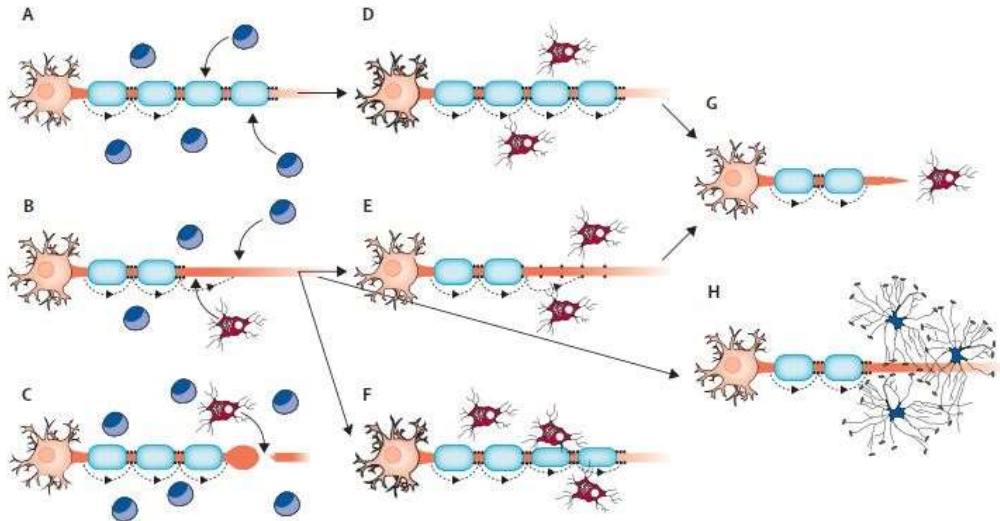


Figure 1. Pathogenesis of multiple sclerosis. The first event is lymphocyte-driven inflammation, which might impede the saltatory propagation of the action potential (arrowheads) in three ways: soluble inflammatory mediators might cause conduction block in structurally intact axons (A), or there may be demyelination (B) or axonal transection (C). Microglia are activated, remove myelin debris and promote remyelination (F). If this fails, persistently demyelinated axons adapt by redistributing ion channels (E: sodium-channel redistribution along denuded axons), which might promote chronic neurodegeneration (G: MS plaque, with a remyelinating oligodendrocyte and degenerating unmyelinated axons). Microglia can become chronically activated, in the absence of lymphocytic inflammation, in areas of normal appearing white matter and also lead to neuronal loss in later stages of the disease (D). In response to chronic tissue injury, astrocytes cause gliosis, which can act as a mechanical barrier for repair (Compston and Coles, 2008).

The consequent disability in MS can be quantified by the gold standard Expanded Disability Status Scale (EDSS), allowing us to grade the disease severity, from 0 to 10 (Table 1), where higher values correspond to a worse disability like a totally helpless bed patient, unable to communicate effectively or eat and swallow (Kurtzke, 1983).

Table 1. Expanded Disability Status Scale. Adapted from Kurtzke (1983)

0	Normal neurologic exam
1.0	No disability, minimal signs in one function system
1.5	No disability minimal signs in more than one function system
2.0	Minimal disability in one function system
2.5	Minimal disability in two function systems
3.0	Moderate disability in one function system or mild disability in three or four function systems, though fully ambulatory
3.5	Fully ambulatory but with moderate disability in one function system
4.0	Fully ambulatory without aid, self-sufficient, up and about some 12 hours a day despite relatively severe disability consisting of one function system. Able to walk without aid or rest some 500 meters
4.5	Fully ambulatory without aid, up and about much of the day, able to work a full day, may otherwise have some limitation of full activity or require minimal assistance; characterized by relatively severe disability, usually consisting of one function system. Able to walk without aid or rest for some 300 meters

5.0	Ambulatory without aid or rest for about 200 meters; disability severe enough to impair full daily activities (eg, to work full day without special provisions)
5.5	Ambulatory without aid or rest for about 100 meters; disability severe enough to preclude full daily activities
6.0	Intermittent or unilateral constant assistance (cane, crutch, or brace) required to walk about 100 meters with or without resting
6.5	Constant bilateral assistance (canes, crutches, or braces) required to walk about 20 meters without resting
7.0	Unable to walk beyond about 5 meters even with aid, essentially restricted to wheelchair; wheels self in standard wheelchair and transfers alone; up and about in w/c some 12 hours a day
7.5	Unable to take more than a few steps; restricted to wheelchair; may need aid in transfer; wheels self but cannot carry on in standard wheelchair a full day; may require motorized wheelchair
8.0	Essentially restricted to bed or chair or perambulated in wheelchair, but may be out of bed itself much of the day; retains many self-care functions; generally, has effective use of arms
8.5	Essentially restricted to bed much of the day; has some effective use of arm(s); retains some self-care functions
9.0	Helpless bed patient; can communicate and eat
9.5	Totally helpless bed patient; unable to communicate effectively or eat/swallow
10.0	Death due to MS

The underlying etiology of MS is still not known, but it is thought to involve a complex genetic attribute, including the HLA-DR15 haplotype, and there are environmental factors that may contribute to MS development such as Epstein–Barr virus infection, vitamin D3 deficiency, smoking and malnutrition (Armon-Omer et al., 2019).

The diagnosis of MS typically involves a combination of medical history assessment, physical examinations, and various diagnostic tests. One of the most important tools of diagnosing MS is a magnetic resonance imaging (MRI) scan that can show evidence of dissemination of MS lesions in time and in space. The diagnosis of MS can be further reinforced by an increase latency of evoked potentials and lumbar puncture (Doshi and Chataway, 2016). Several disease-modifying therapies were approved for MS treatment (Doshi and Chataway, 2016) and a remarkable progress in the development of new MS treatment is being observed in recent years, but still far from finding a definitive drug for MS (Michaličková et al., 2020).

IFN- β is thought to be anti-inflammatory in relapsing-remitting MS (RRMS) and thereby reduces the relapse rate. Unfortunately, only 50% of MS patients respond to and benefit from the IFN- β treatment (Goldmann and Prinz, 2013). There are now several other disease-modifying therapies (DMTs) available with different mechanisms of action (Table 2). Usually, MS therapy prescription is not associated with sufficient information regarding diet and lifestyle. However, dietary factors and lifestyle may exacerbate or ameliorate MS symptoms by modulating the inflammatory status of the disease both in RRMS and in primary-progressive MS (PPMS) (Riccio and Rossano, 2015), two main forms of MS disease that will be explained later in section 2.1.

Table 2. Several Medications approved for the treatment of MS. Adapted from Dobson and Giovannoni (2018)

Medication Class	Examples	Mechanism of Action (Efficacy)
DMTs	Interferons and Glatiramer Acetate	Immunomodulatory (Moderate)
	Teriflunomide	Dihydrorotate dehydrogenase inhibitor (Moderate)
	Dimethyl Fumarate	NRF2 activation and downregulation of NF- κ B (Moderate/High)
	Fingolimod	Selective S1P modulator (High)
Monoclonal Antibodies	Ocrelizumab	Anti-CD20, B-cell depleter (Very High)
	Alemtuzumab	Anti-CD52, non-selective immune depleter (Very High)
	Natalizumab	Anti-VLA4, selective adhesion molecule inhibitor (Very High)

Surveys suggest that most patients with MS show an interest in using dietary supplements and more than half are already doing so (Evans et al., 2018). Mounting evidence indicates that nutrition can play a significant role in MS and most MS patients are trying to change dietary habits, however without the advice of the physician (Schwarz et al., 2008).

2. Multiple Sclerosis

2.1 Main Forms of the Disease

MS is a progressive disease, but the course of this disease can be quite variable, with some people experiencing only mild symptoms and others becoming severely disabled.

From the clinical point of view, the main forms of the disease, characterized by 4 different patterns of disease progression and symptoms are: **(i) RRMS**, the most common form, affecting about 85% of MS patients and characterized by relapses, followed by periods of remission when the symptoms and signs improve or may disappear (Hauser and Cree, 2020). These initial stages of MS with underlying demyelination are thought to have an inflammatory pathological substrate with migration of autoreactive lymphocytes across the blood-brain barrier (BBB). This triggers a cascade of inflammation with T- and B-cell clonal expansion, and microglia activation and oxidative damage, mitochondrial injury, and energy failure, leading to the development of the characteristic sclerotic plaque (Doshi and Chataway, 2016) RRMS usually evolves in secondary-progressive MS (SPMS); **(ii) SPMS** is characterized by further axonal injury and atrophy in both white and grey matter, with worsening symptoms, signs and disability over time; **(iii) PPMS**, affecting about 10-15% of MS patients, is characterized by progressive neurological damages rather than relapses and remissions

(Riccio and Rossano, 2015); Due to spinal cord dysfunction, patients often have a progressive spastic paraparesis. There is brain axonal loss and further microglial activation with atrophy. There is anterograde and retrograde neurodegeneration with oxidative damage and energy failure (Doshi and Chataway, 2016). **(iv) the progressive-relapsing MS (PRMS)**, affecting 5% of MS patients and characterized by continued progressive from onset, with clear acute relapses, with or without full recovery (Hunter, 2016).

Prior to MS diagnosis, about 80% of MS future cases suffer from clinically isolated syndrome (CIS) (Fig.2A). CIS encompasses an acute clinical attack affecting one or more CNS sites and can convert to RRMS. The rate of conversion from CIS to RRMS at 20 years is 21% of patients with a normal MRI scan at baseline versus 82%, if there is one or more clinically silent white matter lesions on MRI (Doshi and Chataway, 2016).

