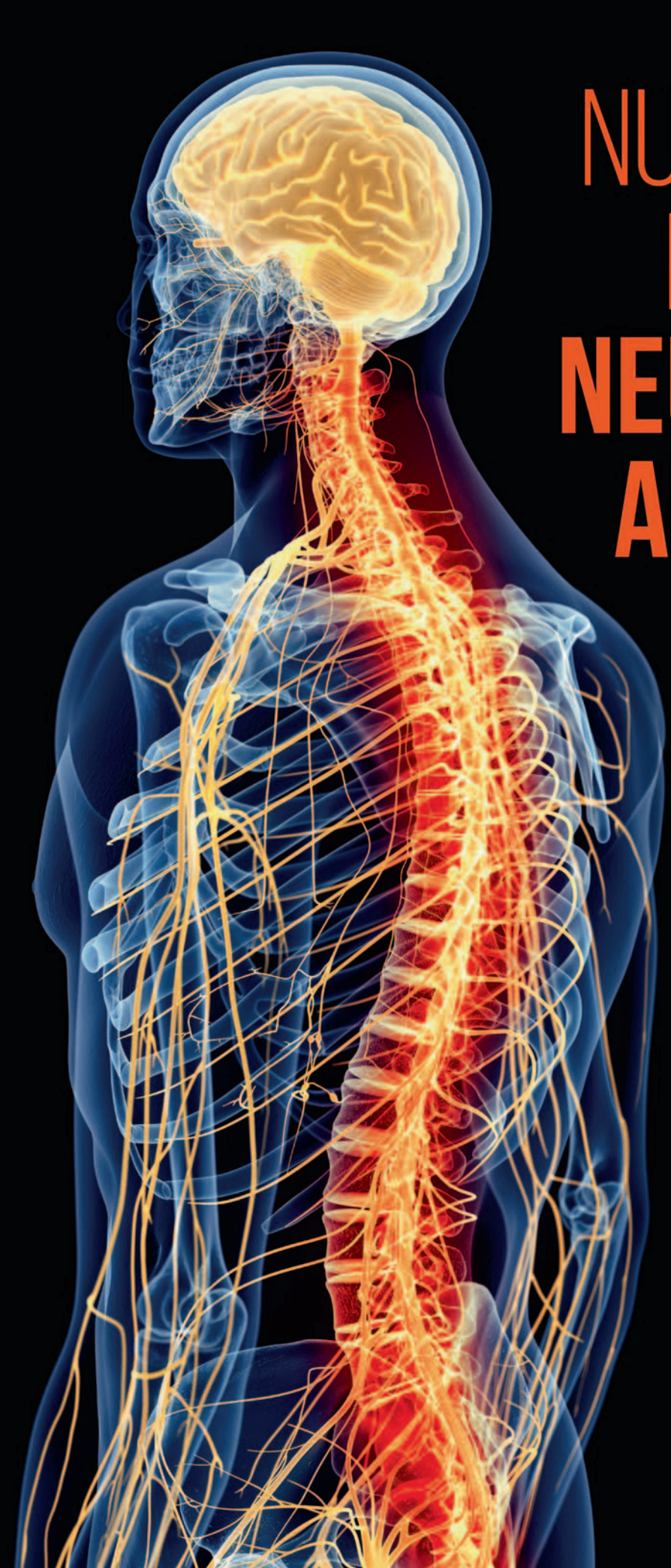

NUTRITION AND LIFESTYLE IN NEUROLOGICAL AUTOIMMUNE DISEASES: MULTIPLE SCLEROSIS

EDITED BY
RONALD ROSS WATSON
WILLIAM D. S. KILLGORE

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SECTION I

MECHANISMS OF MS DISEASE CAUSATION AND INTERVENTION

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CHAPTER

1

Epigenetic Changes in DNA Methylation and Environment in Multiple Sclerosis

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OUTLINE

INTRODUCTION

Epigenetic Regulatory Mechanisms

The first time the term epigenetics appeared in literature dates back to mid-20th century (Conrad Waddington, 1905–1975).[1] However, it has been only in the last decade that epigenetics has become one of the emerging research fields, as a promising source of knowledge, especially in medicine.

Epigenetics has been defined as the study of the mechanisms regulating gene expression without changing the sequence of deoxyribonucleic acid (DNA). This discipline has built a bridge between genetic and environmental influences on the development of a phenotype; that is, it provides the means by which genetic material can respond to the diverse environmental conditions not requiring structural changes. Epigenetic changes allow some genes to be expressed or not, depending on the external conditions, and those changes are essential in cell and tissue differentiation that occurs during embryonic development as well as in adult organisms. Thus, mammalian cells undergo epigenetic changes throughout life. In fact, identical twins with the same genetic background build different epigenetic patterns depending on the environmental factors to which they are subjected, such as smoking, diet, or exercise.[2] In addition, these epigenetics patterns cause observable differences in the phenotype of both twins, either a different behavior or different risk of disease.[3]

The main epigenetic mechanisms include DNA methylation, histone modifications, and action of noncoding RNAs. So far, DNA methylation is the best known of these mechanisms. Most studies have been focused on DNA methylation and how it is associated with the development of a disease. Therefore, our review has been focused on DNA methylation and its role in developing multiple sclerosis (MS).

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DNA Methylation

DNA methylation is a biochemical process by which a methyl group is added to a cytosine residue in the DNA nucleotide chain. This binding occurs in cytosine–guanine dinucleotides (CpG), which are clustered in the genome, building the CpG islands. These are especially abundant in the promoter and other regulatory regions of genes. Methylation is performed by DNA methyltransferases (DNMTs) that catalyze the transfer of a methyl group from S-adenosyl-L-methionine (SAM) to carbon 5 of cytosine.[4] This process may be carried out following two different models: the occurrence of a "de novo" methylation pattern catalyzed by the DNMT3a and DNMT3b[5] enzymes, or by maintaining a methylation pattern in the following cycles of cell replication performed by DNMT1. The latter occurs during DNA replication. Therefore, when a CpG sequence acquires a certain methylation pattern, this modification becomes stable and is inherited as a clonal methylation pattern through subsequent cell divisions.[6]

Hypermethylation of CpG islands in the promoter region of the gene is typically a mechanism of gene repression as it inhibits transcription. This inhibition is basically performed through two processes: (1) by preventing the binding of transcription factors containing recognition sites for CpGs and (2) by means of adhering protein complexes known as methyl-binding domain (MBD) that are bound to the methylated CpG regions and block access to regulatory proteins or transcription factors.[7]

As mentioned earlier, the methyl group donor is the SAM molecule which, once it loses the methyl group, becomes S-adenosyl homocysteine (SAH). This molecule is hydrolyzed to homocysteine and then it is remethylated to methionine by 5-methyltetrahydrofolate cofactor (5mTHF). Finally, methionine is transformed back into a SAM molecule by the action of methionine adenosyltransferase (MAT). DNA methylation potential depends on the ratio between SAM level and SAH in blood. The higher the ratio, the more the methylation potential.[8] Therefore, it can be inferred that for the process of DNA methylation, proper metabolism of homocysteine and methionine is critical, as well as the metabolism of the various enzymes involved in this metabolic route and of other substances, such as folic acid and vitamin B12[9] (Fig. 1.1).

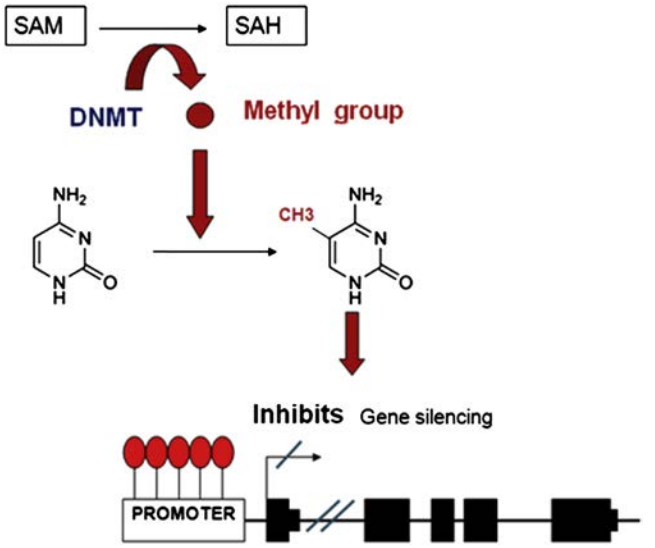

FIGURE 1.1 The process of how DNA methylation within gene promoters regulates transcription. *SAM*, S-adenosyl-L-methionine. *SAH*, S-adenosyl homocysteine. *DNMT*, DNA methyltransferases.

Relevance of DNA Methylation in Clinical Practice

Disruption of epigenetic mechanisms involved in human disease has been, for the last few years, an area of emerging research, yielding positive results in various diseases, especially in oncology. The first tumor related to mechanisms of epigenetic regulation was colorectal cancer (CRC). Initially, a loss of overall methylation was observed in cancer cells of CRC patients compared to healthy controls.[10] Also, the promoters of tumor suppressor genes were shown to be hypermethylated, which caused lower expression of those genes.[11] These findings supported that hypermethylation of tumor suppressor genes was associated with the occurrence of the disease.

However, in other areas of medicine, such as neurological disorders, how disruption of DNA methylation is involved in the disease is not well known yet. In the case of MS, epigenetic changes that might be involved in the pathogenesis of the disease have been identified, which has led to an exciting and new route of research.

MS is considered the leading cause of severe neurological disease affecting young and middle-aged adults. It is a chronic disease causing inflammatory, demyelinating, and neurodegenerative damage in the central nervous system (CNS). Its etiology is still unknown, although an autoimmune and multifactorial origin has been presumed and several genetic and environmental factors of susceptibility have been described for MS. Given the complexity of the disease and the participation of diverse, both genetic and environmental, etiological mechanisms, it is conceivable that there may be an alteration in the epigenetic regulation involved in its progression.[12,13]

RISK FACTORS IN MS AND EPIGENETIC CHANGES

Epidemiological and family aggregation studies suggest that there is a genetic predisposition for MS. However, to date, the only *locus* consistently associated with MS is the major histocompatibility complex (MHC). This predisposition has been associated with DR2

haplotype (HLA-DRB1*1501-DQA1*0102-DQB1*0602), which determines a relative risk of four of having MS.[14] Development of new technologies, such as polymorphism arrays, has helped to identify new candidate genes located outside the MHC region. Therefore, MS is considered as a polygenic disease in which each gene shows a different risk score (usually low or moderate).[15]

Moreover, environmental factors seem essential to the development of MS. For instance, the mismatch rate for disease occurring among monozygotic siblings is 70%, which supports the idea of other variables being involved.[16] Indeed, there is a number of environmental factors described to be involved in the etiology of MS, such as vitamin D levels in serum, animal fats in the diet, injuries, and toxic substances[17] (smoking, heavy metals, organic solvents, etc.). To date, the most consistent risk factors are smoking, vitamin D deficiency, and infection by Epstein–Barr virus (EBV).[18,19]

In the following sections, we summarize the three main environmental risk factors described for MS and the effects these factors may have on the various mechanisms of epigenetic regulation, both in MS and in the development of other diseases (Fig. 1.2).

Smoking and Epigenetic Mechanisms

Smoking is one of the environmental factors influencing the development of MS, as shown in different studies. According to a study conducted by Rodriguez Regal et al., smoking involves an odds ratio (OR) of developing MS of 1.97.[20] Cigarette smoke contains hundreds of potentially toxic elements, including nicotine, carbon monoxide, nitric oxide, cyanides, and polycyclic aromatic hydrocarbons, and some studies have suggested that these toxins might cause gene activation responsible for the MS autoimmune pathogenesis.[21] In fact, smoking has been associated with an increased relapse frequency and with the number of active lesions in the brain MRI of patients with this disease.[22]

On the other hand, when the blood of adolescents whose mothers smoked during pregnancy was analyzed, it was observed that prenatal exposure to tobacco is associated with increased methylation of the promoter of the "brain-derived neurotrophic factor" (BDNF), which promotes the differentiation and growth of new neurons.[23] Likewise, another study conducted by Kjersti Aagaard et al. analyzed the DNA methylation pattern by PCR techniques, in two groups of smokers and nonsmokers. They found changes in DNA methylation in 25 genes in nonsmokers and 438 genes in smokers.[24] Interestingly, epigenetic changes associated with smoking have also been found in oncology. In a study conducted in lung cancer patients, hypermethylation of *CDKN2A*, *DAPK*, and *MGMT* tumor suppressor genes was observed in smokers.[25] Likewise, in a study conducted on cervical cancer among female smokers aged between 15 and 19, hypermethylation of