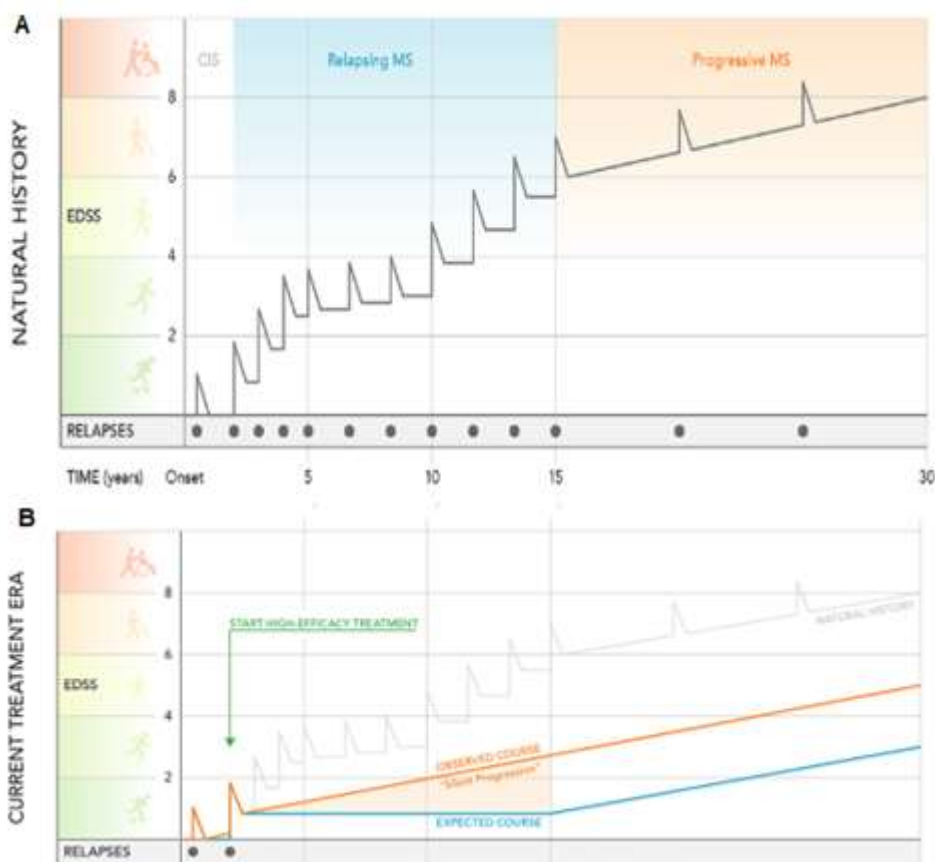


Figure 2. The Evolution of Multiple Sclerosis. A) Natural evolution of relapse-onset MS, without any treatment. During the RRMS, disability accumulation occurs with each flare-up, until it evolves into SPMS. B) The evolution of MS in the current treatment era. With use of effective therapies, attacks are abolished in most patients, but insidious progression independent of relapse activity, termed “silent progression,” is now evident during the relapsing phase. Adapted from Hauser and Cree (2020).

As seen in Figure 2B, the available treatments are only partially effective. This situation leads most patients with MS to use therapies proposed by complementary and alternative medicine - usually diets and dietary supplements. The most common dietary interventions are supplementation with polyunsaturated fatty acids, vitamins, micronutrients and antioxidants such as selenium and change to allergen (gluten and milk) - free diets (Farinotti et al., 2007).

2.2 Inflammatory Impact and Multiple Sclerosis

The adaptive immune system is a key player in the pathogenesis of MS. Inflammation in MS affects the CNS, strongly suggesting that T cells and B cells are selectively recruited by specific target antigens, probably autoantigens, expressed in the CNS (Thompson et al., 2018).

Generation of specific T cell and B cell responses requires professional antigen presenting cells (APCs), such as dendritic cells (DC). In MS, autoreactive lymphocytes, which have the potential to induce CNS autoimmunity, are part of the normal lymphocyte repertoire. The pathogenic immune responses to CNS autoantigens might be initiated in two ways: i) **CNS Intrinsic Model**: the initial event takes place in the CNS, which leads to the release of CNS antigens to the periphery, through lymph nodes or by APCs. Then, an autoimmune response by the microglia is generated that eventually targets the CNS; ii) **CNS Extrinsic Model**: the initial event takes place outside the CNS (example: systemic infection) and leads to an aberrant immune response against the CNS. Several mechanisms (example: reactivity between microbial antigens and autoantigens) might account for the initiation of autoimmune responses.

Both scenarios will lead to tissue damage with the release of antigens to the periphery, which promotes new immune responses in the lymphoid tissue, followed by the invasion of lymphocytes into the CNS (Thompson et al., 2018). In resume, MS is characterized by disseminated and heterogeneous perivascular inflammatory processes at the BBB, with involvement of autoreactive T cells, B lymphocytes, macrophages, and microglial cells against brain and spinal cord white matter (Riccio and Rossano, 2015), which also leads to oxidative damage, mitochondrial injury and energy failure, culminating in the development of the characteristic sclerotic plaque (Doshi and Chataway, 2016).

The innate immune system, mainly consisting of phagocytic cells, also has an important role in the initiation and progression of MS. Macrophages promote the proinflammatory response of T cells and B cells which causes tissue damage. Microglial activation might be one of the initial events in the development of MS lesions. When activated, microglial cells may lead to secretion of free radicals (Fig. 3), proinflammatory cytokines, chemokines, and increased release of glutamate, contributing to disease pathology (Thompson et al., 2018).

Inflammatory cells are a source of extracellular glutamate in the context of neuroinflammation. Monocytes and macrophages, microglia and DC are the most important sources of glutamate in MS. These cells release glutamate through a cysteine/glutamate antiporter and pharmacological inhibition (by sulfasalazine) of this antiporter reduces disease severity in animal models. These findings suggest that glutamate released from activated microglia is sufficient to cause substantial damage to myelin. Glutamate is released when DC contact T cells, which suggests that glutamate produced by DC has a role in T-cell activation induced by APC. Finally, T cells can, in turn, induce the release of glutamate from APC, which is speculated to result in a positive feedback loop (Macrez et al., 2016).

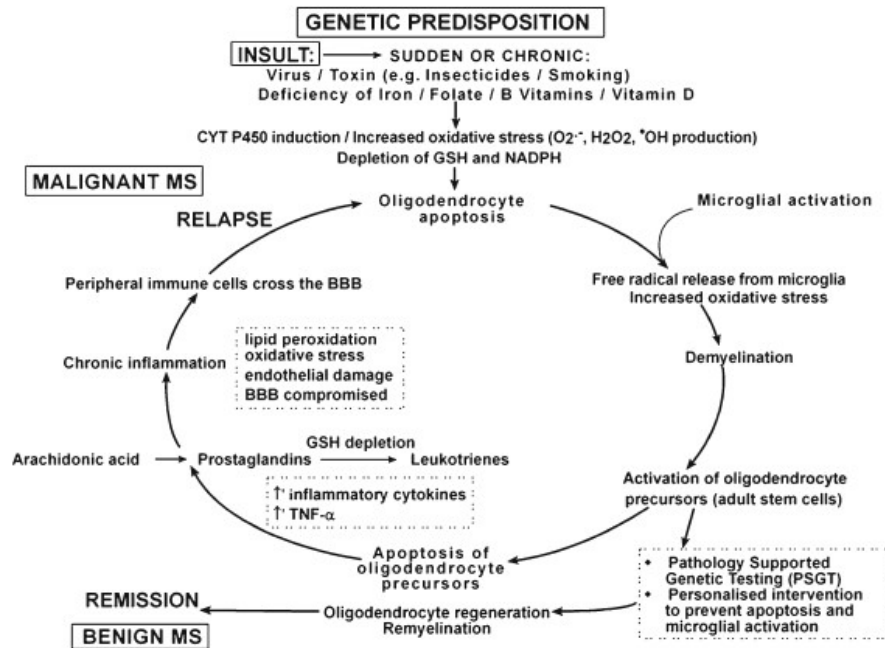


Figure 3. Flow diagram of a hypothesis for genetic and environmental factors determining the etiology and disease progression of multiple sclerosis, ending in Benign Multiple Sclerosis (MS), defined as patients who have an EDSS score of 3 or less after 10 years, and have a >90% chance of remaining free from disability for >20 years without taking DMTs; or Malignant MS, where patients reach a significant level of disability within a short period of time (EDSS score of 6 within 5 years) (Van Rensburg et al., 2012).

It has been established that there exists a significant association between inflammation and neurodegeneration observed in the brain in RRMS, SPMS and PPMS (Frischer et al., 2009). Active lesions during MS relapses are also associated with inflammation (Kutzelnigg and Lassmann, 2014).

2.3 Genetic Susceptibility

Throughout the years, there has been an improved understanding of the genetic factors that contribute to the development of MS. Genetic and epigenetic factors, along with environmental ones (Fig. 3), have a causal role in MS and potentially interact with modifiable risk factors (Thompson et al., 2018).

Within the genes that encode the human leukocyte antigen (HLA) complex, the class II and I genes are relevant modifiers of MS risk. The variants of class II genes present antigens to CD4⁺ T lymphocytes, and the variants of class I present antigens to CD8⁺ lymphocytes (Olsson et al., 2016).

In MS, an association with the serotypes HLA-DR15 and HLA-DR16 are constantly replicated and patients that have the HLA DRB1*15:01 allele are three times more likely to develop MS than are non-carriers (Patsopoulos et al., 2013). The HLA locus accounts for 20-30% of the genetic susceptibility in MS, as estimated from the values of HLA allele sharing by descent in sibships (Thompson et al., 2018).

The classic method for distinguishing the relative contribution of genetic and environmental factors in multifactorial disease is the study of twins. The comparison of concordance rates

between monozygotic and dizygotic pairs is generally considered to measure the influence of genetic factors and the relative concordance rates of dizygotic twins and nontwin siblings measures environmental or other nonheritable factors (Sadovnick et al., 1993).

In their original series (final report on clinical and MRI concordance), after a 7.5-year follow-up, the difference between the monozygotic and dizygotic rates was significant, with a concordance rate of 30.8% (8/26) and 4.7% (2/43), respectively, for clinically definite MS. In the second series (clinical and MRI concordance), the difference between monozygotic and dizygotic rates was striking, with a concordance rate of 26.3% (5/19) and 0% (0/23), respectively. However, the dizygotic twin and nontwin sibling concordance rates were similar. These findings support that, in MS susceptibility, genetic factors play an important role, since both types of twins share an intrauterine environment and identical age. Then, the relative concordance rates between monozygotic and dizygotic twins are a measure of the number of genes involved. MS susceptibility is unlikely to be determined by a single dominant or even a single recessive gene, since the difference in concordance rates suggests the operation of at least two or more genes. If genetic influences on threshold, age of onset and outcome, several loci must be implicated. However, since most monozygotic twins are discordant, the authors have also demonstrated the powerful effect of nonheritable factors (Sadovnick et al., 1993).

3. MS related disease conditions and gastrointestinal findings

3.1 Nutritional Profile in MS Patients

Armon-Omer et al. (2019) observed significantly reduced nutritional intake of several essential minerals in MS patients, including zinc, magnesium, iron, and copper. This is of particular interest as metal homeostasis plays a critical role in regulation of the CNS and is essential for normal functioning. Metal deficiencies in serum of MS patients, specifically iron, magnesium, and zinc, and metal imbalances have been linked to demyelination, involving oxidative stress (OS) (Fig. 3). These authors have observed reduced nutritional intake of complex B vitamins in MS patients including riboflavin (B2) (lower by 27.6%), niacin (B3) (19.7%), pyridoxine (B6) (27%), and folate (B9) (24.1%). Thiamin (B1) mean consumption was lower in the MS group (26.8%) compared with control and significantly below recommended guidelines, indicating that Vitamins of complex B may have a protective role in MS incidence and progression.

Vitamin B12 (cobalamin) plays a key role in myelin formation and is essential for the maintenance of nerve cells; it also has an immunomodulatory effect. Therefore, vitamin B12 deficiency may lead to dysformed myelin and is associated with reduction of immunomodulatory and neurotrophic activities (Farinotti et al., 2007). More about Vitamin B12 deficiency role in MS will be addressed in section 4.3.4.2.

Fatty acids, as structural components of membranes and inflammatory/anti-inflammatory mediators, have important well-known regulatory effects. The only saturated fat that appeared at higher levels in the MS patient group compared with control was palmitic acid (C16_0). Palmitoylation is a reversible post-translational modification of proteins that consists in a

covalent attachment of a palmitic acid to specific cysteine via a thioester bond (Armon-Omer et al., 2019). Palmitoylation of neurotransmitter receptors is critically implicated in the control of a variety of important cellular processes, such as signal transduction and synaptic clustering. More importantly, pathological alterations in palmitoylation are often accompanied by severe neuronal disorders (Naumenko and Ponimaskin, 2018).

3.2 Gut Microbiota

The gut microbiota consists in a large diverse community of microbes that inhabit the mammalian intestine, which are comprised in 6-10 major phyla and about 3,000 distinct species. There are several environmental factors that can alter the gut microbiota composition, and/or its interaction with the host, and can be classified in two categories: microbial or dietary factors (Chassaing and Gewirtz, 2014).

Riccio and Rossano (2015) showed that dietary factors may influence MS symptoms through modulating the inflammatory status of the MS disease, by controlling both the metabolic and inflammatory pathways and the composition of commensal gut microbiota. Eubiosis, the normal state of human intestinal microbiota provides metabolic functions, protects against enteropathogens, and contributes to normal immune functions. When eubiosis suffers a decrease of biodiversity and an increase of pathogenic bacteria, dysbiosis, it will cause an alteration of the mucosal immune system and promote inflammatory, immune, metabolic, or degenerative diseases (Chassaing and Gewirtz, 2014). If a diet favors the change to a dysbiotic gut microbiota, a cycle of gut inflammation, alteration of intestinal immunity, and systemic inflammation and chronic inflammatory diseases may arise.

The food we consume acts on two main targets: (A) the cells of our body and (B) the gut microbiota (Fig. 4). (A) The interaction of dietary factors with enzymes, transcription factors and nuclear receptors of human cells leads to modifications of cellular metabolism. Catabolism or anabolism reactions occur, modulating the inflammatory status; (B) As previously stated, our diet has also an impact on intestinal microflora, where we may find thousands of different microorganisms, the gut microbiota. This complex ecosystem has a strong impact in our immune system and our metabolism (Riccio and Rossano, 2015).

Riccio and Rossano (2015) studied the impact of a healthy diet and physical exercise on oxidative metabolism. As shown on the left side of Figure 4, oxidative metabolism is upregulated by two enzymes, AMP-activated protein kinase (AMPK) and Sirtuins (a group of histone deacetylating enzymes, activated by NAD^+), and a nuclear receptor, Peroxisome Proliferator-Activated Receptor (PPAR). The AMPK-Sirtuins-PPAR pathway is activated by a healthy diet and physical exercise. Molecules like polyphenols, found in vegetables and fruits, and polyunsaturated fatty acids (PUFA) also activate this pathway.

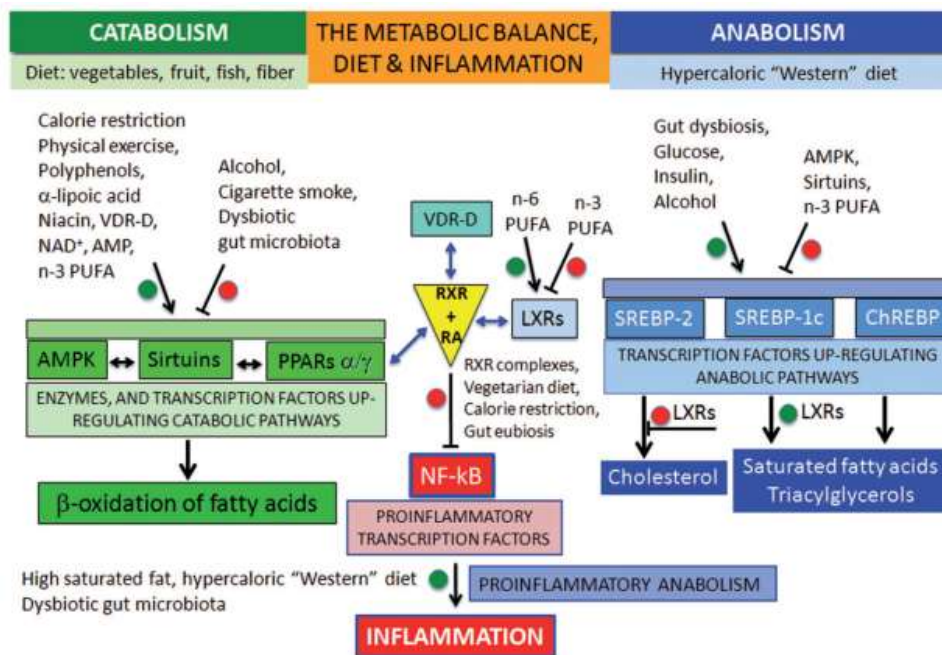


Figure 4. Influence of natural dietary factors on cell metabolism toward oxidative metabolism (left side), biosynthesis (right side), and NF-κB-induced inflammation (bottom) by their binding to nuclear receptors, transcription factors, and enzymes (Riccio and Rossano, 2015).

Conversely, as shown on the right side of Figure 4, “Western” diets and similar, rich in calories, upregulate anabolism reaction through activation of the sterol regulatory element-binding proteins, SREBP-1c and SREBP-2 and the carbohydrate responsive element-binding protein, ChREBP, a transcription factor that is activated by high glucose independent of insulin, and known for playing a key role in glycolysis and lipogenesis (Xu et al., 2013).

Liver X receptor (LXR) isotypes, activated by the cholesterol derivatives oxysterols and glucose, have a relevant role in the synthesis of lipids and triacylglycerols, while inhibiting the synthesis of cholesterol (Riccio and Rossano, 2015). The relation between diet and inflammation are based on two transcription factors, both involved in inflammation and autoimmunity: the nuclear transcription factor-κB (NF-κB) and the activator protein (AP-1). NF-κB has a crucial role in promoting T cells and other cells thought to be involved in MS pathogenesis, including B cells, DC, macrophages, and CNS resident glia (Yan and Greer, 2008).

In MS, activated NF-κB and AP-1 promote expression of proinflammatory genes and molecules. NF-κB is activated by viruses, cytokines, OS and by some dietary components such as saturated fatty acids or trans saturated fatty acids. NF-κB can be inhibited by the Retinoid X receptor (RXR) complexes, vegetarian diet, calorie restriction or/and gut eubiosis (Riccio and Rossano, 2015).

Each of the three nuclear receptors, PPAR, LXR, and vitamin D receptor (VDR), are activated by specific ligands, such as dietary factors. This detail shows how cells respond to changes in nutritional status and represents how nutrients can influence the course of chronic inflammatory diseases (Riccio and Rossano, 2015).

Filippo and coworkers (2010) showed that long-term dietary habits have significant effects on human gut microbiota, after comparing the fecal microbiota of children from Europe (EU) and from Burkina Faso (BF). The effect of food on microbiota has influence on two dominant bacterial divisions: the Bacteroidetes and the Firmicutes, which account for 90% of the gut microbiota.

The BF diet is rich in fibers and low in calories, based on the consumption of plant polysaccharides such as millet, similar to the diet of early human settlements at the time of the birth of agriculture, whereas the Italian diet is Western style, based on protein, animal fat, sugar-sweetened drinks, and refined carbohydrates. Fecal samples of children from BF showed the prevalence of the Bacteroidetes (73%), mainly *Prevotella*, and low levels of Firmicutes (12%). Fecal samples of EU children showed a prevalence of Firmicutes (51%) over the Bacteroidetes (27%) and the Bacteroidetes shifted from *Prevotella* to *Bacteroides*, which use simple sugars, common on Western diets (De Filippo et al., 2010).

The Bacteroidetes/Firmicutes ratio increases in association with a diet rich in complex carbohydrates, because Bacteroidetes, such as *Prevotella*, use complex glycans, producing butyrate, which downregulate the activation of proinflammatory NF- κ B. Conversely, Western diets change the gut microbiota profile and increase the population of Firmicutes, including Mollicutes, often pathogenic (De Filippo et al., 2010).

Riccio (2011) has proposed that the model linking microbiota alteration and the failure of the correct communication between microbiota and the intestine, which leads to low-grade endotoxemia and systemic autoimmune inflammation, might be valid for MS pathogenesis. The possible direct link between gut microbiota and MS has been shown by (Berer et al., 2011). In their studies, it was observed that gut commensal bacteria can trigger a relapsing-remitting autoimmune disease, driven by myelin-specific CD4⁺ T cells, and demyelination, given the availability of the autoantigen myelin oligodendrocyte glycoprotein (MOG). Yokote et al. (2008) studied the possible role of intestinal microflora in the development of autoimmune disease by testing the influence between the composition of gut flora and the autoimmune encephalomyelitis (EAE), the animal model of MS. After the administration of the antibiotic treatment (a mixture of kanamycin, colistin and vancomycin, antibiotics not absorbed through gut mucosa) to mice, the authors observed a significantly suppressed development of EAE along with an altered composition of the gut flora. Histological examination showed less noticeable demyelination at the lumbar region of the spinal cord in treated mice. The authors also observed a lower number of total CNS infiltrating cells at an active stage of EAE. This study was able to link the antibiotic effects with a reduced number of Th17 cells in the gut-associated immune system.

If gut microbiota plays a crucial role in the initial phase of MS, understanding the basis of this association could be a solution for treating chronic diseases, by modifying the composition of gut microbiota through dietary habits. Direct manipulation of the gut microbiota may improve adaptive immune response and reduce inflammatory secretions. Sie et al. (2014) suggested a specific role of intestinal Th17 cells in MS immunopathology. Promoting regulatory T cells (Treg) differentiation, a specialized subpopulation of T cells that act to suppress immune response, and reducing pathogenic Th17 cells might prevent recurrence of autoimmunity in MS patients (Issazadeh-Navikas et al., 2012). The discovery of a defect in the Treg/Th17 balance present in MS patients, could have important clinical implications, as this defect can be modulated by changes in the microbiota composition, which in turn is modulated by dietary changes (David et al., 2014).