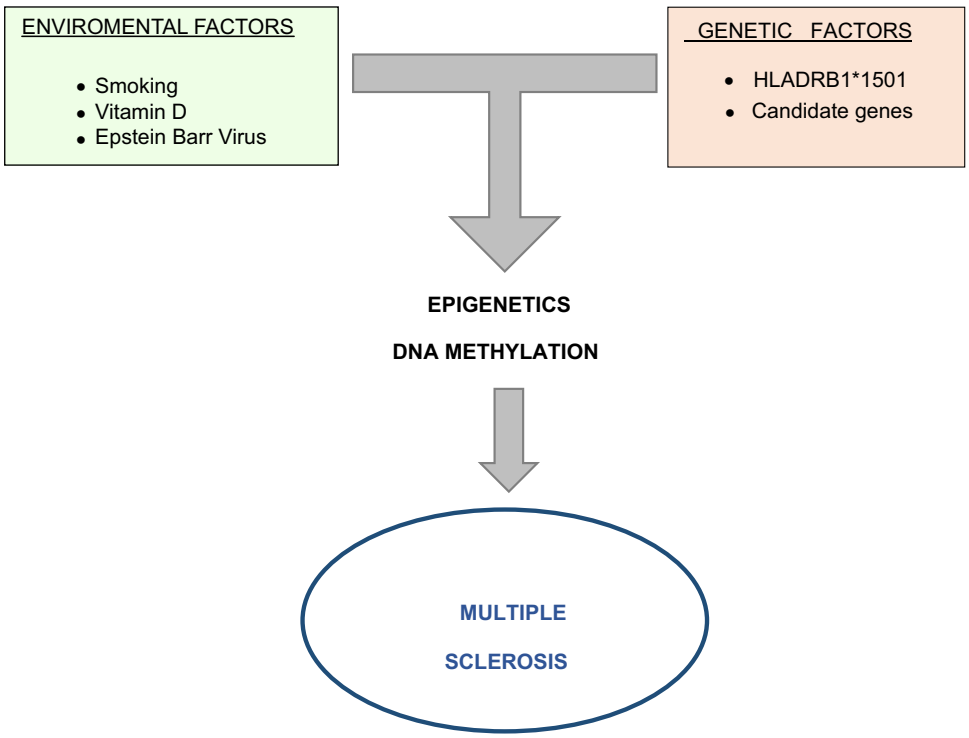

FIGURE 1.2 The interplay between environmental and genetic factors. The interplay is where DNA methylation may play a role to develop multiple sclerosis.

the *CDKN2A* gene in cervical epithelial cells was also observed.[26]

Vitamin D and Epigenetic Mechanisms

Vitamin D deficiency is one of the leading risk factors in the development of MS.[27] This vitamin can be synthesized by ultraviolet radiation or obtained by food, mainly fish oil. Many studies have been conducted studying the role of solar radiation as a protective factor in MS. The decrease in the amount of effective ultraviolet radiation in countries of higher latitude, involves an increased risk of MS.[28,29] This is because vitamin D is a powerful regulator of the inflammatory and immunomodulatory response, working on both adaptive and innate immunity.[30]

Although the mechanism by which vitamin D causes these changes is still uncertain, a study conducted by Joshi et al. suggests that it might be due to epigenetic modifications. In this study, the effects of 1,25(OH)2D3 (active form of vitamin D produced in the skin after exposure to ultraviolet light) on human *IL-17A* production by T CD4+ cells were analyzed. They observed that 1,25(OH)2D3 directly inhibits the *IL-17 locus*, responsible for the transcription of proinflammatory cytokines, by means of a modification of the histone deacetylase 2 (HDAC2) in the promoter region of the *IL17A* gene.[31] Other studies had previously shown that vitamin D is able to cause epigenetic changes, as in the case of colon cancer in which 1,25(OH)2D3 has been observed to be able to induce expression of the gene encoding the *JMJD3* (lysine-specific demethylase).[32]

Epstein–Barr Virus and Epigenetic Mechanisms

To date, several infectious agents have been serologically and pathologically associated with MS. As an example, Sundstrom et al. analyzed the evidence to support whether a viral infection occurs in the preclinical stage of MS and only the EBV antigen showed a direct pathological correlation with the onset of MS.[33] Moreover, it has been observed that a history of infectious mononucleosis (symptomatic form of EBV infection) doubles the risk of an individual to develop MS, while seronegativity for this virus is associated with a very low risk of developing MS (OR 0.06 compared with seropositive patients).[34]

Interestingly, EBV infection has been associated with epigenetic changes in the infected cells, and several tumor types have been related to EBV infection due to the hypermethylation of tumor suppressor gene promoters.[35] This occurs in nasopharyngeal cancer and Hodgkin lymphoma induced by EBV, where the promoter hypermethylation has been observed to be triggered by an increase in *DNMT1*, *DNMT3a*, and *DNMT3b* enzymes, and carried out by the LMP1 viral protein.[36] MS epigenetic changes associated with EBV are also associated with the expression of microRNAs (miRNAs). The expression of miRNA-142-3p in MS patients has been linked to an increased immune tolerance, whereas miRNA-155 expression is associated with increased T cell differentiation and CNS inflammation.[37]

DNA METHYLATION IN MS

MS etiology remains not fully understood. However, the most accepted hypothesis postulates that MS is an immune disease mediated by autoreactive T cells, which are activated by exposure to one or more environmental factors in individuals with certain genetic predisposition. This disease is progressive with the occurrence of focal inflammatory (mainly white matter), demyelination (with relative preservation of axons in early stages) lesions, and neurodegeneration (in later stages). The exact pathophysiological mechanism mediating between environmental risk factors and genetic susceptibility to develop MS is unknown.[38] It is precisely at this intersection where DNA methylation may provide new insights.

Inflammation and DNA Methylation

Several authors have linked the degree of methylation in specific genes with the occurrence of MS. In this regard, Kumagai et al. have found that the promoter of the sphingosine-1-phosphate enzyme (SPH-1) gene, which is involved in negative regulation of inflammatory signaling, is hypomethylated in MS patients compared to healthy controls. Promoter methylation of *SPH-1* leads to a decreased expression of this enzyme and consequently to increased activity of inflammation mediated by lymphocytes.[39]

Furthermore, Janson et al. have analyzed the CD4 + T cells from a series of patients with relapsing–remitting multiple sclerosis (RRMS), and have found that such patients have a demethylation of the *FOXP3* gene encoding for the scurfin protein, whose deficiency is associated with autoimmune disorders. *FOXP3* gene demethylation can inhibit the differentiation to Th1 and Th2 cells and at the same time can promote regulatory T cells (Treg) and Th17 cells. The Th1/Th2 and Treg/Th17 balance influences the disease status so that changes therein may lead to the appearance of a new lesion or to its repair, and DNA methylation is one of the factors regulating this balance.[40,41]

Additionally, promoter hypomethylation of the gene encoding IL-17A, proinflammatory cytokine secreted by activated T lymphocytes, has also been observed. This finding has been linked to the development of autoimmune diseases and plays a core role in the MS pathogenesis.[42]

Demyelination and DNA Methylation

A study conducted by Mastronardi et al. shows that during the white matter demyelination in MS patients, the promoter of the peptidyl arginine deiminase 2 (*PAD-2*) is demethylated and therefore *PAD-2* is overexpressed in the brain. This enzyme makes the myelin basic protein (MBP) to be less stable as a result of enzymatic conversion of arginine into citrulline. Moreover, citrullination triggers the MBP to behave as an antigen to T lymphocytes. In this analysis, the methylation of *PAD-2* promoter in the white matter of MS patients is reduced by 25% compared with healthy controls. Furthermore, this change only occurs in MS patients and not in patients with other neurological disorders, such as Alzheimer's disease, Parkinson's disease, or Huntington's disease.[43]

Moreover, genome-wide methylation studies performed on CD4, CD8, and brain have revealed a number of genes to be differentially methylated in MS compared to controls.[44,45] In this regard, Graves provides the first evidence for the association of DNA methylation at *HLA-DRB1* with MS risk.[46]

Neurodegeneration and DNA Methylation

To date, no studies are available that have specifically analyzed the involvement of epigenetic mechanisms in the neurodegeneration of MS patients. However, changes in DNA methylation during neuronal death have been reported. For instance, Castaños et al. have analyzed cells from patients with amyotrophic lateral sclerosis (ALS) showing that overexpression of the *DNMT3a* enzyme triggers cell degeneration and death, whereas enzyme inhibition protects those cells, and DNA methylation is the mechanism that regulates *DNMT3a* expression.[47] These results support that such a mechanism might be involved in the neurodegeneration occurring in MS patients, but this remains an open question for further research.

CONCLUSIONS

MS is a neurological disease with a major health, social, and family impact. Despite the significant advances in recent years, the exact etiopathogenic mechanism that causes MS remains unknown and a definitive therapy is not yet available.

Epigenetic changes, such as DNA methylation, are mechanisms by which environmental factors can influence individual gene expression. Epigenetic disruption is a research field of major interest in medicine, especially in the study of those diseases where combined genetic and environmental risk factors influence disease development, such as MS.

Although the number of studies conducted to date in patients with MS is low, the results are encouraging to further deepen this area. Currently available data show a relationship between regulation of DNA methylation in candidate genes that are key in the development of MS and autoimmune processes.[48,49] While the results point in that direction, conducting cohort studies with more patients and controls is necessary.

Knowing epigenetic changes involved in the MS pathogenesis will help us to clarify the mechanisms causing the disease, and thus the way will be open to identify potential biomarkers and search for new therapeutic targets.

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CHAPTER

2

EBV Infection and Vitamin D in Multiple Sclerosis Patients

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OUTLINE

MULTIPLE SCLEROSIS AND ENVIRONMENTAL FACTORS

Multiple sclerosis (MS) is a chronic, inflammatory, and debilitating autoimmune disease of the central nervous system (CNS) with unknown etiology. The inflammatory phenotype of MS is expressed as a relapsing–remitting course of neurological dysfunction, although the clinical symptoms vary between individuals.[1] MS mostly occurs in young adults, although MS risk appears to decline largely after the age of 50.[2,3]

As documented for a variety of autoimmune diseases, MS is more prevalent in females.[4,5] The female to male ratio ranges from 2:1 to 3:1 based on region,[6] and it is tempting to speculate that the female bias in immune complex diseases such as MS might be a consequence of sex hormones. However, it is not clear whether sex-related factors potentially exert deleterious effect or protective effect, although results from animal models remain conflicted, with some research showing no effect[7] and other findings indicating a slight worsening of clinical experimental autoimmune encephalomyelitis (EAE) following ovariectomy.[8] A number of MS-related genetic variations have also been reported,[9] highlighting the potential role of gene–environment interaction as well as the epigenetic phenomenon for female predominance.[10] Indeed, the female to male ratio for MS susceptibility is shown to be higher in individuals with certain human leukocyte antigen (HLA) haplotypes. In addition, the female to male ratio is higher in MS patients with *HLA-DRB1*15* haplotypes than those who are negative for *DRB1*15*.[9]

Both infectious and noninfectious factors have been implicated in MS development.[11,12]. Rather than a sole trigger, the initiation of MS disease seems to be reliant on the elaborate interactions between infectious or noninfectious environmental risk factors with shared susceptibility genes.[13]

MS AND INFECTIONS

Infectious agents are considered an important environmental risk factor for autoimmune diseases. In this regard, viruses have long been considered as possible etiological agents of MS.[14] As far back as 1946, the rabies

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virus was the first to be considered as having an association with MS.[15] Furthermore, aberrant immune reactivity against several viral infections has been reported.[16]

In an attempt to assess the temporal relationship between viral epidemics and MS relapses in the general population, the epidemics of influenza A and human herpesvirus-4 or Epstein–Barr virus (EBV) were shown to be temporally linked to the number of relapses in MS patients, supporting the notion that viral infections may influence MS development.[17] Although infection with several viruses is considered as a potential candidates involved in MS development[14,15] and no single virus has yet been proven solely responsible, infection with EBV and human herpesvirus 6 (HHV-6) has gained considerable attention.[18,19] Like other members of herpesviridae family, these viruses establish lifelong latent infections and are neurotropic and lymphotropic.[20]

The correlative evidence for an MS association generally includes amplifying viral genome with polymerase chain reaction (PCR), assaying virus-specific antibodies in serum and cerebrospinal fluid (CSF), and spotting virus antigens within tissue sections obtained from brain and MS plaques.[21,22] An etiological role for EBV infection in MS is supported by the most consistent findings including elevated EBV-specific antibody titers, dysfunctional EBV-specific $CD8^+$ T cell responses, higher tendency to induce spontaneous transformation of peripheral blood B cells, and accumulation of EBV-infected B cells and plasma cells in the brain of MS patients.[22]

MS AND EBV INFECTION

EBV is a ubiquitous double-stranded DNA virus that establishes a lifelong persistent infection in over 90% of the adult population worldwide.[20] Infection with EBV is usually asymptomatic in the first years of life; however, the risk of infection increases dramatically during adolescence or adulthood. Infectious mononucleosis (IM) is one of the most self-limiting clinical presentations of primary infection with this virus, which is characterized by fever, fatigue, pharyngitis, lymphadenopathy, and massive expansion of virus-specific T lymphocytes.[23]

EBV can infect both B cells and epithelial cells. At least three forms of EBV latency known as latency program I, II, and III have been described, and each program is reflected by a distinct set of proteins as well as the state of EBV-associated diseases or malignancies.[20] In the blood, the virus establishes latency in memory B cells where there is usually no expression of viral proteins, except for EBV nuclear antigen 1 (EBNA-1) and sometime latent membrane protein 2a (LMP-2a).[24]

In general, EBV infection is vigilantly controlled by immune responses especially by EBV-specific $CD8^+$ T cells.[25] However, the virus can exploit the immune system through encoding a number of proteins that have the ability to subvert the host's immune surveillance.[26] One of these proteins is the viral homolog of IL-10 (vIL-10), which is encoded by the EBV gene BCRF1 and has anti-inflammatory properties similar to human IL-10.[27,28] The virus not only modulates B cell differentiation and function but also can elicit a vigorous and persistent cytotoxic T cell response. Infection with EBV can also interfere with the normal process of autoreactive B cell neutralization or control at several tolerance checkpoints.[26,29,30]

The role of EBV infection in patients with MS was first highlighted by studies reporting an increased tendency of spontaneous transformation of EBV-induced B lymphocyte in vitro[31] and higher serum levels of anti-EBV antibodies in MS patients compared with healthy controls.[32] The association between EBV seropositivity and risk of MS has been thoroughly confirmed by compelling evidence collected since mid-1990s demonstrating that virtually all MS patients are EBV seropositive compared with 90–95% of matched healthy controls.[11,32–37] Seroconversion from EBV negative to EBV positive is associated with a higher risk of developing MS[38] particularly in pediatric cases where around 80% of MS cases were shown to be EBV seropositive compared with 50% of matched healthy controls.[39]

Although elevated levels of serum or plasma IgG antibodies against different EBV antigens have been described,[19,34,40–42] EBNA-1 is the only antigen that elicits the most robust antibody responses as supported by compelling evidence showing a significant association between elevated anti-EBNA-1 antibody titers and increased MS risk.[19,35] This association has also been reported by magnetic resonance imaging (MRI)[43] as well as clinical and radiological features of disease activity,[44] although results remain conflicted[45,46] and longitudinal studies are warranted to assess the serological profiles of anti-EBNA-1 in connection with the progression of MS.[47]

Evaluating anti-EBNA antibodies in sera collected prior to MS onset provided further evidence in which the relative risk of developing MS was found to be 30–36 times higher among those individuals who were EBV positive and had anti-EBNA titers ≥320 compared with those with titers <20.[48,49] The increase in anti-EBNA-1 antibody titers seems to be age dependent at least before clinical onset of MS. In addition, coinfection with other herpes viruses or a new EBV strain is assumed to alter the host immune control on the latent EBV infection,[50] which may support the relevance of EBV-specific immunity, particularly immune responses against EBNA-1 in MS pathogenesis.[47]

The antibody response to EBNA-1 is directed at multiple different epitopes, and further analysis highlighted that antibodies directed against particular epitopes especially within its glycine–alanine repeat are

of importance.[51–53] While IgG antibodies against all EBNA-1 epitopes were shown to be associated with MS risk, IgG antibody against EBNA-1 epitopes 385–420 showed strong association to MS when compared with total EBNA-1 IgG levels.[54]

Patients with MS also have elevated levels of anti-EBV IgG antibodies in the CSF,[42,55] which may reflect the intrathecal synthesis of EBV-specific antibody or might simply be an indication of transport from the blood to the CSF.[22] The intrathecal synthesis of viral-specific antibodies found in the CSF of MS patients might be an explanation for the occurrence of polyspecific humoral immune responses directed against viruses.[56–59] Further evidence supporting the association between EBV and increased risk of MS came from studies where the relative risk of MS has shown to be 2–3 times higher in individuals with a history of IM than those without EBV infection.[60–66] While consensus results have emerged from a serological point of view, studies investigating the EBV load have provided conflicting results, with some research showing no difference,[67–72] and others reporting a significant difference of EBV load in MS patients.[37,73] Despite conflicting results, the association of EBV reactivation and disease activity in MS patients was reported.[74,75]

Defective immune control of EBV in MS patients was suggested from the observation of increased spontaneous EBV transformation of B cells in vitro.[31] Indeed, spontaneous EBV-induced transformation of peripheral blood B cells and production of EBV particles have been observed in the culture of lymphoblastoid cells obtained from patients with MS,[31,76] which could be due to an increased frequency of EBV-infected B cells or impaired T cell function.[22] Furthermore, in 2013, an increased frequency of EBV-infected B cells was reported in CSF and peripheral blood of MS patients.[77]

POTENTIAL MECHANISMS UNDERLYING EBV INFECTION IN MS

While there is compelling evidence for the epidemiological associations of MS and EBV infection, the underlying mechanisms by which EBV infection could contribute to MS development are yet to be understood. Therapeutic approaches using B cell depletion not only support the clinical efficacy but also the functionality of B cells in the pathogenesis of MS.[78]

Impaired regulatory immune functions,[79–81] transactivation of human endogenous retrovirus element (HERV),[82,83] impaired $CD8^+$ T cell control of EBV-infected B cells,[84–87] breakdown of immunologic self-tolerance to CNS antigens,[88] molecular mimicry or cross-recognition of EBV gene products particularly EBNA-1-specific T cells with CNS antigens such as myelin basic protein (MBP) peptide,[89–93] bystander damage to the CNS,[94] activation of innate immunity,[95] expression of αB-crystallin in B cells,[96] accumulation of EBV-infected autoreactive B cells,[29] and inhibition of the activation-induced T cell apoptosis[97] are all plausible explanations through which EBV infection may contribute to the induction of autoimmunity and MS development either directly or indirectly.

The pattern of increase in anti-EBV IgG antibodies in MS patients may reflect an altered T cell response to EBV-transformed B cells in MS, because anti-EBNA-1 titers were found to be positively correlated with the precursor frequency of T cells recognizing autologous EBV-transformed B cells.[98] Although CSF T cells from MS patients are shown to recognize autologous EBV-transformed B cells[99] and cross-recognize EBV and MBP,[91,92] it is not clear how EBV infection drives local CNS inflammation.[16]

A significant increase in the EBV DNA load could result from either a large increase in the frequency of latently infected B cells or an increased number of EBV genomes in B lymphocytes or it may reflect a global effect on B cell activation, which may lead to increased EBV production.[22] Increased frequency of EBV-infected plasma cells and plasmablasts in the peripheral blood of MS patients was described in 2013.[77] Moreover, it has been shown that EBNA-1 is the only EBV protein that can be expressed by proliferating EBV-infected memory B cells,[100] suggesting that higher expression of EBNA-1 as a consequence of higher viral load could result in stimulation and expression of larger number of EBNA-1-specific B cells. This may lead to higher serum levels of EBNA-1 IgG. It is interesting to note that predominant EBNA-1-specific clonal expansions of T cells recognizing myelin antigens have been documented in MS patients, again suggesting that the increased EBV antigen load in these patients can hyperstimulate humoral- and cell-mediated immune responses specific to EBV.[16,92] The inflammation observed in a majority of MS patients may also reflect the combination of EBV infection and a dysregulated inflammatory response, although this needs to be addressed in larger studies.[37]

Numerous genetic variations associated with MS risk are described,[101,102] and analysis of genome-wide association data support a causal role for the interaction between EBV and MS-associated gene variants,[103] although the overall contribution of genetic factors seems to be modest and cannot explain how EBV infection increases MS susceptibility.[104,105] To support this notion, statistical interactions between HLA alleles and EBV infection were carried out, suggesting that the risk factors may share a common biological pathway.[54] The association found between *HLA-DRB1*15* haplotypes and anti-EBNA-1 IgG levels[54] and IM history[106] are shown to increase the risk of MS disease, suggesting that HLA genes may influence the immune control of EBV infection. Given the fact that epigenetic changes such as

DNA methylation and histone acetylation can be influenced by environmental factors,[107] any disruption of epigenetic regulation can contribute to the developing MS via a complex interplay between genetic and EBV infection.[108] Genetic variations of EBV may also influence the MS susceptibility perhaps through affecting B cell homeostasis and evoking humoral and cellular immune process.[22] Interestingly, both EBV strain variation and high frequency of coinfection by EBV types 1 and 2 have been documented in MS patients.[50,109]

MS AND VITAMIN D STATUS

The notion that vitamin D deficiency is a potential risk factor for MS was originally proposed by Goldberg to explain the geographical distribution of MS in relation to ultraviolet radiation (UVR) and vitamin D insufficiency.[110] Compelling epidemiological evidence has been provided over the past years, strengthening the link between low vitamin D uptake and increased MS risk particularly in younger individuals.[111–114] Indeed, the relative risk of MS was shown to be 40% lower among women with regular vitamin D intake >400 IU/day than those with no supplemental vitamin D intake.[112] These findings were further supported by two large and prospective studies conducted in the United States and Sweden where compared with patients who had serum 25-hydroxyvitamin D (25[OH]D) concentration less than 75 nmol/L, the risk of MS were reported to be 50–60% lower among individuals with serum levels of 25[OH]D more than 100 nmol/L and 75 nmol/L, respectively.[111,113]

Vitamin D metabolites have also been linked with clinical and MRI outcomes[115] as well as MS relapse and degree of disability.[116] In patients with clinically isolated syndromes (CISs), low vitamin D concentration was not only associated with increased MS risk[117] but was also considered as a strong risk factor for long-term MS activity and progression in patients mainly treated with interferon beta-1b (IFN-β1b).[118] Furthermore, the inverse correlation found between seasonal fluctuations in vitamin D uptake and lesion activity in MS patients may suggest an immunomodulating effect on CNS inflammation.[119]

While patients with MS usually receive adequate nutritional vitamin D intake, only a fraction of these patients show suboptimal levels of vitamin D with unknown reason.[37] However, it should be noted that reduced outdoor activity, seasonal variance, long-term glucocorticoid use, age, thyroid dysfunction, pregnancy, obesity, and even month of birth can influence vitamin D levels.[120–124]

Vitamin D exerts its action when the active form binds to its cognate nuclear vitamin D receptor (VDR). Upon interaction with its ligand, VDR heterodimerizes with the retinoic X receptor (RXR) and subsequently binds to vitamin D–responsive elements (VDREs) in the promoter region of target genes to exert its regulatory effects.[125] Studies using chromatin immunoprecipitation and parallel DNA sequencing of 1,25D-treated B cells also indicate that over 2700 different VDREs can bind to VDR.[126,127]

MECHANISMS UNDERLYING VITAMIN D IN MS

Accumulating data obtained from human and EAE models support the beneficial role of vitamin D supplements in MS.[128–132] However, the exact molecular mechanisms underlying the effect of vitamin D yet remains obscure and it is not clear how vitamin D may contribute to the pathogenesis of MS.

It is commonly thought that genetic susceptibility together with environmental factors are required for MS development,[133] and the interplay between MS susceptibility genes and vitamin D may be a plausible scenario for the correlation observed between low levels of vitamin D and increased risk of MS. Given the fact that genome-wide association studies have revealed a large number of novel loci that may influence susceptibility to many common diseases such as MS,[127,134] the interaction of VDR alleles and a number of autoimmune disease–associated genes has been reported.[135–137] Early evidence for an effect of vitamin D on HLA gene expression came from a study that showed treatment with 1,25-dihydroxyvitamin D3 (1,25[OH]2D3) can specifically alter HLA-DR antigen expression and antigen presentation,[138] although it did not show direct interaction of vitamin D and HLA haplotypes. Identifying a putative VDRE in the promoter region of *HLA-DRB1*[139,140] further support the view that genetic variation of VDR may be associated with susceptibility to MS together with HLA particularly *HLA-DRB1*1501* haplotype,[141] which is a susceptibility gene for MS.[142]

It is thought that a particular VDRE can induce *HLA-DRB1*1501* expression upon addition of vitamin D as documented by specific increase of the cell surface expression of *HLA-DRB1* in lymphoblastoid cells bearing only *HLA-DRB1*15*. It has also been suggested that vitamin D insufficiency in early life might affect *HLA-DRB1* expression in thymus and finally lead to a defective thymic T cell selection by allowing autoreactive T cells to escape central thymic deletion.[139] However, this idea has been challenged by the data obtained from Sardinian population where MS susceptibility was found to be independent of VDREs in the HLA-DRB1 promoter.[133]

Comprehensive studies since 2013 have identified a vast majority of single-nucleotide polymorphisms (SNPs) which are located in noncoding regions of the genome and considered as MS-risk loci.[102,143] The

potential association found between the VDR gene variation and MS susceptibility[144] suggests that genetic variations within the VDR gene may be involved in MS risk under certain conditions such as vitamin D deficiency or lack of exposure to sunlight.[145] Considering genetic variation within the VDR gene, several SNPs were found to be associated with the risk of relapse or with vitamin D levels,[146] although only a few polymorphisms are shown to have functional impact.[147] As an example, rare variants in the CYP27B1 gene, which encodes the 1-alpha-hydroxylase enzyme to convert 25[OH]D to the biologically active form of vitamin D (1,25-dihydroxyvitamin D, 1,25[OH]2D),[148] have associated with increased risk of MS,[149,150] although these findings were not replicated by other studies.[151,152]

ChIP sequencing of human primary $CD4^+$ T cells from healthy volunteers has shown that VDR binding in response to physiological levels of vitamin D occurs predominantly in a VDR motif–independent manner.[153] However, in vitro analysis of vitamin D responsiveness of MS-associated genes in $CD4^+$ T cells suggest that the presence of VDRE and binding of VDR is not sufficient to induce gene expression in vitro. While the majority of MS-associated genes were found to have one or more VDRE in their regulatory region, only few VDRE-containing genes including IL-2RA displayed vitamin D responsiveness.[154]

Different immune cell types express VDR,[155,156] highlighting the important role of vitamin D and VDR in immunity[157,158] as well as metabolism and homeostasis.[159] To support this notion, VDR expression is shown to be important for optimal T cell activation.[160] In addition, vitamin D seems to exert its full potential through paracrine calcitriol signaling to T cells within tissues.[161] The emerging data from studies targeting VDR gene support the view that vitamin D is important to control T cell–mediated autoimmune responses.[162,163] Considering the immune regulatory function of vitamin D[164] and its positive link with regulatory T cell function in MS patients,[165] vitamin D may contribute to the emergence of an autoimmune disease phenotype by both increasing effector $CD4^+$ T cell sensitivity to extrinsic cell death signals and influencing specific gene regulation particularly pro-inflammatory and anti-inflammatory genes.[163,166,167] Prolonged vitamin D insufficiency might also perturb the complex network of VDR target genes (VDRTGs) in immune cells, which probably are responsible for the induction of an autoimmune response.[168]

Vitamin D can also exert antimicrobial responses,[169] and supplementation with vitamin D was reported to diminish EBV reactivation[170] and sensitize EBNA-1-specific T cells in MS patients.[171] It is tempting to speculate that vitamin D might indirectly influence the pathogenesis of MS although more functional studies are needed to address this issue.