4. Oxidative Stress

Oxygen, although crucial to our organism, is at the same time one of the most reactive and life-threatening agents known. To defend our organism, intracellular homeostasis is maintained by a balancing of oxidation and reduction (redox) reactions. When pro-oxidants outbalance the anti-oxidative counterparts, a state of OS is reached, resulting in damage of macromolecules (Ohl et al., 2016). OS causes demyelination and neurodegeneration by oxidation of lipids (Fig. 3), proteins, and DNA but also by favoring the state of pro-inflammatory response (Michaličková et al., 2020). OS is one of the most important components of the inflammatory process of MS, leading to degradation of myelin and axonal damage (Ricchio and Rossano, 2015).

The thioredoxin system, which is composed of thioredoxin (Trx), thioredoxin reductase (TrxR) and NADPH, and the glutathione system, which is composed of NADPH, glutathione reductase (GR) and glutathione (GSH), are two cellular disulfide reductase systems (Ren et al., 2017) that play crucial roles in the antioxidant defense, nutrient metabolism, and the regulation of crucial pathways associated with homeostasis. Conversely, glutathione deficiency contributes to OS, and, therefore, may play a key role in the pathogenesis of many diseases, including MS (Wu et al., 2004).

Thioredoxin and glutaredoxin (Fig.5) are small proteins containing an active site with a redox-active disulfide; they function in electron transfer via a reversible oxidation of two protein-SH groups to a disulfide bridge. Both can supply ribonucleotide reduction and other reactions with electrons from NADPH via their specific reduction mechanism (Holmgren, 1989). These two disulfide reductase systems are active in various areas of the brain and are considered critical antioxidant systems in the CNS (Ren et al., 2017).

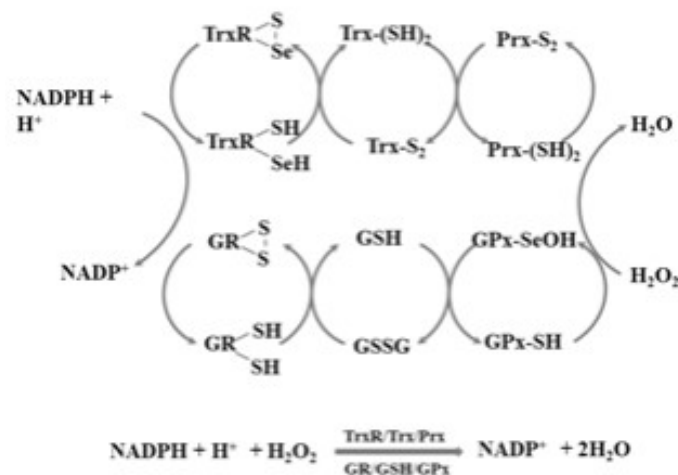


Figure 5. Thiol-dependent redox systems. The disulfides in oxidized Trxs/GSH are converted to thiols by consumption of NADPH through a redox cycling via TrxRs/GRs (Ren et al., 2017).

The CNS produces relatively high levels of ROS due to the large energy demand and is more susceptible to the damaging effects of ROS compared with other tissues. The Trx and GSH systems are crucial to maintain the redox balance in the CNS. Both systems are dysfunctional in neurodegenerative diseases (Ren et al., 2017) and probably MS.

4.1 Endogenous Factors

4.1.1 Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS)

The agents inducing OS are chemical compounds classed as reactive oxygen species (ROS) or reactive nitrogen species (RNS), both unstable (Ohl et al., 2016). The free radical group includes compounds such as the superoxide anion radical ($O_2^{\bullet-}$), nitric oxide radical (NO^{\bullet}), nitric dioxide radical (NO_2^{\bullet}), hydroxyl radical (OH^{\bullet}), alkoxyl (RO^{\bullet}) and peroxy (ROO^{\bullet}) radicals. Most typical non-radical reactive species relevant to biological systems are singlet oxygen (1O_2), ozone (O_3), hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), peroxynitrite ($ONOO^-$), organic peroxides and aldehydes (Michaličková et al., 2020).

OS plays a significant role in the pathogenesis of MS, as a mediator of demyelination and axonal damage. The fast activation of microglia followed by the activation of astrocytes, is most frequently linked to the expression and release of oxidative-stress related molecules (Ohl et al., 2016).

4.1.2 Redox Systems Efficacy

Within the cell, there are two diverse sources mainly responsible for ROS production: mitochondria (byproduct of cellular respiration) and ROS-producing enzymes. The latter are highly expressed in macrophages, neutrophils, and microglia, and are used to kill invading pathogens. During MS lesion formation, the expression of several ROS-generating enzymes, including nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (NOXs), myeloperoxidase (MPO) and inducible nitric oxide (NO) synthase (iNOS) were found increased in microglia and macrophages (Carvalho et al., 2014).

Impaired mitochondrial function is commonly associated with enhanced production of ROS and implicated in both early and chronic stages of MS pathology, actively contributing to both axonal and neuronal injury. Moreover, mitochondrial dysfunction in the cortical neurons of PRMS patients is extensive, contributing to OS in advanced stages of the disease. Given the susceptibility of the CNS to ROS, OS may significantly contribute to neurodegeneration in MS (Carvalho et al., 2014).

Antioxidant protection consists of **enzymatic** and **non-enzymatic antioxidants**, which act in three ways: i) they prevent the formation of free radicals or ii) react with the free radicals before their interaction with the essential biomolecules, and iii) participate in the repair of oxidative damage, aiding in the restoration of cellular components affected by oxidative stress. **Non-enzymatic** antioxidants are low molecular weight compounds, synthesized in the body (glutathione, bilirubin, uric acid, ferritin, ceruloplasmin) or ingested through foods (vitamins A, C, E, carotenoids, α -lipoic acid, flavonoids) (Michaličková et al., 2020).

CNS has antioxidant defense mechanisms consisting of antioxidant enzymes, such as thioredoxin system, superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and catalase (CAT) that counterbalance the detrimental effects of ROS (Fig. 6).

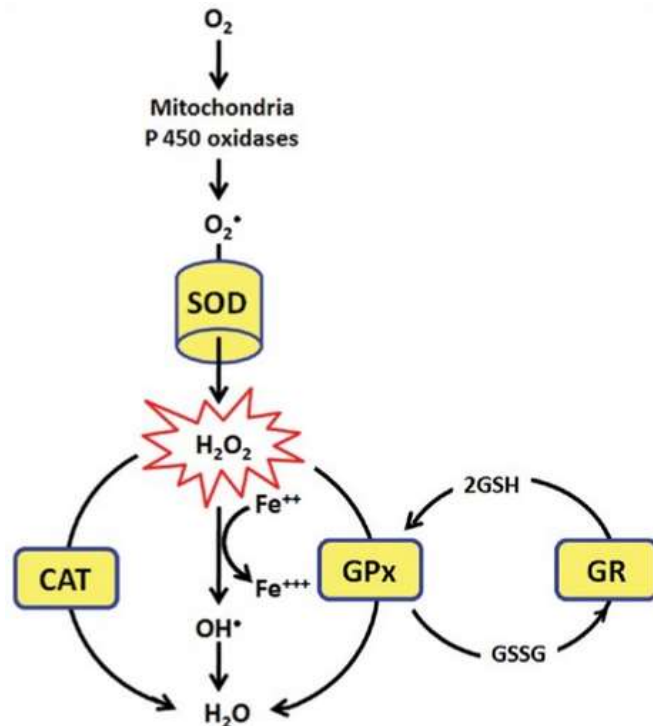


Figure 6. Antioxidant enzymes that belong to the antioxidant defense mechanisms of CNS. Superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and catalase (CAT) are the main endogenous enzymatic defense systems, by directly scavenging superoxide radicals and hydrogen peroxide, converting them to less reactive species. SOD catalyzes the dismutation of superoxide radical ($\bullet O_2$) to hydrogen peroxide (H_2O_2). Although H_2O_2 is a neutral molecule, it is rapidly converted by Fenton reaction into $\bullet OH$ radical which is very reactive. GPx reduces hydrogen peroxide by taking hydrogens from two GSH molecules resulting in two H_2O and one GSSG. GR then regenerates GSH from GSSG. CAT dismutate H_2O_2 into H_2O . Pandey and Rizvi (2010).

Glutathione participates in many cellular reactions: i) GSH effectively scavenges free radicals and other ROS; ii) GSH is essential for the activation of T-lymphocytes and polymorphonuclear leukocytes as well as for cytokine production that contribute to the successful immune responses when the host is immunologically challenged. However, cellular GSH concentrations decrease in response to malnutrition – especially protein malnutrition, resulting in a limited supply of cysteine, a crucial component of GSH – and OS (Wu et al., 2004). GSH depletion can potentially influence the production of prostaglandins and leukotrienes, in the arachidonic acid pathway (Fig. 3).

4.1.3 Endogenous Inflammation

Free radicals and OS have crucial roles in inflammatory processes. Some markers of oxidative damage, such as diene conjugate levels (a measure of lipid peroxidation), total antioxidative activity and total antiradical activity, which contribute to myelin and axonal injury, are increased in MS patients. In these patients, products such as $ONOO^-$ and $O_2^{\bullet-}$, both ROS, are highly toxic to neurons (Gilgun-Sherki et al., 2004; Koch et al., 2006).

The reason for using antioxidants in MS is based on the observation that OS is one of the most important components of the inflammatory process (Bagur et al., 2017). The main regulator of the cellular antioxidant protective mechanisms is the transcription factor nuclear factor erythroid 2-related factor2 (Nrf2) (van Horssen et al., 2010).

Several studies have already linked inflammation to OS: Nrf2 deficient mice have a worse disease outcome in several inflammation-mediated animal models, including sepsis (Thimmulappa et al., 2016) or T cell-mediated hepatitis (Osburn et al., 2008). Late adult Nrf2^{-/-} female mice are prone to develop autoimmune syndromes that closely resemble the human disorder systemic lupus erythematosus (Ma et al., 2006).

Also, Nrf2 knockout mice are more sensitive to the neurotoxic consequences of acute exposures to environmental agents and drugs, inflammatory stresses, as well as chronic exposures to cigarette smoke (Kensler et al., 2007).

4.2 Redox-active Enzyme and Transcriptional Factors

Antioxidant enzymes catalyze the ROS neutralization reactions or the regeneration reaction, for example reduction of oxidized antioxidants. These enzymes include: i) SOD, an enzyme that neutralizes the superoxide anion and represents the first line of defense against OS; ii) GPx, which catalyzes the reduction of hydrogen peroxide and organic hydroperoxide in the presence of reduced GSH as an electron donor, while GSSG is formed at the same time; iii) GR, which participates in regeneration of glutathione, i.e. reduction of GSSG in the presence of reduced NADPH; iv) CAT, an enzyme that has multiple functions, of which the most important is the elimination of hydrogen peroxide from the biological system by decomposition into water and oxygen (Michaličková et al., 2020).

GSH is the main antioxidant in the brain and plays a key role in the detoxification of reactive species (Carvalho et al., 2014). An important phenomenon in MS is an altered glutathione homeostasis (Fig. 6), especially decreased GR and GPx (Michaličková et al., 2020).

Key transcription factors regulated by ROS include p53, NF- κ B and Nrf2. The latter is part of the intrinsic cellular mechanism that neutralizes excess ROS and protects against oxidative injury. It plays a vital role in maintaining cellular homeostasis through its ability to regulate basal and inducible expression of a multitude of antioxidant proteins, detoxification enzymes and xenobiotic transporters (Kensler et al., 2007).

In anti-oxidative stress responses, Nrf2 upregulates detoxifying enzymes and antioxidant proteins. This Nrf2-induced enzymatic machinery includes enzymes mediating GSH synthesis and the thioredoxin (Trx) system (Ohl et al., 2016). Cells are protected by reduced GSH which acts over SOD and ONOO⁻ (Forman et al., 2009), where cysteine molecule is the key to the protection afforded by GSH. Its sulfur atom scavenges peroxides and free radicals, converting them to neutral compounds, such as water. Nrf2 also contributes to differentiation, proliferation, inflammation and lipid synthesis, and there is increasing evidence of an association between aberrant expression and/or malfunctioning of Nrf2 and diverse pathologies including neurodegeneration (Ohl et al., 2016).

Under normal physiological conditions, Nrf2 is sequestered in the cytosol and constitutively targeted for ubiquitination and proteasomal degradation. This interaction keeps low basal expression of Nrf2 regulated genes. OS disrupt the Nrf2-Keap1 complex, allowing translocation of Nrf2 to the nucleus. Nrf2, after dimerizing with small musculoaponeurotic fibrosarcoma (Maf) proteins (sMaf), binds to antioxidant response elements (ARE) in the promoter region of target genes (Fig. 7). Nrf2 controls the expression of a various cytoprotective proteins, including most antioxidant enzymes, such as thioredoxins and enzymes that participate in the synthesis and metabolism of glutathione, such as γ -

glutamylcysteine ligase (GCL), glutathione-S-transferases, GR, GPX. In addition, Nrf2 also regulates the expression of antioxidant proteins like ferritin (Ohl et al., 2016).

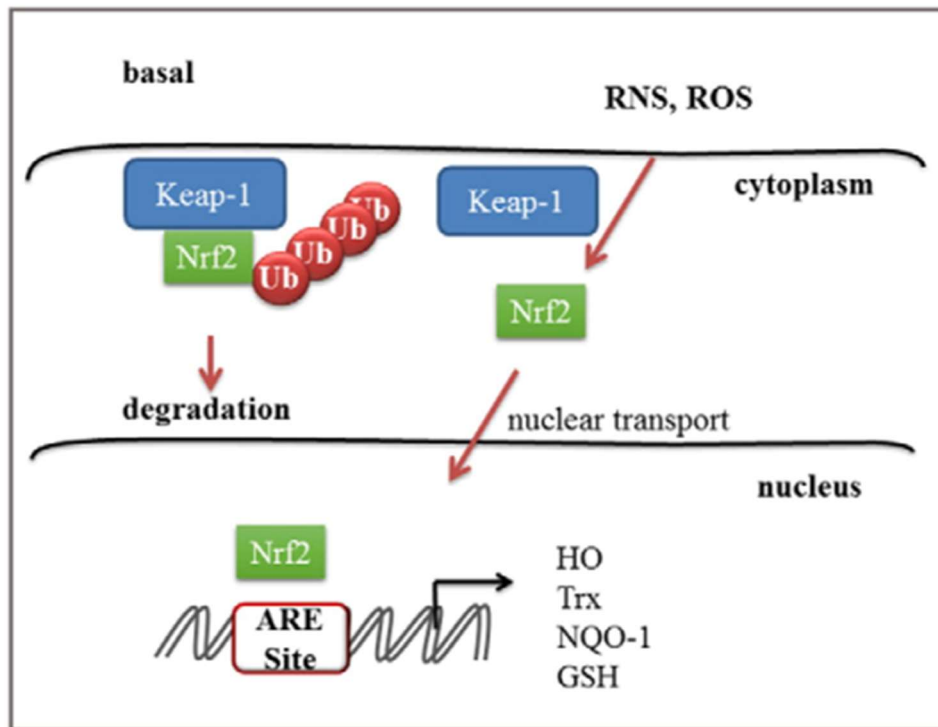


Figure 7. Scheme of Nrf2 activation. Under normal conditions, Nrf2 interacts with Keap-1, which results in degradation of Nrf2. Under cellular stress, higher levels of RNS or/and ROS will promote Nrf2 releasing from its inhibitor and translocation into the nucleus. Here, Nrf2 binds to antioxidant response elements (AREs) in the promoters of target genes, coding for antioxidant or detoxifying enzymes (Ohl et al., 2016).

Pandit et al. (2009) have observed that decrease in a transcription factor complex containing Nrf2 in MS grey matter is correlated with increased oxidative damage. Moreover, Nrf2-deficient mice in the model of EAE, the most used pre-clinical MS model, expressed a more severe phenotype and augmented microglial activation. Although higher expression of Nrf2 are found in astrocytes and infiltrating macrophages in active lesions (van Horssen et al., 2010) and in oligodendrocytes (OL) at lesion edges (Licht-Mayer et al., 2015), neurons have a lower Nrf2 expression (van Horssen et al., 2010) and this may explain the lower capacity of neurons to cope with OS.

4.3 Exogenous Factors

Around a quarter of the risk of developing MS is genetic, suggesting a pivotal role for environmental factors in determining disease course. Determining which modifiable risk factors are the most important is fundamental to any preventive approach. Lack of sun exposure and low vitamin D, and smoking, are important modifiable risk factors (Fig. 3).

4.3.1 Environmental factors: smoking and toxics

Cigarette smoking is one of the few environmental factors that has been consistently associated with MS in epidemiological studies. Hernán et al. (2005) have estimated that the risk of developing SPMS was more than three times higher in smokers than in non-smokers RRMS patients and confirmed that smokers have a moderately increased risk of developing

multiple sclerosis compared with non-smokers. Together, these findings suggest that not only cigarette smoking is an exogenous factor for multiple sclerosis but can also potentiate the progression of this neurological disease (Hernán et al., 2005).

Nicotine, the addictive substance present in cigarettes, is released in the form of a vapor, which is inhaled by the smoker. It is already known that nicotine induces the production of NO in the CNS (Smith et al., 1998), through the activation of certain pathways associated with nicotinic acetylcholine receptors.

Hence, Hernán et al. (2005) suggested that cigarette smoking increases the NO concentration at the sites of multiple sclerosis inflammatory lesions and contributes to axonal degeneration and thus to the permanent deficits observed in the SPMS.

As increased levels of the NO metabolites nitrite and nitrate (NO_x) were found in cerebrospinal fluid (CSF), serum, and urine of MS patients (Rejda et al., 2004). These authors studied if there was any correlation between these findings and the activity and clinical progression of MS. MRI showed a correlation between CSF nitrite and nitrate levels with the volume of Gd-enhanced lesions on MRI, but only in mildly disabled patients with MS. The expression of NO_x was detected within acute lesions in multiple cell types including astrocytes, macrophages, inflammatory cell infiltrates, endothelial cells, and adjacent normal-appearing white matter, a possible explanation for the lack of direct correlation between CSF and serum NO_x levels with undisrupted BBB since both nitrite and nitrate are small molecules, existing as charged anions, which effectively impedes their movement across the BBB (Rejda et al., 2004).

Besides nicotine, other components of cigarette smoke may have direct toxic effects on the CNS, like cyanide and its main metabolite, thiocyanate (Lundquist et al., 1987).

There exists a reverse pathway where thiocyanate oxidase converts thiocyanate to cyanide. This enzyme is found only in erythrocytes but its presence, ensuring a trace of cyanide in the blood, shows that the latter is an essential metabolite (Smith et al., 1963). Cyanide has an important role in the possible etiology of demyelination. In their experimental work, these authors injected potassium cyanide in one group of adult Wistar albino rats for 22 weeks, keeping another group as a normal control. It was observed noticeable cellular changes in the cortex, hippocampus and in the cerebellum, with neuronal degeneration and cell loss in the brains of the animals receiving cyanide. However, the implication of a disorder of cyanide metabolism in demyelination diseases within a picture of vitamin B12 deficiency is still unknown (Smith et al., 1963).

Possible links between incidence of MS and passive smoking, particularly in children, have been analyzed for the first time by (Mikaeloff et al., 2007). In a population-based, case-control study in children, the authors observed that the risk of MS occurrence was higher in the children of parents who smoked at home than in the children of parents who did not smoke at home. The duration of exposure also had an effect on this risk.

Smoking may increase the risk of MS by increasing the frequency and persistence of respiratory infections (Sriram et al., 1999).

4.3.2 Virus and bacteria

Recent studies have shown that bacterial and viral infections are risk factors for various neurodegenerative diseases such as MS, Amyotrophic lateral sclerosis and Alzheimer's disease. Several bacterial and viral pathogens have been reported for neurodegeneration, such as OS, disorders or diseases that primarily affect the synapses, and neuronal death (Alam et al., 2017).

Sriram et al. (1999) identified *Chlamydia pneumoniae* in the CSF of a patient with rapidly progressive MS, which led to a study to verify if there was a possible link between chronic persistent CNS infection with *C. pneumoniae* and MS. *C. pneumoniae* may provide an initial inflammatory insult as well as a chronic stimulus, thereby establishing a chronic state of immune activation.

PCR assays demonstrated the striking presence of *C. pneumoniae* MOMP gene in the CSF of 97% of MS patients versus 18% of other neurological diseases controls (Sriram et al., 1999). Although *C. pneumoniae* could represent the pathogenetic agent of MS, it may simply represent a secondary infection of damaged CNS tissue. Regardless of the exact role of *C. pneumoniae* in patients with MS, the authors' study demonstrates a strong association between these two entities (Sriram et al., 1999).