JOINT EFFECTS OF EBV INFECTION AND VITAMIN D STATUS IN MS

Given the fact that there is considerable evidence in favor of an etiological role for both EBV infection and low vitamin D concentration in MS,[172] it is not yet clear whether they interact in concert or independently or whether EBV exerts its potential in the presence of suboptimal vitamin D levels or whether vitamin D supplementation has an effect on antiviral immune responses. While it is possible that vitamin D and EBV influence MS disease independently,[173] another scenario would also be plausible, by which vitamin D status and EBV infection contribute jointly in genetically predisposed individuals, which suggests a potential biological interaction of these risk factors in the development of MS.[174–176]

Previous studies addressing the link between the immune response against EBV infection and vitamin D found no evidence of statistical interaction between these risk factors, suggesting the independent action of vitamin D and EBV in MS.[177] However, early support for a potential interaction between these two risk factors came from studies where a weak but significant association was observed between serum concentration of vitamin D and anti-EBNA-1 antibodies, indicating a potential interaction between them particularly among young MS patients.[175,176]

Further support came from studies showing an inverse correlation between anti-EBNA-1 titer and vitamin D concentration. Indeed, daily supplementation of vitamin D3 in relapsing–remitting MS (RRMS) patients were found to decrease the level of anti-EBNA-1 antibody titers when compared with the baseline titers measured before vitamin D supplementation.[174] In addition, weekly supplementation of vitamin D3 for 6 months were found to limit the augmentation of IgG antibody titers against EBNA-1 and viral capsid antigen (VCA) in MS patients.[178] In 2015, an inverse correlation between vitamin D level and EBV load was also reported in RRMS patients, supporting the beneficial effects of vitamin D and a plausible interaction between these risk factors.[37]

However, the inverse correlation might be driven by cytokine production, persistent antigen stimulation, or both.[37] Moreover, the immune response against EBV infection during seroconversion might also be influenced by genetic susceptibility[54,176] as supported by the findings that indicate an important role for MS-associated *HLA-DRB1*15* haplotype and its association with IM history,[104] reactivity to EBV-related epitopes,[54,105] and anti-EBNA-1 IgG responses.[179] To support this notion, studies conducted in twins and their relatives imply that the environmental risk factors exert their effects in the context of underlying genetic susceptibilities, indicating that risk factors in MS are mainly interactive rather than being independent.[180,181] Given the modulatory role of

epigenetic phenomenon such as changes in methylation, vitamin D is also thought to interact with *HLA-DRB1*15* via VDREs.[182] Indeed, a preferential interaction of VDR with disease susceptibility regions were found in lymphoblastoid cell line (LCL)[126] and other immune cell types.[153]

As reported in 2007, massive interactions were identified among EBV proteins and between EBV and human proteins.[183] While these findings provide a broad perspective on EBV strategies for replication and persistence in the host,[183] it is not clear which and how EBV protein(s) interact with other risk factors particularly vitamin D and genetic. This figure becomes even more complicated when considering the high number of EBV-encoded proteins[20] and virus genetic variants that has shown to be associated with MS disease.[184] However, Epstein–Barr nuclear antigen 2 (EBNA-2) seems to be a potential candidate for interplaying between EBV infection and other risk factors. EBNA-2 is a highly polymorphic gene and the polymorphisms found in this gene may also have functional consequences in these patients.[185,186] It has also been shown that a single amino acid in EBNA-2 gene can regulate superior growth maintenance of B LCL.[187] Interestingly, EBNA-2 expressing cells were found in affected brains of MS patients.[94] EBNA-2 also regulates the transformation of virus-induced B lymphocytes[188] through other cell transcription factors particularly via a recombination signal–binding protein for immunoglobulin kappa J region (RBPJ).[189]

It is thought that EBNA-2 may be involved in virus interaction with both vitamin D and MS-associated susceptibility genes. Indeed, a striking overlap between EBNA-2 distribution and VDR-binding site has been described in B cell lines. In addition, a significant enrichment of EBNA-2-binding motifs was found in MS-associated genomic intervals. Interestingly, EBNA-2 and VDR were shown to have similar genomic localization with MS regions.[190] Although data remain conflicted, it has been suggested that EBNA-2 and vitamin D exert potential antagonistic activity, and they may compete for MS susceptibility regions in immune cell subsets.[190] While EBNA-2 was found to drive proliferation and activation of B cells,[189] vitamin D down-regulate B cell function,[191] indicating the opposed action of these factors.

However, EBNA-2 may also interact with VDR inside DNA-binding sites where it might down-regulate VDR-responsive genes perhaps through forming a complex with other regulatory proteins as described for the interplay between EBNA-3 and VDR.[176,192] Indeed, ChIP-sequencing data obtained from LCLs indicate the enrichment of cell transcription factors such as RBPJ within EBNA-2 genomic sites,[189] and the tendency of EBNA-2 and RBPJ to bind MS-associated genomic intervals might be relevant to the pathogenicity of MS disease.[193]

Although it is important to differentiate between immune effects by UV exposure independent of vitamin D, and immune effects by vitamin D per se, vitamin D deficiency is assumed to influence the immune response directed against EBV infection early in life.[194] It has also been hypothesized that viral IL-10 (vIL-10) produced during EBV infection can elicit a host immune response that may compete with human IL-10 and undermine the protective function of vitamin D.[162] The lack of IL-10 production at the mRNA expression level and protein level has been well documented in MS patients.[37,195–198] Compared with healthy controls, these patients were also found to have impaired IL-10 regulatory function[81,199] and lower frequency of IL-10 producing T cells.[200,201] Given the fact that 1,25[OH]2D3 could reversibly prevent the progression of EAE, a model of MS,[130] and on the other hand, IL-10 signaling was shown to be necessary for the inhibition of EAE mediated by (1,25[OH]2D3),[202] an acquired IL-10 deficiency induced by EBV infection might also contribute to MS disease.[162]

In general, the complex interplay between viral genetic variants and both host and other risk factors, such as vitamin D, support the notion that infection with EBV may facilitate predisposing a person to MS through the gene–environment interactions.

CONCLUSION

The emerging, but mysterious, role of EBV infection and vitamin D status in MS pathogenesis has been emphasized. Given the fact that a growing set of genetic susceptibility factors such as certain HLA class II haplotypes also confer the risk of developing MS, it is not yet clear how MS-associated genetic predisposition is affected by EBV infection and vitamin D status. While individual risk factors may exert independent effects, the complex interplay between these environmental triggers and genetics in pathogenically relevant immune cell subsets is highly likely. The interplay between EBV infection and vitamin D status in genetically predisposed individuals remains an intriguing concept. However, it would be of interest to investigate the global imprint that EBV infection and vitamin D can have on immune signature in these patients. A detailed understanding of the biological actions of these risk factors via genetic and epigenetic factors not only provides insights into MS disease pathogenesis but also offers a rationale for investigations of both infectious and noninfectious risk factors for management of MS patients with clinical complications. More generally, the answer to these concerns would also strengthen the evidence base in support of controlled clinical trials targeting both sides of virus–host interaction in complex autoimmune patients including MS.

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CHAPTER

3

White Matter Abnormalities in MS: Advances in Diffusion Tensor Imaging/Tractography

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OUTLINE

A BRIEF OVERVIEW OF THE NEUROPATHOLOGY OF MULTIPLE SCLEROSIS

Multiple sclerosis (MS) is an acquired progressive inflammatory demyelinating condition affecting the central nervous system (CNS) that often presents with a relapsing and remitting course. To understand the symptoms and presentation of MS, it is crucial to first understand the basic neuropathology and associated neuroanatomy that is affected by the disease. MS generally involves neuropathology affecting three primary features of the neuron and surrounding tissue. These features are lesions, inflammation, and damage to the myelin sheath that surrounds the axons of a neuron. As shown in Fig. 3.1, a neuron is composed of cell body with branch-like dendrites and a longer fiber projection called an axon. It is the axons that permit neural communication over significant distances within the nervous system. A neural signal originating in the cell body travels along the axon and terminates at the synaptic bouton, where neurotransmitters are released into the synapse to stimulate adjacent neurons. The terms gray matter (GM) and white matter (WM) are often used to describe various aspects of these neuronal tissues. Specifically, brain tissue such as the cerebral cortex is often labeled as GM because it comprises dense clustering of the cell bodies of neurons, leading to a characteristic grayish appearance to the naked eye or when seen on standard T1 magnetic resonance imaging (MRI) scans. WM comprises the axons and their surrounding myelin insulation. The axon is a protoplasmic projection from the cell body that allows rapid transduction of an electrochemical signal, known as an action potential, across longer distances of the nervous system. In humans, axons are insulated by a fatty white-appearing covering called myelin. The layer of myelin is produced by the attachment of glial cells to the axon (oligodendrocytes in the CNS and Schwann cells in the peripheral nervous system, PNS). The myelin sheath covering is discontinuous and the gaps between the myelin sheath on axon are known as nodes of Ranvier. These gaps allow exchange of ions with the extracellular space which helps regeneration of action potential across the axon. The myelin covering enables faster conduction

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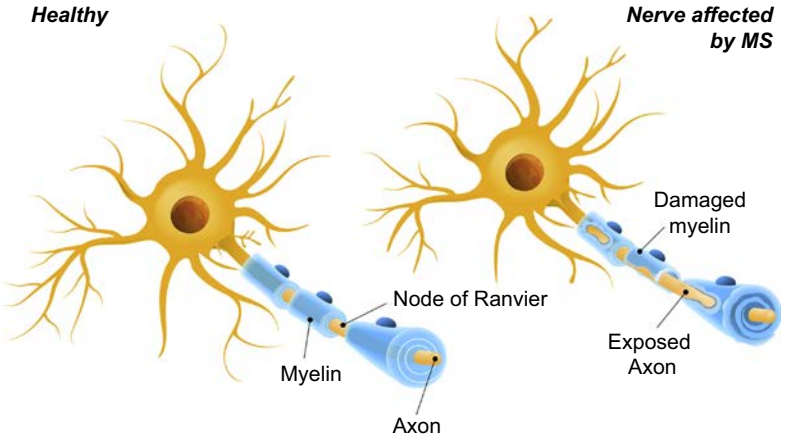

FIGURE 3.1 A graphical representation of the anatomical structure of a neuron and comparison between a healthy neuron and a neuron affected by multiple sclerosis (MS). As shown in the figure, the myelin sheath surrounding the axon is damaged in MS. *Reprinted with permission from www.123rf.com; designua © 123RF.com.*

of the action potential across neurons by permitting the neural impulse to propagate rapidly from node to node.

In brief, the pathology of MS involves damage to the myelin sheath, which results in disturbances in conduction of nerve impulses, which in turn affects motor, sensory, visual, and autonomic systems.[11] These disturbances may manifest in several ways. First, lesions (or plaques) to the WM, brain stem, basal ganglia, optic nerve, and spinal cord are among the most commonly observed.[12] These lesions are a result of demyelination and subsequent attempts of remyelination, which eventually builds up plaques along the damaged axons.[12] MS is also associated with the loss of oligodendrocytes, which are responsible for the production of myelin in the CNS.[12] Second, MS can lead to a disruption of the blood–brain barrier, which allows T cells to enter the CNS and initiate a cascade of other immune responses, which in turn commences inflammation.[12] There are four clinical subtypes of MS[8]: (1) relapsing remitting (RR) type—which is the most common pattern and involves periods of flair-ups followed by periods of relative dormancy; (2) secondary progressive (SP) type—which involves a slow worsening of symptoms over time, often with a relapsing and remitting progression; (3) primary progressive (PP) type—which involves a slow but fairly consistent worsening of symptoms over time, without a clear relapse/remission pattern; and (4) progressive relapsing type—which involves a progressive worsening of symptoms with acute periods of exacerbations without clear remissions.

NEUROIMAGING IN MS

MS is a challenging disease when it comes to diagnosis and treatment. Over the past decade, the development of new imaging modalities such as MRI has revolutionized the management of this disease, particularly with regard to diagnosis and monitoring disease progression. In this chapter, we briefly outline the use of standard clinical MRI scans for diagnosis and monitoring, and introduce the investigational use of newer cutting edge neuroimaging technologies, such as diffusion tensor imaging (DTI) and fiber tractography, which hold the promise of rapidly advancing understanding of this debilitating disease.