Operskalski et al. (1989) have investigated whether the development of MS was associated with exposure to uncommon viruses or common viruses. The most striking finding was a strong positive association for history of infectious mononucleosis (IM), where Epstein-Barr virus (EBV) is the most common etiologic agent. Compston and Coles (2008) referred to the hygiene hypothesis whereby, due to a clean environment, individuals not exposed to infections early in life have aberrant responses to infections when encountering these challenges as young adults. Martyn et al. (1993) had already stated that the consequences of infection with EBV depends on the age at which the virus is first encountered, since infection until early childhood is usually mild or even asymptomatic while the exposure that happens in adolescence or adult life, is frequently associated with more severe clinical signs and symptoms.

4.3.3 Inflammation Status

Multiple sclerosis is primarily an inflammatory disorder of the brain and spinal cord in which focal lymphocytic infiltration leads to damage of myelin and axons (Compston and Coles, 2008). The authors have observed that inflammation is necessary for new lesion formation and conditions axon degeneration.

Vitamin D3, besides regulating bone metabolism, participates in cell signaling and many metabolic processes, and for this reason it might explain why low levels of vitamin D3 are associated with chronic inflammatory diseases (Riccio and Rossano, 2015).

Dietary habits and lifestyle and vitamin D levels can be modified to provide beneficial effects on the course of the disease. In fact, physical exercise, the assumption of "healthy" dietary factors, and restoration of optimal concentrations of vitamin D, have an anti-inflammatory action and may ameliorate the course of the disease (Riccio and Rossano, 2015).

4.3.4 Diet factors

4.3.4.1 Vitamin D

Vitamin D is the most discussed antioxidant supplement for MS patients and many studies suggest correlations between a low vitamin D serum level and onset and progression of the disease (Haindl et al., 2023).

Vitamin D (cholecalciferol) is a lipid-soluble vitamin synthesized by the conversion of 7-dehydrocholesterol to vitamin D in the skin. Exposure to ultraviolet (UV) B radiation is required for this reaction. Although the etiology of MS is complex, decreased exposure to UV radiation and low vitamin D appear to play a role (Brown, 2006).

In 1967, Leibowitz et al., studied 20 geographical variables and their coefficients of correlation with the prevalence rates of MS in various countries. Among them, the mean annual duration of sunshine gave the highest correlation with the prevalence of MS. The correlation was negative suggesting that the more sunshine in a given area, the lower the prevalence of MS. The authors have observed that MS is more prevalent in areas reporting fewer than 2000 hours of sunshine annually.

In fact, worldwide epidemiological studies have since shown that the MS prevalence rate is nearly zero in equatorial regions and increases to more than 50 per 10⁵ population at latitudes greater than 45° in both the northern and southern hemispheres (Hayes et al., 1997).

Auer et al. (2000) have demonstrated, for the first time, an annual rhythm of enhancing MRI lesions in RRMS and SPMS patients, indicating seasonal fluctuations in subclinical disease activity, with a clear biphasic seasonal fluctuation of the disease activity, which was highest in spring and early summer and lowest in autumn. However, the geographic anomaly in Norway, where sunlight exposure is very low and surprisingly so is MS prevalence rate, may reflect differences in vitamin D intake attributable to dietary preferences (Hayes et al., 1997). Most foods have little vitamin D content but in Norway fish oil is a rich source of vitamin D. Norwegians living in coastal fishing districts consumed fish and margarine that provided about 1300 IU of vitamin D daily. The consumption of margarine in the Norwegian communities is important since Norwegian margarine contains fish oils (Goldberg, 1974).

Vitamin D receptors are found in most body cells including neurons, oligodendrocytes, and astrocytes in the CNS. Vitamin D has many functions including influencing the immune response through the production of cytokines. Activation of vitamin D receptors decreases production of proinflammatory cytokines such as interleukin-2, interferon-gamma (IFN- γ), and tumor necrosis factor-alpha (TNF- α). Conversely, production of anti-inflammatory cytokines, such as interleukin-4, is increased by vitamin D (VanAmerongen et al., 2004). Hence, Vitamin D may be beneficial in patients with MS due to its regulatory effects on the immune system.

In a large longitudinal study among MS patients, Ascherio et al. (2014) found that higher serum 25-hydroxyvitamin D levels, marker of vitamin D status, is associated with a lower number of T2 lesions, a lower degree of brain atrophy and clinical progression during the 5 years of

follow-up. The authors have also provided evidence that low vitamin D is not a consequence of the disease process itself but rather its predictor.

VanAmerongen et al. (2004) suggested that MS patients living in temperate climates should have their serum 25OHD concentration checked in winter and use a vitamin D3 supplement, if needed. Based on the geographic pattern and seasonal variation seen with MS, vitamin D supplementation and adequate exposure to sunlight should be recommended for healthy patients. However, further studies should be done to determine the effectiveness and the optimal dose of vitamin D supplementation needed (Sherrill J Brown, 2006).

4.3.4.2 Other Important Vitamins in MS Progression

Beside vitamin D, mentioned in section 4.3.4.1, there are other vitamins relevant in MS management, such as B12, A, C and E.

Cobalamin (vitamin B12) and folate (vitamin B9) are biologically intertwined. Deficiency of vitamin B12 results in functional folate deficiency. B12 deficiency is classically associated with neurologic complications, namely myelopathy, peripheral neuropathy, and cognitive and affective symptoms. Folate is important for nucleoside synthesis and DNA repair. Folate deficiencies are linked to DNA instability and breakdown of phospholipid membranes and thus might affect myelin integrity (Evans et al., 2018).

Reynolds et al. (1991) studied the possible association of MS with pernicious anemia (PA), after several patients described neurological benefits for being treated with injections of vitamin B12 for placebo purposes. He has observed an overlap of MS and PA and a subgroup of patients with this association. There are, at least, three possible explanations for the combined occurrence of MS and vitamin B12 deficiency: i) their autoimmune nature (Reynolds, 1992) Even though age range is so different, the sex, racial and geographical distribution of MS and PA are similar. The female-to-male ratio in both disorders is 3:1; ii) that vitamin B12 deficiency renders some patients more vulnerable to the putative viral/immunological mechanisms widely suspected in MS; iii) that chronic immune reactions or recurrent myelin repair processes increase the demand for vitamin B12.

Evidence of a probable role for vitamin B12 in myelin formation comes from studies of children with inborn errors of vitamin B12 and methylation metabolism. An important aspect of the neuropathology of these children is a failure of myelination, or even demyelination (Surtees et al., 1991). However, none of the relationships between MS and vitamin B12 have been confirmed (Miller et al., 2005).

Vitamins A, C, and E, all of them with antioxidant properties, may decrease free radical-induced cellular injury and this is the rationale for their use in MS (Farinotti et al., 2007).

Vitamin A (retinol, or β -carotene) is an antioxidant. One active metabolite, retinoic acid, participates in neuroplasticity and immune regulation. Vitamin A deficiency causes changes to the skin and eyes (Evans et al., 2018).

A randomized clinical trial (RCT) of 101 patients reported, for the first time, that the use of vitamin A 25000 IU daily for 6 months, followed by 1000 IU daily for 6 months, reduced fatigue (physical, cognitive and psychosocial) and depression, common psychiatric signs in MS patients, through the modulation of inflammatory conditions (Bitarafan et al., 2016).

Vitamin C is an antioxidant involved in fatty acid neurotransmitter synthesis its supplementation was not beneficial in EAE. One small observational study found lower vitamin C levels in patients with MS than in controls. Further trials of vitamin C supplementation in MS would be a bigger perspective (Evans et al., 2018).

Vitamin E, also known by α -Tocopherol, has antioxidant properties and is one of the main 'chain-breaker' antioxidants. Interestingly, vitamin E levels have been reported to be decreased in the plaques of demyelination of MS brains (Jiménez-Jiménez et al., 1998).

Vitamin E deficiency, more common with malabsorption disorders, can cause neuropathy and ataxia syndrome. Blood vitamin E measurements should be adjusted for lipid levels.

Observational studies found lower serum vitamin E levels in patients with MS vs controls but found no difference in CSF. Further studies of isolated vitamin E supplementation in MS would elucidate if its antioxidant properties have a relevant impact on MS (Evans et al., 2018).

4.3.4.3 Essential Minerals

Armon-Omer et al. (2019) found significant reduced nutritional intake of several essential minerals in the MS group, including zinc, magnesium, iron, and copper. This is of particular interest as metal homeostasis plays a critical role in regulation and normal functioning of the CNS.

Zinc plays roles in modifying neuronal excitability and synaptic plasticity in the CNS (Armon-Omer et al., 2019). Being a cofactor of more than 300 enzymes including metalloproteinases (MMPs), such as SOD, and a component of a large number of proteins including Myelin Basic Protein (MBP), zinc emphasizes its important role in the nervous system (Bredholt and Frederiksen, 2016). Zinc is mainly found in presynaptic vesicles in neurons so when there's zinc deficiency occurs induced apoptosis in neural cells. Conversely, elevated levels of zinc are neurotoxic, so the efficient zinc homeostasis in the brain is crucial (Bredholt and Frederiksen, 2016). It remains a matter of debate to what extent zinc deficiency could play a critical role in MS, especially as existing studies in MS patients reveal a large span of blood zinc concentrations, ranging from slightly lower to higher levels in contrast to healthy controls (Pawlitzi et al., 2018).

Magnesium is neuroprotective in various animal models and is known to improve neurological outcome in patients with neural damage, although the mechanism of neuroprotection remains uncertain: increasing magnesium concentration reduces presynaptic release of the neurotransmitter glutamate, blocks glutamatergic N-methyl-D-aspartate receptors, potentiates adenosine action, improves mitochondrial calcium buffering, and blocks calcium entry via voltage-gated channels (Lees et al., 2004).

Yasui et al. (1990) published a post-mortem study that showed a significantly lower content of magnesium in CNS tissues, especially in CNS white matter including the demyelinated plaques of MS patients. The implications of the changes in the magnesium metabolism in the demyelinating process remains unclear.

Selenium is an essential micronutrient with a narrow range of optimal concentrations and during periods of insufficient intake, the selenium stored in visceral organs, like the liver, is redistributed to the brain (Ren et al., 2017).

Vitamin E and selenium work together to protect against the harmful effects of free radicals and selenium can also bind cadmium, mercury, and other metals, acting as an anti-toxic element (Fathi et al., 2013). These authors have studied serum levels of selenium in MS patients and observed that selenium levels were significantly lower than the ones observed in the healthy controls.

Selenium is also essential for activity of glutathione peroxidase (Fathi et al., 2013) and selenocysteine and selenium-methionine, can provide direct antioxidant benefits and can also be incorporated into the synthesis of antioxidant enzymes, as glutathione peroxidase and thioredoxin reductases. Selenium supplementation can induce a regulatory phenotype in Th cells and polarize macrophages to an anti-inflammatory profile. Its beneficial effects depend mainly on its incorporation into proteins that will mediate the activation, proliferation and differentiation of innate and adaptive immune components (Toledo et al., 2020).

Iron is considered a key factor in the pathogenesis of MS, as it may cause neuronal damage by stimulating OS. However, contradicting results associated to iron levels and MS progression may explain that inadequate iron levels (both low and high) may be harmful in MS since iron excess might increase free radicals, which may elevate oxidative stress, while iron reduction could decrease immune system function and cause an energy deficit due to loss of mitochondria membrane potential (Armon-Omer et al., 2019). Iron is also crucial so catalase can play its function as an antioxidant enzyme. The iron in its heme group is essential for its catalytic activity in neutralizing hydrogen peroxide.

The theory that iron deposition in the brain contributes to MS pathogenesis has caused uncertainty among MS patients as to whether they should, or not, avoid iron. However, the fact that a subgroup of people diagnosed with MS show clinical improvement when they are on iron supplementation emphasizes the importance of individualized therapy (Fig. 3), based on genetic and biochemical determinations (Van Rensburg et al., 2012).

Copper is an essential cofactor for several antioxidative enzymes and is necessary for iron absorption and transfer. The experimental animal model of MS in which administration of cuprizone as a copper chelator leads to pathology by affecting OL, probably by mitochondrial injury, OS, and subsequent apoptosis (Armon-Omer et al., 2019). In contrast, elevated copper levels have been found in MS patients, probably due to serum ceruloplasmin reduction of ferroxidase function. It remains unclear if this could be the consequence but also the triggering factor of the higher OS status found in MS subjects (De Riccardis et al., 2018). The precise roles of copper in MS pathology require further investigation (Armon-Omer et al., 2019).

4.3.4.4 High Saturated Fats

The higher prevalence of the disease in Western countries and in territories distant from the Equator (above 40 degrees of latitude) might be linked to regional dietary habits, besides limited sunlight exposure, suggesting a contribution of hyper-caloric diets typical to those areas (Esposito et al., 2018). Armon-Omer et al. (2019) referred that those countries with high abundance of MS are characterized by high-fat, high-carbohydrate and hypercaloric “Western” diets, with high saturated fats and processed foods.

Saturated fatty acids of animal origin, found in foods such as whole milk, butter, cheese, meat, and sausages, are the components of the diet considered more frequently for their deleterious influence on the course of MS (Riccio and Rossano, 2015).

Swank and Dugan (1990) suggested that the frequency of MS was related to fat consumption, based on the relative frequencies of MS and the calculated fat consumption of different populations. One study, in which MS patients were followed up for 34 years, the lowest saturated fat consumers (20 grams/day; mean 17 grams/day) showed little worsening of disability, whereas an increase in the fat intake to an average of 25 grams/day or 42 grams/day was accompanied by a striking increase in disability, to a need for bed care. (Swank and Dugan, 1990) studied the effects of consumption of low-fat diets in relation to severity of MS in patients from the same study. Their findings showed that a diet of 20 grams of saturated fat daily was the one that keep patients with MS ambulant and working. Under these limited consumption of saturated fats about 95% of MS patients remained only mildly disabled for approximately 30 years. Dropping the diet, even after 5-10 years was, frequently, followed by reactivation of the disease. The authors have also observed that patients consuming 10-15 grams/day or less, by removing red meat from the diet and reducing intake of the dark meat found in poultry, had even better improvement in energy and fatigue levels.

However, it was only in 2003, that Swank and Goodwin discovered a link between a lower intake of animal fat and remission of MS. High-fat diets lead to the synthesis of storage lipids and cholesterol, consequently decreasing the membrane fluidity and leading to capillaries obstruction, and the onset or increase of inflammation (Swank and Goodwin, 2003).

Inflammation is increased by hypercaloric diets, characterized by animal fat, red meat, sugar-sweetened drinks, fried food and low fiber. The persistence of this type of diet leads to a dysbiotic gut microbiota, alteration of intestinal immunity, and low-grade systemic inflammation (Riccio and Rossano, 2015).

4.3.4.5 Carbohydrates

Dietary sugars are mainly hexoses, naturally present in fruits and some vegetables, with the molecular formula $C_6H_{12}O_6$. Simple sugars fructose and glucose are sweet sugars and high-fructose corn syrup (HFCS) is a sweetener and preservative made from the latter ones, most used in sugary soft beverages and baked goods (Ma et al., 2022). In Europe, sugar consumption in different countries is between 7% and 25% of total energy intake (Lelis et al., 2020). Dietary sugars and mixed processed foods may be a key factor leading to the occurrence and aggravation of inflammation (Ma et al., 2022).

Coe et al. (2015) in a preliminary study to assess and compare the dietary pattern of MS patients, used a small sample size of 10 MS patients and 10 healthy controls. The authors observed that MS patients ate more carbohydrates than the control group, but there was no difference in body mass index (BMI) between the two groups. However, a further study with a larger sample size of MS patients and control group could return interesting results. Autoimmune diseases such as MS are multifactorial etiological diseases, involving T cell-mediated inflammatory pathology and, later, Cao et al. (2017) did observe that a high-sugar diet stimulated Th17 cell differentiation and exacerbated EAE by altering the gut microbiome.

High-glucose diet can directly act on CD4+ T cells, by inducing T cells to differentiate into Th17 cells, thereby increasing the proportion of Th17 cells. Zhang et al. (2019) also showed that high glucose intake exacerbates autoimmunity by inducing Th17 cells, through a ROS-dependent pathway.

These studies have focus on the deleterious effects of high sugar diets and found that high sugar intake increased the proportion of CD4+ cells in EAE mice and exacerbated neuroinflammation in the brain and spinal cord.

4.3.4.6 Polyunsaturated Fatty Acids

Omega 3 and omega 6 fatty acids are essential fatty acids (FA), that is, they cannot be synthesized and are essential components of the human diet. Omega 3s are found in fish and seafood, particularly in oily fish, and in some plants, like flaxseeds. Omega 6s are present particularly in vegetables and grains. While both omega 3 and 6 FA have positive structural properties when incorporated into cell membranes, in simple terms, omega 3s are essentially anti-inflammatory and omega 6s pro-inflammatory. Ideally, the ratio of omega 6 to omega 3 in the human diet should be around 4:1 to 2:1, although modern Western diets are typically deficient in omega 3s, with a ratio closer to 25:1 (Bagur et al., 2017).

People with MS show lower levels of PUFAs, in cells and higher levels of saturated fatty acids (SFAs). Omega 3 FA have immunomodulatory effects in people with MS and are neuroprotective (Jelinek et al., 2013). From a large international cohort of people with MS - 2469 respondents, where 1493 (60.5%) had RRMS, the authors have observed that those consuming fish more frequently and those taking omega 3 supplements had significantly better quality of life and less disability, lower relapse rates and reduced disease activity. Flaxseed oil supplementation was associated with over 60% lower relapse rate over the previous 12 months. Their findings that this oil has stronger associations with disease activity and disability than fish oil, suggests that flaxseed oil is a preferred method of omega 3 supplementation for people with MS.

The intakes of hydrogenated FAs and SFAs need to be reduced to avoid increase in the MS inflammatory process. A low-fat diet with antioxidant supplementation led to significantly lower C-reactive protein concentrations than did placebo. The oxidative stress and inflammatory markers 8-iso-prostaglandin F2a and IL-6 also decreased after dietary intervention, whereas catalase activity increased. These results suggest that diet and dietary supplements of long-chain omega-3 PUFAs, such as eicosapentaenoic acid and docosahexaenoic, may modulate cell metabolism and MS-related inflammatory processes. A low-fat diet supplemented with omega 3 FAs decreased the rate of relapse and reduced fatigue by 60%, generating significant improvements in the EDSS (Table 1) and decreased the risk of developing the disease. The consumption of sources of long-chain PUFAs when exposure to sun is low can offer protection against the risk of MS: for example, the intakes of sesame seeds, hemp, and/or evening primrose oil, cod liver oil, or fish 3 times/week during childhood and adolescence (Bagur et al., 2017).

4.3.4.7 Milk Products

In the 1970's, a few authors suggested a relationship between dairy products consumption and MS prevalence (Agranoff and Goldberg, 1974).

In 1974, Agranoff and Goldberg, suggested that the geographical predisposing factor in MS could reflect regional dietary differences like milk consumption. One of the ideas that emerged was that substituting human milk for cow's milk beyond the normal nursing period was detrimental since this is a period of rapid brain development.

Their studies in the United States gave a correlation between latitude and MS mortality ratio of 0.90 and a correlation between milk consumption and MS mortality ratio of 0.82, being both highly significant. Then, the authors performed a similar analysis using food-consumption data for countries in the Organization for Economic Cooperation and Development (O.E.C.D.), obtaining the values observed in Table 3. The contrast between the positive correlation of MS with animal-product fats and the negative one with products containing polyunsaturated fats is striking.

Tabel 3. Consumption of Animal and Crop Product Fats and MS Levels for the O.E.C.D Countries in 1974 (Austria, Belgium, Canada, Denmark, U.K., Finland, France, Germany, Holland, Italy, Ireland, Japan, New Zealand, Norway, Portugal, Spain, Sweden, Switzerland, ex-Yugoslavia, United States). Adapted from Agranoff and Goldberg (1974)

Per-caput daily consumption	MS scored low to high on a scale from:		
	0 to 1	1 to 3	1 to 5
All dairy fats except butter	0.83	0.85	0.80
Butter fat	0.70	0.67	0.61
All meat fat	0.82	0.82	0.67
Other fats of animal origin	0.44	0.44	0.53
Total animal product fats	0.92	0.91	0.88
Cereal fats	-0.62	-0.46	-0.55
Fruits, vegetable, and potato fats	-0.58	-0.41	-0.50
Pulse, nut, and seed fats	-0.70	-0.72	-0.74
Fish fats	-0.21	-0.37	-0.25
Derived vegetable fats	-0.05	0.08	0.08
Total crop product fats	-0.19	-0.04	-0.06
Animal fats/total fats	0.82	0.77	0.75

Leibowitz et al. (1967) noted a high rate of MS among native-born Israelis, probably reflecting the high consumption of dairy products in Israel relative to Middle Eastern countries.