MRI is a widely used imaging modality that provides excellent resolution of the lesions common to MS. Standard MRI scans work on basic principles of quantum mechanics. In brief, during a typical MRI scan, the body part of interest is placed within a strong magnetic field, which aligns a large number of the hydrogen protons in the direction of the magnetic field. By applying a radio frequency (RF) pulse to the body part, the orientation of the protons can be momentarily reoriented. After cessation of the RF pulse, the realignment of the protons with the magnetic field will lead to a change in magnetic flux which can be captured by the receiver coil in the scanner and used to reconstruct three-dimensional images of the body part. Depending on the pulse sequences and imaging parameters used, the MRI can produce various sequences such as T1-weighted (T1WI), T1 contrast-enhanced (T1C), T2-weighted (T2WI), fluid-attenuated inversion recovery (FLAIR), DTI, and magnetic resonance spectroscopy (MRS), each providing meaningful information about the health and anatomy of the tissues and structures being imaged. Fig. 3.2 shows examples of T2WI scans showing MS lesions. MRI scans can be used clinically to make a diagnosis of MS. The McDonald criteria,[13] currently considered the most reliable method of MS diagnosis, rely upon MRI to demonstrate the dissemination of lesions in time and space. Table 3.1 represents the most recent (2011) version of these criteria for using T2WI MRI images to diagnose MS.[18] In the next

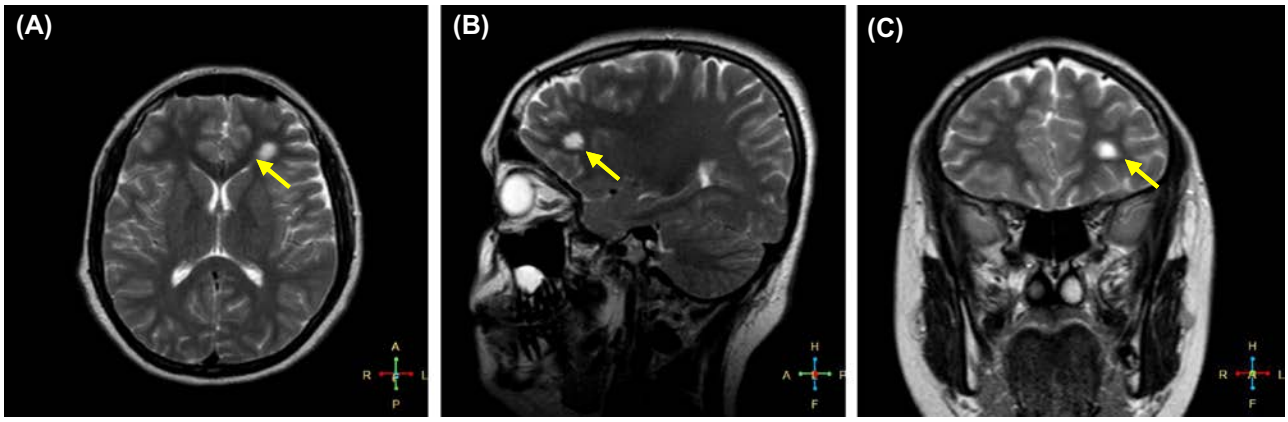

FIGURE 3.2 T2 weighted structural scans showing an oval-shaped hyperintense lesion in the left forceps minor region on (A) axial view, (B) sagittal view, and (C) coronal view. *Reprinted with permission from* www.radiopaedia.org*; image courtesy of Dr. Ahmed Abd Rabou.*

TABLE 3.1 Revised McDonald Criteria[18]

Dissemination in Space	Dissemination in Time
≥1 T2 lesions in two or more of the following locations: • Periventricular • Juxtacortical • Infratentorial • Spinal cord • If a patient has a brain stem/spinal cord syndrome, the symptomatic lesion(s) are excluded from the criteria, not contributing to the lesion count	• A new lesion on follow-up MRI-T2 lesion and/or gadolinium enhancing or • Presence of asymptomatic gadolinium-enhancing lesion and a nonenhancing T2 lesion on any one scan

few paragraphs, we outline some of the major findings on each type of MRI scan in patients with MS.

T1-Weighted Imaging

While T1WI scans provide exquisite detail of the brain and show clear demarcation between GW and WM, they are not as sensitive as T2WI for detecting MS. In general, T1WI findings vary on the basis of duration and severity of the disease. Axonal loss or destruction in early stages of disease can appear as hypointense or isointense ovoid, rounded or linear shaped lesions, appearing as dark spots on the scan. These are usually seen along the callososeptal interface or periventricular area and are referred to as T1 *black holes*. Sometimes, as the disease progresses the black holes may be marked by a peripheral rim of hyperintensity due to macrophage infiltration and lipid peroxidation of the surrounding tissues. This gives the lesions a *beveled* or a *lesion-within-lesion appearance*. In advanced stages of disease, thinning of the corpus callosum (CC) with or without generalized brain atrophy can be seen on T1WI.

T1-Weighted Contrast Imaging

Adding a contrast agent to an MRI scan can help in identifying certain lesions or pathologies. In the case of MS, gadolinium contrast can be used with a T1 sequence to highlight the actively demyelinating lesions. The lesions can appear as punctate, nodular, or rim-shaped contrast-enhancing lesions in the cerebral WM. An incomplete rim with the open nonenhancing end facing toward the cortex resembling a horseshoe is a characteristic finding of MS seen on this sequence. The "horse shoe sign" represents active stage of disease. Treatment with steroids drastically suppresses the enhancement and appearance of these lesions.

T2-Weighted Imaging and FLAIR

The T2 sequence, especially FLAIR, is considered to be the most sensitive MRI scan for detecting MS plaques. These images are helpful for identifying lesions because they suppress the appearance of cerebrospinal fluid, which allows for greater resolution in detecting lesions in the periventricular regions. Multiple hyperintense lesions, sometimes surrounded by hypointense peripheral rim with perilesional edema, can be seen. The lesions can be ovoid (as shown in Fig. 3.2), linear, circular, or triangular in shape. A triangular shaped lesion with the base of triangle adjacent to the lateral ventricle and apex pointing toward the cortex is one of the typical findings of MS. Perivenular collection of inflammatory cells along medullary veins can be seen as hyperintensities perpendicular

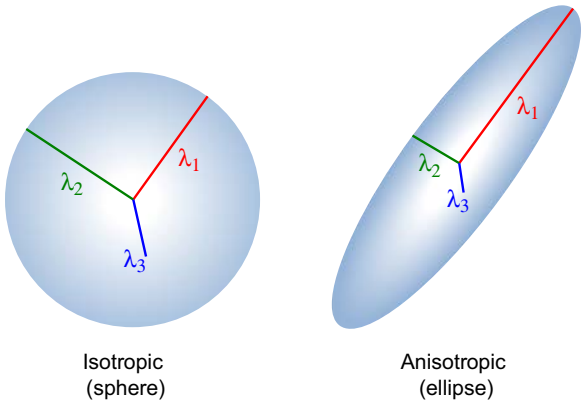

FIGURE 3.3 Illustrative example of prototypical water diffusion. Isotropic diffusion means that water molecules can diffuse equally in all directions, as illustrated by a spherical pattern. Anisotropic diffusion means that water molecules are constrained and diffuse more readily in one direction (λ_1) than in the other two directions (λ_2 and λ_3). *Reprinted with permission from* www.dotromp.com*; image courtesy of Do Tromp.*

to the lateral ventricles on axial and sagittal views. This finding is referred to as *Dawson fingers*. The callososeptal interface may show alternate areas of hyperintensity and hypointensity on FLAIR sagittal view giving a dot–dash appearance. This is known as the *dot–dash sign* and is one of the earliest characteristic finding of MS.

Magnetic Resonance Spectroscopic Imaging

Proton MRS is one of the unique applications of the MRI technique. It yields information about the chemical composition of different metabolites in the tissues rather than information about anatomical structure or function. Biochemical changes are common within a tissue that is affected by certain disease states. These changes are then compared with the normal distribution of the chemicals to assess the degree and extent of damage within that tissue. While the range of neurochemicals that can be assessed with MRS is limited, there are some that may be particularly important in the case of MS. In particular, N-acetyl aspartate (NAA) is an extremely abundant chemical in the brain, particularly within myelin, so it could be an indicator of WM damage in MS. In fact, evidence reported in 2014 supports the suggestion that in primary and SP type of MS the MRS shows decreased levels of NAA, suggesting a biomarker of axonal damage.[27] Other neurochemicals have been found to be elevated in acute lesions of MS, including the levels of myoinositol, choline, and glutamate.[25]

Diffusion Tensor Imaging

DTI is a relatively new neuroimaging technique that has been used to study WM alterations in a great variety of conditions, ranging from depression, to traumatic brain injury, to MS. DTI measures the movement of water molecules within the living tissue,[2] permitting inference regarding the underlying structure of the tissues and their membranes. The motion of water molecules can be described in geometric terms as either resembling a sphere or an elongated ellipsoid and is characterized as being either isotropic or anisotropic in nature, respectively. Isotropic movement occurs when water molecules are unconstrained and free to move in any direction equally, and would thus be best defined as a spherical diffusion pattern. On the other hand, water moving in a tube or garden hose would move preferentially in one direction much more than in other directions, and would therefore be better characterized as anisotropic (i.e., an ellipsoid) pattern of diffusion.[2] For instance, due to the lack of axons within the brain ventricles that would have restricted the movement otherwise, the water is free to move in any direction and hence the movement within these structures would be described as being isotropic. In the brain WM, on the other hand, the presence of axons restricts the movement of water molecules in a particular direction and therefore movement within WM regions is predominantly anisotropic in nature.

Axons are not always perfectly aligned along one axis and in order to avoid having to measure diffusion along an impractically large number of axes, a concept of diffusion ellipsoid has been developed.[15] The diffusion ellipsoid is defined using three eigenvectors that have three corresponding eigenvalues (λ_1, λ_2, and λ_3) that correspond to their physical length.[16] The longest, middle, and shortest eigenvectors are represented by λ_1, λ_2, and λ_3, respectively.[16] Fig. 3.3 shows the relationships between these three eigenvectors for isotropic and anisotropic shapes.

A number of diffusion measurements have been developed in an attempt to characterize diffusion patterns within the brain WM. Fractional anisotropy (FA) is a global diffusivity measure that measures the degree of anisotropy and is used to evaluate WM integrity. FA is defined by the following formula[15]:

$$FA = \sqrt{\frac{1}{2}} \frac{\sqrt{(\lambda_1 - \lambda_2)^2 + (\lambda_2 - \lambda_3)^2 + (\lambda_3 - \lambda_1)^2}}{\sqrt{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}}$$

FA values range from 0 to 1, with higher values indicating higher anisotropy (i.e., water diffuses more along one axis relative to the others). Mean diffusivity (MD) has also frequently been used to measure the overall diffusivity and represents the average of the three eigenvalues[29]:

$$MD = \frac{(\lambda_1 + \lambda_2 + \lambda_3)}{3}$$

Two other DTI metrics that have been proposed to further explain changes in the global measures (i.e., FA

and MD) are radial diffusivity (RD) and axial diffusivity (AD). RD is used to measure diffusion across the axon whereas AD describes movement of water molecules along the axon. Changes within these metrics have been attributed to demyelination and axonal damage, respectively. In their pioneering studies, Song and colleagues showed that loss of myelin following retinal ischemia in mouse optic nerve was associated with increased RD and unchanged axial diffusivity. [22–24] Moreover, they showed that axonal degeneration observed during histological analysis was concurrently associated with reduced AD and unaltered RD.[22] Therefore, these metrics have been used to describe potential reasons for changes within the global diffusivity measures. RD is defined in the following way[23]:

$$\lambda\perp = \frac{(\lambda_2 + \lambda_3)}{2}$$

AD is represented by $\lambda\| = \lambda_1$[23].

DTI Findings in MS

Using conventional MRI, earlier studies were able to demonstrate macrostructural damage, such as WM lesions, that underlie the physical and cognitive disturbances that are commonly observed in MS. With application of DTI to a wider range of illnesses including MS, both physicians and scientists were able to better understand this condition on a microstructural level. One of the earliest studies by Werring, Clark, Barker, Thompson, and Miller[28] showed reduced FA and high MD in normal-appearing white matter (NAWM) in frontal, parietal, temporal, and occipital regions. Based on the earlier description, this suggests that MS is associated with regions of greater spherically shaped diffusion, potentially suggesting poorer axonal integrity or disruption of myelin (see Fig. 3.4B and C). An important implication from these findings is the notion that WM changes may start occurring before clinical symptoms emerge and remain undetectable using conventional MRI and hence potentially delay clinical interventions that could affect the onset of the illness or reduce its severity.

More recent studies have rectified this earlier limitation by investigating individual WM fiber bundles with the advent of WM tractography (Fig. 3.4A), an outgrowth of DTI procedures. This technique allows a more accurate identification and description of WM architecture. As shown in Fig. 3.4, it is possible to use the FA values at individual locations throughout the brain to determine the probable fiber pathways representing large bundles of axons and plot them for visual representation. Fink et al.[5] have investigated coherence within a number of WM regions including the uncinate fasciculus (UF), superior longitudinal fasciculus, fornix, and cingulum in a group of MS patients. The left UF showed reduced FA and increased MD while the right UF was characterized by increased RD. Increase in RD has been frequently interpreted to signal demyelination.[22] In addition, there was a bilateral reduction in FA within the fornix. Similar to the UF findings, increased RD was observed in the left cingulum.

Similarly, Hecke et al.[7] used voxel-based morphometry that implements whole-brain approach to studying brain WM to examine WM microstructure in RR and SP MS. They have demonstrated reduced FA in a number of WM tracts including the inferior longitudinal fasciculus (ILF), capsula interna, and forceps major in MS patients. There were also changes in AD that were consistent with the FA findings such that lower AD was observed in the ILF and capsula interna, as well as in the body of the CC and corona radiata (CR). Increased MD and RD were observed in the ILF, the capsula interna and externa, genu, body, and splenium of the CC, forceps major, and CR. These findings therefore indicate that MS is characterized by both axonal damage and demyelination, although the precise location of the damage varies by tract.