Milk fatty acids are characterized by a great diversity. Over 140 fatty acids have been isolated from milk, many of which are either unique to milk, or are present in milk in higher amounts than in any other dietary component. Such fatty acids include those with branched chains or odd-numbered chain lengths and hydroxy fatty acids. Most of these unusual fatty acids are produced by the rumen bacteria and are not found in human milk (Fig. 8).

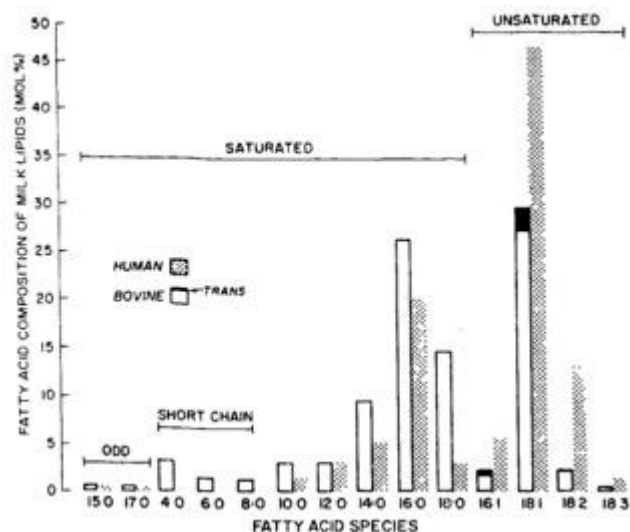


Figure 8. Comparison of fatty acids present in bovine and in human milk. Bovine milk lipids contain butyric (4:0), hexanoic (6:0), and octanoic (8:0) acids, all absent from human milk. Bovine milk contains higher amounts of longer-chain saturated fatty and lower amounts of the unsaturated fatty acids than human milk. It also contains trans fatty acids, primarily vaccenic (18:1, 11-trans) and traces of various positional and geometric isomers of linoleic (18:2) and linolenic (18:3) acids. Adapted from Agranoff and Goldberg (1974).

Linoleic acid and linolenic acid are reduced in the rumen to a variety of substances, the most common of which is vaccenic acid (18:1, 11-trans), a trans fatty acid, which is the most appealing candidate to be the toxic lipid in milk that predisposes to MS, according to the authors.

Malosse et al. (1992) found that coincidence intriguing enough to examine it in different world areas, taking into consideration the countries for which MS national prevalence and milk consumption were known and believed to be reliable. The authors found a highly significant correlation ($p < 0,0001$) between cow milk consumption and MS prevalence, a low correlation between butter consumption and MS prevalence and no significant correlation between cheese consumption and MS prevalence. The authors believed that those findings indicated that milk processing destroyed partly or completely factor(s) which facilitate or trigger the appearance of clinical MS in subjects at risk.

4.3.4.8 Gluten-containing Grains

Shatin (1964) reflected upon how the diet alterations through millenniums could have had an impact on an individual's health. When man started to produce food, instead of hunting, it led to changes in his diet. The time lapse between prehistory and nowadays is a brief period in the biological history of man. This transition could have been too sudden to develop an adequate adaptive response to the drastic changes in his dietetic habits. The challenge to man's metabolism by the protein-complex of wheat and/or rye could have led to complex syndromes. The author concluded that consumption of gluten-containing grains, as wheat and rye, may increase the susceptibility to MS. Measures of the wheat yield in different countries gave a positive correlation with the distribution of MS. Per capita calorie consumption also correlated positively with the distribution of MS, although the coefficient of correlation was not quite as high as with wheat yield. It was observed that a diet rich in cereals containing gluten are much more common in areas characterized by elevated MS prevalence.

In another study, the author observed that a high-protein, gluten-free diet, with supplements, led rheumatoid arthritis patients to enter remission status. Rheumatoid arthritis also has an inflammatory, immune, and chronic characteristics similar to MS. (Shatin, 1964) introduced the concept of a fundamental etiological lesion in the small intestine, related to gluten consumption resembling the relation between gut microbiota alterations and MS inflammatory grounds. So, particular attention has focused on the hypothesis that gluten sensitivity may play a role in the neurological manifestations of MS. Patients with celiac disease have neurological manifestations resembling those that occur in MS on clinical or MRI grounds. Celiac disease should be considered in the differential diagnosis of MS (Ghezzi and Zaffaroni, 2001).

4.3.4.9 Phytate-rich Grain Foods

Less well-known, but of importance is the fact that certain food grains can exert an antagonistic effect to the action of vitamin D. Phytate ion counteracts the action of vitamin D and reduces its effectiveness by forming an insoluble salt with calcium. It retards calcium metabolism when supplies of vitamin D are limited. Depressive effects of phytate on free calcium have been observed in infants, children, and adults, despite increased intake of vitamin D (Goldberg, 1974). The calcium balance of a living body depends on different variables such as growth, calcium intake, vitamin D and hormones. Vitamin D is undoubtedly the most dominating single factor in the regulation of the absorption of calcium (Nicolaysen et al., 1953).

Wills and Fairney (1972) have observed that rats were unable to maintain plasma-calcium concentration in the face of a high dietary phytic-acid intake while on an adequate cholecalciferol diet. The results also show that despite a fivefold increase in cholecalciferol, the high dietary intake of phytic acid was associated with the development of hypocalcemia.

Oats and corn are the most antagonistic to vitamin D. It is noteworthy that rice is especially low in phytate. The phytate contents of various grains are given in Table 4. Phytate-rich grains, particularly barley and oats are consumed in Europe. The high consumption of oats in northern Scotland, combined with a dietary low in vitamin D (low in fish and fish oils) and extraordinarily little sunshine is an example where we can find one of the highest MS rates (Goldberg, 1974).

Table 4. The phytate content of food grains. Adapted from Goldberg (1974).

Food Grain	Weight phytate phosphorus per 100g of grain (mg)
Oats	180 – 200
Oatmeal	230 – 330
Wheat	233 – 260
Corn (yellow)	212 – 225
Corn (white)	180
Rye	168
Barley	198
Yellow corn meal	230
Rice (polished)	36

Japan's reliance upon rice (exceptionally low in phytate) as the principal grain, and in fish consumption in Japan, are factors that tend to protect the population against vitamin D deficiency and MS does occur with low prevalence in this part of the world (Goldberg, 1974).

5. Diet and Nutritional Effects

Diet and inflammation are linked, at least, through two transcription factors involved in inflammation and autoimmunity: the NF- κ B and the AP-1 (Yan and Greer, 2008). In MS, both NF- κ B and AP-1 are activated and induce the expression of several proinflammatory genes and the production of proinflammatory molecules. As shown in Figure 4 for NF- κ B, this can be activated not only by viruses, cytokines, and OS but also by some dietary components such as saturated fatty acids or trans unsaturated fatty acids, which therefore can be considered proinflammatory. The production of proinflammatory molecules during relapses is sustained by hypercaloric diets and counteracted by low-calorie diets. In principle, what favors anabolism will promote the inflammatory processes (Ricchio and Rossano, 2015).

A correlation between limited intake of animal fat and MS remission was reported only in 2003 (Swank and Goodwin, 2003), indicating a potential causal relationship. Meat contains arachidonic acid (the omega-6 (n-6) PUFA, which is the precursor of proinflammatory eicosanoids [prostaglandins, thromboxanes, and leukotrienes]) and activates the Th17 pathway (Ricchio and Rossano, 2015).

The most representative milk fat globule membrane (MFGM) protein (40% of total MFGM proteins), butyrophilin (BTN), is suspected to have a role in MS, as it is very similar to myelin oligodendrocyte glycoprotein (MOG), a glycoprotein that is faced as a puttable candidate autoantigen in MS. BTN and MOG share the same behavior in MS experimental models, and MOG/BTN cross-reactive antibodies have been found in MS, in autism and in coronary heart disease (CHD)(Ricchio and Rossano, 2015).

Dietary antioxidants can counteract the negative effects of saturated or trans fatty acids, downregulating the expression of proinflammatory molecules, OS, and angiogenesis. Among them, the most important compounds are the polyphenols and carotenoids from vegetables, n-3 PUFA from fish, vitamins D and A, thiol compounds such as lipoic acid, and oligoelements such as selenium and magnesium. In general, a nutritional intervention with anti-inflammatory food and dietary supplements decreases the biosynthesis of proinflammatory compounds and might alleviate the symptoms of chronic fatigue syndrome, and favor patient wellness (Ricchio and Rossano, 2015).

6. Conclusion and Future Perspectives

Multiple sclerosis is a neurodegenerative disease that, despite advances in drug development still has no cure. Given that its etiology is not yet known, the extremely diverse evolution of this disease from patient to patient and its autoimmune characteristic being questioned, the treatment should be individualized. Each patient should have individual therapy focused on the objective of achieving a benign form of the disease, through the regeneration of oligodendrocytes and remyelination. Hence, it is necessary to understand the processes that lead to the apoptosis of oligodendrocytes, myelin lesions and chronic inflammation, characteristic of maligned Multiple Sclerosis.

In this work, some of the environmental factors, in addition to genetic predisposition, that may trigger Multiple Sclerosis were enunciated and described. It has been found that certain harmful factors, such as exposure to tobacco smoke and deficient levels of essential vitamins and minerals, may play a crucial role in the development of the disease, due to oxidative stress and the inability of enzymes, such as glutathione reductase, to replace reduced glutathione homeostatic balance. Due to this imbalance, reactive oxygen species released by microglia, will damage myelin and cause apoptosis of oligodendrocyte precursors. The glutathione depletion will result in the formation of prostaglandins and leukotrienes, in the presence of inflammatory cytokines. Lipids are macromolecules affected by this oxidative stress, meaning that the blood brain barrier may be compromised, allowing cells of the peripheral immune system to access the central nervous system and cause a relapse.

In conclusion, there are dietary factors that influence the level of inflammatory response, so Multiple Sclerosis patients should be counseled regarding their dietary choices. There are several reasons why this strategy has gained scientific relevance: i) the consequences that an unbalanced diet can have on the microbiota and, consequently, on the inflammatory response; ii) the hypothesis that MS is an autoimmune disease is being replaced by the metabolic model; iii) the existence of biochemical markers, making it easier to individually analyze each patient regarding deficiencies they may present and, through taking supplements, correct these deficiencies; iv) Multiple Sclerosis patients are open to alternative therapies, like taking vitamin supplements and changing their diet, so clinical advice in this area would be crucial. Possibly, it would even have an impact on the evolution of the disease, so further studies should be designed to explore the importance that the microbiota may have in MS, and the relevance of each vitamin and essential mineral.

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