Kern, Sarcona, Montag, Giesser, and Sicotte[9] studied the relationship between WM integrity and motor function in RR MS using whole-brain DTI analysis as well as probabilistic tractography. This study observed 7.1% decrease in FA in the CC, CR, cingulum, and internal capsule, with concurrent 24.95% increase in RD within these regions, thus suggesting demyelination. Other regions with reduced RD included the cortico-spinal tract, right cerebellar peduncle, right external capsule, and left cerebellum. These changes in WM metrics were related to performance of motor tasks. In particular, reduced FA and increased RD in the body of the CC and mid-posterior CR were associated with reduced right-hand performance on the nine-hole peg test (NHPT). Increased RD in cortical WM adjacent to the left motor and right frontal cortices also predicted poor right-hand performance on the NHPT. Furthermore, worse left-hand performance was related to the reduced FA in the body of the CC and a region of occipital WM. These results suggest that at least motor dysfunction observed in MS is differentially affected by WM compromise due to asymmetry. Finally, increased RD at baseline predicted decreased performance on the NHPT[9].

In 2015, Asaf, Evan, and Anat[1] studied a large sample of RR MS participants using whole-brain analysis approach in order to examine temporal timeframe of WM degeneration. This study included participants with MS at different stages of the disease duration: less than 1 year (short duration), 1 year (medium duration) and over 1 year (up to 6 years; long duration). Compared to medium disease duration, long disease duration was characterized by diffuse reduction in FA, especially in the body of the CC, by 22%. In the short disease duration

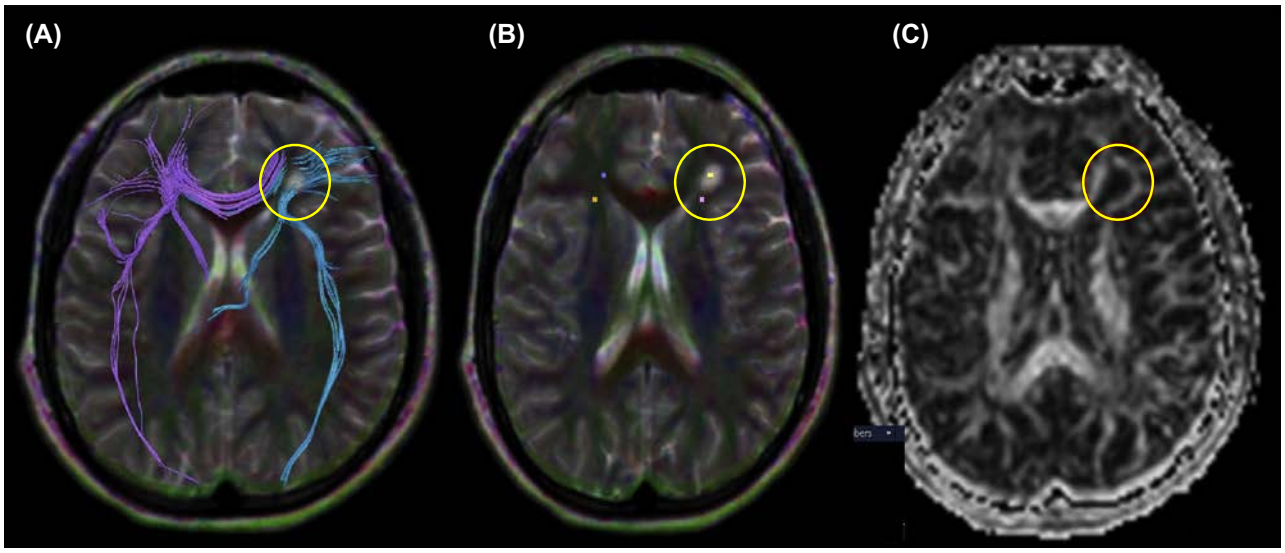

FIGURE 3.4 DTI findings for a plaque located in the left forceps minor region. (A) A tractographic image revealing destruction of white matter fibers at the site of plaque (*circled*). (B) A combination of anatomical color map with T2WI image with ROI markings at the site of plaque (*circled*) and normal appearing white matter. The circled ROI at the site of plaque shows decreased FA and increase ADC indicating increased diffusion of water molecules. (C) The FA map. White matter fibers appear bright except at the site of the plaque which appears dark as the diffusion becomes isotropic (*circled*). *Reprinted with permission from* www.radiopaedia.org*; image courtesy of Dr. Ahmed Abd Rabou.*

group, FA was reduced by 31% compared to healthy controls, especially in the ILF. There was no difference between the short disease duration and medium disease duration. Overall, disease duration negatively correlated with FA. This study provides evidence for a time-dependent WM atrophy that affects different tracts to a variable degree.

Similarly, Sigal, Shmuel, Mark, Gil, and Anat[21] showed an association between disease duration and changes in diffusivity measures. Specifically, this study observed a positive correlation between disease duration and rate of relapse and average diffusivity coefficient (ADC). Moreover, lower FA and increased AD and RD were observed in the MS group compared to healthy controls in the whole CC but not within its subregions. These findings further suggest that WM degeneration is temporally contingent. Taken together, these observations led researchers to explore the association between this trend and corresponding cognitive deterioration.

Relationship Between DTI Measures and Cognitive Profile of MS

Following the initial investigations into the WM changes in MS, researchers became interested in examining the effects that these neural changes have on the cognitive profile associated with this condition. Koenig et al.[10] used probabilistic tractography to investigate the relationship between the WM and cognitive function in RR and SP MS. This study observed reduced FA and increased RD, AD, and MD in the posterior cingulum bundle in the MS group compared to controls. The findings also indicated that episodic memory, as measured by the Brief Visuospatial Memory Test-R (BVMT), was a significant predictor of RD in the posterior cingulum bundle. Moreover, speed of processing, as measured by the Symbol Digit Modalities Test (SDMT), was a strong predictor of RD in the posterior limb of the internal capsule and posterior cingulum bundle. Taken together, these findings indicate that MS is associated with WM abnormalities within tracts that have traditionally been implicated in emotion, attention, and memory. These alterations were, in turn, manifested by memory and attention problems.

Memory problems are frequently observed in MS and have therefore been studied in relation to WM microstructure. Hecke et al.[7] studied working memory in a group of RR MS patients using whole-brain voxel-based morphometry. They observed reduction in FA in the group of MS patients compared to healthy controls in a number of major WM tracts, including the ILF, capsula interna, and forceps major and concurrently reduced AD in the ILF, capsula interna, body of CC, and CR. Additionally, there was an increase in RD and MD in the ILF, capsula interna and externa, genu, body, and splenium of the CC, forceps major, and CR. These diffusion measures were also shown to be related to performance on working memory tasks, such as Paced Auditory Serial Addition Test (PASAT). In particular, there was a significant positive correlation between PASAT and FA in the left ILF, forceps minor, the capsula interna and externa, genu of the CC, left cingulum, superior longitudinal fasciculus (SLF), and CR. This pattern of results was also observed in a study by Syc et al.[26] who used a continuous

tractography method to study the microstructure of the cingulum and fornix. This study observed 19% reduction in FA in a group of RR, SP, and PP MS in the fornix, with a concurrent increase in RD, AD, and MD. There was also an increase in RD, AD, and MD within the left and right cingulum, with no significant changes within FA. In the left cingulum, there was a significant association between the diffusivity measures and performance on the PASAT of information processing and attention, where lower scores on the test were associated with lower FA and higher MD and RD.

Contrary to Syc et al.,[26] using the same tractography method, Ozturk et al.[17] studied microarchitecture of the subregions of the CC in relation to performance on the PASAT in a sample of RR, SP, and PP MS patients. The findings of that study showed reduced FA and increased RD and MD in the whole CC in MS compared to healthy controls. When subregions of the CC were studied individually, a positive correlation was observed between FA and the body and splenium of the CC. This finding not only suggests the involvement of multiple tracts in performance of PASAT but is also indicative of heterogeneous changes within different portions of the CC in this condition. Caligiuri et al.[3] have examined the role of the callosal subregions in cognitive function in MS. They observed an association between FA in the genu and splenium of the CC and cognitive function where cognitive impairment was significantly related to reduction in FA. Since the study by Caligiuri et al.[3] used a compound score to measure cognitive function, it cannot be directly compared to the results of the study by Ozturk et al.[17] who observed change in different subregions of the CC in relation to performance on the PASAT.

Another test that is frequently used to assess cognitive difficulties observed in MS is California Verbal Learning Test (CVLT), a task specifically designed to assess short- and long-term verbal memory. Performance on this assessment has recently been studied in conjunction with WM damage observed in MS. Using tractography, Fink et al.[5] studied microarchitecture of the UF, SLF, cingulum, and fornix and observed that RD within the UF predicted performance on the encoding subscale of the CVLT. Moreover, this study also showed a significant positive correlation between the recognition subscale of the CVLT and PD in the right fornix. These results indicate that in this clinical population, different aspects of verbal memory are differentially affected depending on the specificity of WM damage as assessed by DTI techniques.

Relationship Between DTI Measures and Psychiatric Profile of MS

Apart from the cognitive complaints, emotional problems are also often observed in patients with MS. In particular, depression is one of the most frequently reported psychiatric sequelae. The lifetime prevalence of depression in MS is estimated to be 25–50%.[14] Pujol, Bello, Deus, Marti-Vilalta, and Capdevila[19] studied structural alterations in the frontal and temporal regions in depressed MS patients. Their results showed an association between lesions in the arcuate fasciculus and greater depressive symptoms. These lesions predicted approximately 17% of the variance in depression scores. Feinstein et al.[4] studied NAWM in MS patients. Their results showed greater reduction in FA in the left anterior NAWM in the depressed MS compared to nondepressed MS. Additionally, increased MD was observed in the right inferior frontal lobe.

In a DTI study reported in 2014, Gobbi et al.[6] performed a whole-brain analysis looking at both PP and SP forms of MS. They observed reduced FA in the forceps minor in the depressed subgroup compared to the nondepressed participants. This finding is of a particular significance given that this region of the CC connects parts of the dorso-medial prefrontal cortex (DMPFC) and has been implicated in the pathogenesis of depression.[6] Pujol et al.[19] studied the microstructure of the arcuate fasciculus in patients with MS and showed that lesions within this tract were associated with cognitive expression of mood in these patients. After controlling for cognitive deficits, lesions in the arcuate fasciculus predicted 26% of the variance in Beck Depression Inventory (BDI) scores.[19] Shen et al.[20] used whole-brain analysis to examine the association between WM architecture and the Hamilton Rating Scale for Depression (HAM-D). This study showed a positive association between the scores on HAM-D and FA in a number of WM regions including the right precentral gyrus, cingulate gyrus, and posterior cingulate. This is inconsistent with past research showing decreased WM integrity with increased depressive symptoms. This finding may be attributable to the compensatory mechanisms that have been previously observed.

CONCLUSIONS

MS is a progressive and debilitating disease that affects the myelin sheath of axonal pathways. Traditional clinical imaging, particularly T2-weighted MRI, has revolutionized the ability of researchers and clinicians to diagnose and track disease progression. These types of MRI scans provide clear evidence of the characteristic lesions of MS. Nonetheless, advances in MRI technology, particularly DTI and fiber tractography are providing even greater resolution and understanding of how MS affects specific fiber tracts and may allow an even more precise monitoring of disease progression. While these

newer DTI methods are still primarily investigational, they hold great promise for furthering understanding of MS and its underlying pathology.

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CHAPTER

4

Palmitoylethanolamid and Other Lipid Autacoids Against Neuroinflammation, Pain, and Spasms in Multiple Sclerosis

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OUTLINE

INTRODUCTION

In this book it needs no extensive introduction to make clear that multiple sclerosis (MS) is a chronic neurological disorder based partly, if not totally, on a complex chronic neuroinflammatory pathogenesis. We gathered clinical experience with the anti-neuroinflammatory autacoid palmitoylethanolamide (PEA) since 2010 and also started reviewing all published clinical data at that time.[1] PEA is available since 2005 as a supplement (nutraceutical).[2] As of 2016 there are only two high-quality patent-based formulations developed and available (without prescription needed); one product is developed and produced in Italy (Normast, 300 and 600 mg tablets), and other in the Netherlands (PeaPure, 400 mg capsules). In our Dutch clinic for neuropathic pain we have worked with both formulations in patients suffering from a number of neuroinflammatory disorders, mainly in neuropathic pain, including in patients suffering from MS, and documented our findings in various case-report series.[3,4]

In this chapter, we review the following: (1) the pathogenetic approaches in MS; (2) the principle of natural inhibition of neuroinflammation; (3) the lipid autacoids as natural breaks on pathological inflammation; (4) lipid autacoids in neuroinflammation; and (5) the role of PEA in neuroinflammation, its neuroprotective and neurorestorative effects, and its role to reduce pain and spasms and muscle cramps. We conclude this chapter by describing our current treatment protocol for the treatment of neuropathic pain in MS patients. PEA is the example of the lipid autacoids we focus on most, as the compound had been identified already in 1950s, and a great number of preclinical and clinical studies are available supporting its clinical potential and safety.

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PATHOGENESIS OF MS: DISTURBANCE OF THE INFLAMMATORY BALANCE

Since 2000s, a number of anti-inflammatory drugs are available to either decrease the frequency of clinical exacerbations or delay the increasing intensity of physical disability in relapsing MS. However, the magnitudes of therapeutic effects are moderate and a number of varieties of MS, such as the primary-progressive and progressive-relapsing types, remain difficult to treat.[5] Moreover, side effects of current treatments can very much interfere with quality of life and can also be quite severe and thus reduce compliance or, worse, patients find them unbearable and stop such treatments. Immunotherapy, therefore, has its dark side.[6] Apparently, as of 2016, none of the therapeutic inroads have led to a clear mitigation of the underlying complex and differing pathogenetic and neurodegenerative processes in all classes of MS.[7] It is important to use as a vantage point the fact that the pathogenesis of MS is extremely complicated and intertwined. Sadly though, under the influence of different pharmaceutical companies, focusing on specific isolated pathways related to the mechanism of action of the New Chemical Entity (NCE) they develop, we underestimate the interactions between all these pathways with their many upstream and down-stream targets. One need to go one step further and realize that such a pathogenesis cannot be defined as a discrete number of interacting pathways, but rather as an entire complex network of a higher order than any number of linear pathways. This modern inroad via a systems approach implying polypharmacology to target multiple etiopathogenic pathways is slowly gaining more interest.[8]

However, the clinical evaluation of such polypharmacological and often multimodal treatment approach will be quite difficult, as we are accustomed in our clinical trials to evaluate each intervention one by one. As most isolated interventions will not have high efficacy due to the mono-target approach followed, we are in great need to modify the way we try to solve such issues. Undoubtedly, regulatory bodies such as the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) will also need to work jointly with the pharmaceutical industry to tackle these issues. This is especially relevant for a disorder like MS, where we try to add to the strict pharmacological factors—such as interferons, copaxone, fingolimod, and natalizumab—dietary factors, such as the omega-3 fatty acids, vitamins such as D and B12, and food supplements like PEA. These cofactors might indeed boost the efficacy of the strict pharmacological treatments when administered together as a multifactorial cocktail. For the time being, the solution of the problem will not be one compound focused on one upstream target only, but a multimodal way of interfering with the various and different neuroimmunological pathways.

What seems clear is that at least two main patterns can be recognized in the pathogenesis of MS: an overshoot of pro-inflammatory factors and a reduction of anti-inflammatory factors and inflammation-resolving factors. For instance, many experiments point out that there is an abundance of activated pro-inflammatory cytokines playing a role in the pathogenesis of MS, such as interleukins (1 and 6), interferons, tumor necrosis factor alpha (TNF-α), and macrophage migration inhibitory factor (MIF). Hand in hand with such abundance of pro-inflammatory factors, there is a reduction in activation or concentration of anti-inflammatory and pro-resolving factors or receptors, such as cytokines (IL-10, IL-4), peroxisome proliferator-activated receptors (PPARs) and transforming growth factor-b, and most probably also of the less explored "breaks for inflammation," the N-acylethanolamides (NAEs), endocannabinoids, lipoxins (Lxs), resolvins (Rvs), protectins (Pts), and maresins (Mss).[9,10] All these classes of lipid autacoids have more or less comparable global properties and exert protective effects against damage induced by inflammation in various organs and tissues.[11–15]

The imbalance in pro- and anti-inflammatory effects creates great damage on many different levels, due to a number of activated down-stream targets, leading to the production of free radicals by activated immune competent cells from monocytes up to glial cells in the brain.[16] This all subsequently leads to compartmentalized damage in MS and such compartmentalization further complicates the treatment, as some compartments are quite difficult to reach with conventional drugs and formulations.[17]

INHIBITION OF NEUROINFLAMMATION BY "FOLLOWING WHERE NATURE LEADS"

In 1986, the eminent neuroscientist professor Erminio Costa (1924–2009) delivered a keynote lecture in Washington, and the title of his presentation was *To follow where nature leads*. In this lecture, Costa talked with great vision about how nature itself can become our tutor in developing new therapeutic inroads.[18]

The modern lipid autacoids, such as the Lxs, Rvs, Pts, Mss, as well as the NAEs of which PEA is the prototype, are classes of compounds following nature in their action, and their mechanisms of action have been perfected during millions of years of evolution in animals (Fig. 4.1).[19] The first molecule entering the clinic from this group of lipid autacoids which was developed according to Costa's vision was indeed PEA,

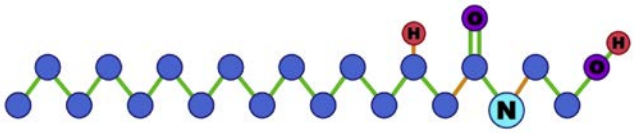

FIGURE 4.1 Molecular structure of palmitoylethanolamide, first determined in 1957.

starting with its clinical development in the early 1990s.[20] It was the famous Nobel laureate professor Rita Levi-Montalcini (1909–2012) who identified the anti-neuroinflammatory activity of PEA and pointed out that such activity would indicate MS as a possible indication for PEA.[21–23] Levi-Montalcini was a close friend of Ermine Costa and both worked together at that time with some pharmaceutical and chemical experts in bringing forward PEA, as this natural compound clearly was the best model for Costa's vision of *to follow where nature leads*. Levi-Montalcini spearheaded a small group of dedicated scientists of a small but highly scientific Italian company, Life Group Spa, led by Dr. F. Della Valle, and conducted the first in vitro and in vivo experiments supporting PEA's central role as a natural break on pathological inflammation.[22,23] In 1993, Levi-Montalcini and coworkers published the first findings related to the mechanism of the anti-inflammatory effects of PEA in the article titled *A proposed autacoid mechanism controlling mastocyte behavior*.[23] In this publication, her group demonstrated that lipid amides of the N-acylethanolamine type (such as PEA) are naturally occurring molecules capable of modulating immune cells such as the mast cells. In 2002, this concept of local anti-inflammatory autacoids was "rediscovered" for the class of lipid autacoids called the resolvins.[24] Levi-Montalcini, however, remains the first scientist to have stipulated this important concept of inflammation inhibiting lipid mediators, and she coined these molecules ALIAmides, from autacoid local inflammation antagonism.[22] The importance of these insights lies in the fact that up to the beginning of this century it was generally thought that once the inflammatory trigger vanishes, inflammatory processes slowly phase out. The work on the modulatory effects of PEA on overactive mast cells formed the base of a new paradigm in inflammation biology. We quote Levi-Montalcini: "Evidence is provided here supporting the existence of a novel autacoid mechanism negatively modulating mast cell behavior in response to noxious stimuli in vivo; hence, the denomination 'autacoid local inflammation antagonism' (ALIA) in particular, as lipid amides of the N-acylethanolamine type have been reported to accumulate in tissues in degenerative inflammatory conditions."[23] The leading idea of Levi-Montalcini was that tissue accumulation of N-acylethanolamines is a biologically significant response during pathological degenerative conditions in order to control such inflammation. Since this millennium, we know that PEA indeed acts as a negative feedback molecule preventing escalation of neuroinflammation via its activation of the nuclear receptor PPAR-α.[25,26] When PEA docks on the PPAR-α, genes which activate inflammatory cascades (NF-kappa-B) leading to the production of TNF-α and other pro-inflammatory cytokines are switched off. When given orally, PEA has almost no clinically relevant side effects nor relevant drug–drug interactions, though it has clear pain and probably some muscle cramp/spasm-reducing properties, as documented in a number of clinical studies and case reports in several pain states and muscle cramps.[1,3,4] Before we go into details related to PEA as a co-treatment modality for MS, we will explore the relevance of the class of lipid autacoids, which in fact are all ALIAmides, in more detail.

LIPID AUTACOIDS OF ALIAMIDES: NATURES BREAK ON PATHOLOGICAL INFLAMMATION

Let us start by giving a definition of autacoids. "Autacoids are locally produced modulating factors, influencing locally the function of cells and/or tissues, which are produced on demand and which subsequently are metabolized in the same cells and/or tissues."[27] There are many classes of lipid autacoids, among which the groups of the NAEs, Lxs, Pts, Rvs, and Mss seem currently most important. The key function of autacoids belonging to these lipid classes is to inhibit overactive and activated immune cascades and thus act like a "stop" signal in inflammation processes otherwise becoming pathological—like a break. Such autacoids are already referred to in literature as *nature's way to resolve inflammation*, clearly supporting the vision of *to follow where nature leads* of Ermine Costa in 1988.[28]

NAEs are derived from membrane phospholipids, N-acylphosphatidylethanolamine (NAPE).[29] PEA is one such NAE. Rvs are metabolites of the polyunsaturated omega-3 fatty acids: eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and docosapentaenoic acid (DPA). The metabolites of EPA are termed E resolvins (RvEs), those of DHA are termed D resolvins (RvDs), and those of DPA are termed resolvins D (RvDsn-3DPA) and resolvins T (RvTs). Pts and Mss are also derived from omega-3 fatty acid DHA. Lxs are synthesized from arachidonic acid (see Fig. 4.2).[30]

The inflammation-resolving Lxs were first isolated by Sherhan et al. in 1984.[31] As reported in 2012, the putative value of many of these classes as therapy for a great number of different and complex inflammatory disorders has been recognized: "Recent studies demonstrate that human and animal cells convert ω-3 polyunsaturated fatty acids into resolvins (Rvs), which are novel,

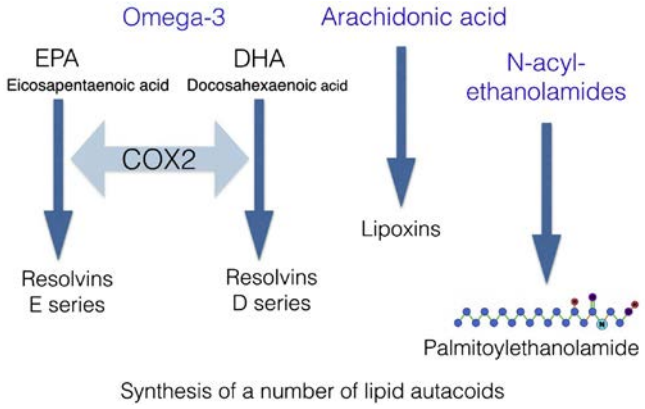

FIGURE 4.2 Synthesis of lipid autacoids.

highly potent, short-lived, anti-inflammatory agents that control the duration and magnitude of inflammation in models of complex diseases."[32] Various forms of these compounds have been tested in a number of different animal models for acute and chronic inflammation, such as asthma, mucoviscidosis, dry eyes, ischemia and reperfusion injury, hyperangiogenesis, conjunctivitis, retinitis, periodontitis, peritonitis, and skin inflammation.[33–35] Moreover, as of 2016, some companies have been developing new formulations of omega-3 fatty acids in order to locally boost the synthesis of, for instance, the Rvs. Such a specific eye formulation has been developed based on a microemulsion of polyunsaturated fatty acids and moisturizing polymers (registered as a medical device). Perhaps certain liposomal omega-3 formulations, creating a retard and reservoir effect, could be developed too, in order to further stimulate endogenous lipid autacoid synthesis. Another possibility is to adapt diet in such a way that its lipid profile enhances lipid autacoid synthesis and, for instance, the efficacy of PEA.

LIPID AUTACOIDS IN CENTRAL NEUROINFLAMMATION

Models for central nervous system (CNS) neuroinflammatory indications supporting the use of the lipid autacoids are among other models of MS, amyotrophic lateral sclerosis, Alzheimer disease (AD), Parkinson's disease, Huntington's disease, depression, and meningitis.[36–40] Importantly, most of the lipid autacoids defined in this chapter are effective in the nanomolar range, and for many the upstream targets have been identified.[41] In a number of models for central neuroinflammation, lipid autacoids proved to have a clear anti-inflammatory effect. This also holds true for the precursors, such as DHA. DHA treatment in an inflammation paradigm could reduce the inflammation-induced activation of microglia, phosphorylation of p38 mitogen-activated protein kinase (MAPK), and reduce the production of pro-inflammatory cytokines, such as TNF-α and interleukin-1β (IL-1β).[42] Alzheimer's is currently seen as a disorder very much related to a central neuroinflammatory pathology. Zhu and colleagues have explored the entorhinal cortex, an area often affected early in AD pathogenesis, and found that the levels of the lipid autacoids, such as maresin 1 (MaR1), protectin D1 (PD1), and resolvin (Rv) D5, were all lower in concentration in the brain regions explored in AD patients as compared to the same brain regions of healthy volunteers, indicating a reduced "break" in the inflammatory axis in AD.[43]

PALMITOYLETHANOLAMIDE AS A NEURORESTORATIVE AND NEUROPROTECTIVE COMPOUND

Central nervous autoimmune pathology and neuropathic pain syndromes induced by lesions to the peripheral or CNS are difficult to treat, and current analgesics lack neuroprotective and neurorestorative effects. PEA as an anti-neuroinflammatory autacoid, however, holds great promise for treating a variety of CNS disorders, including MS and its symptomatology (neuropathic pain), due to its extra neuroprotective and neurorestorative properties.[44] This also holds true of new series of PEA homologs tested in in vitro models of neurodegeneration based on oxidative stress and excitotoxicity. PEA itself can partially prevent toxicity-induced cell death. By chemical modification of the amide bond, derivatives of PEA could be synthesized with improved neuroprotection.[45] There are quite some clinical and preclinical studies supporting PEA's neuroprotective and neurorestorative capacity, and its various upstream and down-stream mechanisms of action are known[38,47–50] (see Fig. 4.3, PEA's anti-inflammatory mechanisms of action).[38,46–50]

The pure PEA compound clearly has sufficient efficacy to measure in animal models as well as in clinical trials.[1] For instance, in a chronic nerve constriction model daily treatment with 30 mg/kg PEA prevented pain threshold, decreased and reduced the presence of edema and macrophage infiltrate in the compressed nerve, and led to a significantly higher myelin sheath and a greater axonal diameter, while a greater number of fibers were conserved. In PPAR-α null mice PEA treatment had no effect (see Fig. 4.3). The PPAR-α activation of PEA thus seems to have both an analgesic effect, as well as a neuroprotective and neurorestorative effect.[51] Such neuroprotective effects of PEA via the PPAR-α receptor have been documented in different paradigms.[52–54] Furthermore, comparable effects were seen in the clinic, where PEA could protect nerves against compression, both in carpal tunnel syndrome, as well as in sciatic compression.[55] In 2011, we conducted a qualitative meta-analysis of all studies in nerve compression syndromes, and conducted a classical Numbers Needed to Treat analysis on the raw

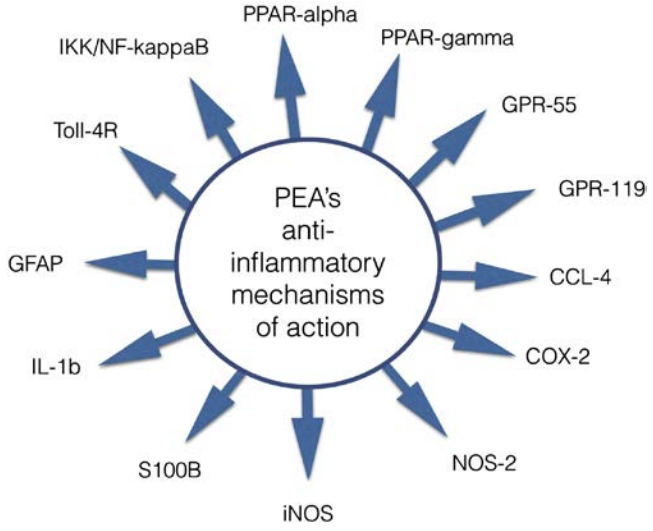

FIGURE 4.3 PEAs upstream (PPAR and NF-kappa-B) and downstream targets.

database of one of the pivotal trials, analyzing PEAs analgesic capacity in a double blind, placebo controlled RCT of 636 patients suffering from sciatic pain. A total of 636 patients were entered, and treated for 3 weeks with either placebo or two different dosages of PEA. High dose was proved significantly better than low dose, which was significantly better than placebo. The calculated NNT for high dose PEA was 1.5.[55]

There are also a number of clinical studies supporting the anti-neuroinflammatory action of PEA, as well as its neuroprotective effects related to other indications. PEA, for instance, inhibits neuroinflammation in the retina in diabetes, in other retinopathies, as well as in glaucoma, and it preserves vision.[56] In our practice we have gathered some special cases, supporting such protective effects, as well as the hypotensive effects of PEA in glaucoma. In a number of cases patients could even reduce topical beta-blockers and other prescribed drugs, and intraocular pressure (IOP) also normalized without these classical therapies.

PALMITOYLETHANOLAMIDE IN MS

PEA has been extensively explored in a great number of inflammatory animal models, as well as in many clinical trials. Already in the 1970s a number of RCTs were published, supporting the efficacy and safety of PEA in the treatment and the prophylaxis of flu and common cold.[57] This led to the registration of PEA for respiratory indications around 1976, under brand names, Impulsin and Palmidrol. The compound has clear analgesic and anti-inflammatory properties, and analgesic effects in chronic pain (neuropathic pain and arthritic pain) resulting in evidence for its safety as well as for its efficacy.[1] The active dose range is 300–2400 mg daily and no dose-limiting side effects or clinically relevant drug–drug interactions are documented. In animal models the compound has been administered orally as well as via infusions; PEA has analgesic and antidepressant properties, it prevents neurotoxicity and neurodegeneration, and inhibits peripheral and central inflammation and mast cell degranulation. It is interesting to note that the first insights into the mechanism of action, as presented by Levi-Montalcini, was already focused on PEA's modulating effect on hyperactive mast cells.[22,58,59] During 2015–2016, these cells were again referred to as an important factor in the pathogenesis of MS.[60,61] PEA even has antimicrobial properties, although not sufficient for a stand-alone therapy.[40,62] As mentioned, the key anti-inflammatory effects of PEA are explained via its high affinity of the PPAR-α.[48–51] Already in the 1980s it was found that PEA accumulates in tissue threatened by damage, for instance, by inflammation or ischemia.[63] This response was at that time already seen as the result of an activation of a protective mechanism, and subsequent studies supported that hypothesis.

In 2000, Baker and colleagues explored the control of spasticity in an MS model [the mouse chronic relapsing experimental allergic encephalomyelitis (CREAE) model] by lipid autacoids, and they selected PEA in addition to various endocannabinoids. At that time, PEA was still wrongly seen as an endocannbinoid. Subsequent studies on its receptor binding profile demonstrated the absence of any affinity, for the CB1 as well as for the CB2 receptor. Baker et al. described a marked increase (~200%) of certain endocannabinoids ($P < 0.01$) and PEA ($P < 0.05$) within the spinal cord of spastic mice (see Fig. 4.3). Upon administration of PEA (10 mg/kg i.v.) spasticity was significantly and maximally inhibited within 10–30 min.[64] In 2008 in an animal model for MS (mice) PEA was shown to reduce immunocompetent cell activation and pro-inflammatory cytokine expression in the CNS.[65] Recently, in a small pilot clinical trial in MS, PEA was demonstrated to have positive effects on the cognitive function and health in the quality-of-life questionnaire, could reduce pain at the injection site of IFN-β1a, and reduced IL-17 and TNF-α serum levels in patients with RR-MS treated with IFN-β1a. This study is the first clinical study to demonstrate the ability of PEA to reduce the pro-inflammatory cytokine profile in RR-MS. Such results should be translated in positive clinical outcome scores in full-powered clinical trials in future.[66] In a second small pilot trial (open) 20 patients suffering from MS and with neuropathic pain were treated with PEA. Of these, 16 patients had a pain score, on the numerical rating scale (NRS), above 3 at base line and 12 patients had a score of 8 or higher. These 12 patients with high pain scores all responded well to treatment with PEA, 6 had pain reductions greater than 75%, and the other 6 had pain reductions between 50% and 25%.[67] Such pain reductions obtained after treatment with PEA have also been seen

in different central neuropathic disorders, such as syringomyelia.[68] Furthermore, MS patients treated with PEA in our clinic sometimes spontaneously report a decrease in number and intensity of spasms. This is in line with other observations, that PEA is able to reduce muscle cramps by a hitherto not identified mechanism. Recently we reported on three patients with such treatment-refractory muscular cramps who indeed responded favorably to treatment with PEA.[69]

RECOMMENDATIONS BASED ON CLINICAL EXPERIENCE

The last paragraph in this chapter is not based on sound scientific foundations, but is practice based and you will find practical recommendations perhaps of use for your patients.

In our clinic for neuropathic pain, we frequently treat patients suffering from MS and neuropathic pain. Based on our experience and the literature reviewed in the preceding sections we have constructed a protocol for the treatment of patients suffering from MS and neuropathic pain. In the beginning of our work in this field, around 2000–2010, we frequently prescribed medical Cannabis. There is a wealth of data supporting efficacy and safety of Cannabis in MS, spasms, and neuropathic pain, not discussed in this chapter. Basically, RCTs provide class 1 and 2 evidence for cannabinoids treating spasticity and pain in MS.[70] We ourselves instruct patients to prepare a drink from flos cannabis, by adding hot boiling water and hot, full-fat milk to a tablespoon of the cannabis flowers and letting it simmer for a while (at least 10 min). They are instructed to start with half a cup and titrate upward. One can store the drink after cooling down in a refrigerator for few days. After 2010, we increasingly substituted "cannabis tea," as we called it, by PEA. This is because many patients are slightly unhappy with the feeling of high on Cannabis. PEA is free of such a side effect. However, there are patients who prefer to use Cannabis together with PEA. Mostly, we see that these patients can reduce the amount of Cannabis and still obtain good analgesia or better control of the spasms. This is a well-known property of PEA; it boosts the efficacy of other analgesics, such as opioids, NSAIDs, and co-analgesics as pregabalin and gabapentin.[1,71] It has even been suggested that one can wean off addicted patients or delay tolerance under the umbrella of PEA.[72] During the period 2010–2012, we mainly worked with the Italian PEA formulation, Normast. Since 2012, we started working with a PEA formulation manufactured in the Netherlands, PeaPure. This is because Normast was officially notified in a special class of compounds in Italy, namely diet food for medical purposes, and the Dutch authorities did not agree with that category pointing out that for them PEA was a supplement. PeaPure was introduced in the Netherlands in 2012 as such a supplement. We always recommend to start with 400 mg capsules three times a day, and double the dose if there is less than 30% improvement on the NRS after 1 month. We advise patients to use this double dose for another 1–2 months, as we found out that a significant number of patients fully respond to PEA only after 4–12 weeks. If after 3 months the response is still not clinically relevant, we advise the patient to stop PEA, or to boost PEA with analgesics such as pregabalin or gabapentin. In our experience we reach relevant pain reductions with much less dose of such analgesics than we do normally. Hand in hand with the PEA regimen we advise the patient to take at least 2000 IU vitamin D, preferably together with a high-dose omega-3 capsule, and use such capsules also three times daily to enhance putative synergies.[73] Since 2015, a new formulation of PEA has become available, based on new production procedure, to enhance the amount of ultrafine PEA crystals in the capsule, together with a low-dose regime of various selected vitamins B, PeaPlex. Such ultrafine particles are suggested to be relevant for PEA's clinical efficacy.[74] Since its introduction we have seen patients responding more favorably to this combination, which contains, as in PeaPure, also 400 mg PEA in each capsule. Omega-3, PeaPlex, and vitamin D together are the "dietary" core of our treatment protocol. On top of this protocol various other compounds can be administered, as PEA does not lead to troublesome interactions.[1] This all leads to the combination between our basic protocol and various analgesics and co-analgesics, including cannabis. In certain cases, we add a special compounded topical cream that we have developed based on 5% baclofen.[75] Some patients claim that this cream relieves some spasticity, and once a nurse, who was the sister of the MS patient in question who used the cream, had claimed that she could transfer her sister from bed to chair and vice versa with much more ease after applying the cream to the legs of her sister.

CONCLUSION

In MS there is a disturbed balance between overactivated inflammatory factors and inflammation-resolving and -inhibiting factors. Much is known about the various inflammatory markers in MS, but much less is known about the putative natural breaks on these overactive inflammation processes. The body can initiate such breaks via a series of lipid inflammation-resolving and -inhibiting endogenous factors. In line with the vision of professors Ermine Costa and Rita Levi-Montalcini during 2000s and more recently attention has been given to

these natural corrective mechanisms. The key molecules able to mitigate inflammatory responses belong to the lipid autacoids. Autacoids are endogenous molecules, produced on demand, and acting directly in tissue where they were produced. There are a number of relevant families of these molecules, the NAEs to which PEA belongs, the Lxs, Rvs, Pts, and Mss. The molecules of all these classes together can be described as ALIAmides, from Autacoid Local Inflammation Antagonism. Omega-3 fatty acids are the precursors for the last three classes. Relevant (high) dosages of these polyunsaturated precursors are supposed to stimulate enhanced synthesis of these specific autacoids. The NAEs are formed from membrane phospholipids. There are no diet precursors know at this time, but PEA itself is available as a food supplement. PEA can be dosed easily and up to 2400 mg/day (for instance, three times two capsules), and troublesome side effects or drug–drug interactions are unknown, while the compound has been evaluated in a number of RCTs, in a population over 5000 patients.[1]

Lipid autacoids might become an important new inroad in treating MS and its symptoms. Boosting endogenous lipid autacoid synthesis with high-dose and high-quality omega-3 formulations seems possible and logical. Synergies with PEA are to be expected and such supplements and dietary factors can easily be combined with more classical pharmacological approaches without fear for negative interactions. Clearly, ALIAmides seem to be compounds that can modify MS via diet and diet supplements.

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CHAPTER

5

Gateway Reflexes Are Stimulated by Neural Activations and Promote the Pathogenesis of Multiple Sclerosis Models

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OUTLINE

INTRODUCTION

Multiple sclerosis (MS) is a common autoimmune disease that affects the central nervous system (CNS). There are about 2.5 million patients with MS around the world, with most people diagnosed between the ages of 20 and 40 years, and a sex ratio of patients of 2:1 (female:male).[1,2] Symptoms of MS are diverse, such as numbness, weakness, visual impairment, dizziness, loss of balance, fatigue, and depression. Based on the course of the disease, patients can be categorized into four groups: (1) relapsing–remitting MS, (2) secondary progressive MS, (3) primary progressive MS, and (4) progressive-relapsing MS.

Researchers have investigated MS treatments by focusing on environmental, genetic, and epigenetic causes. However, the pathogenesis of MS involves a combination of these factors, which is consistent with other autoimmune diseases. Many candidate genes have been identified, and the major histocompatibility complex (MHC) region on chromosome 6p21 has shown significant linkage to the disease.[3] Since mid-2000s, genome-wide association studies (GWAS) have detected genetic variants that confer even a modest risk of the disease. In particular, GWAS identified specific alleles of MHC class II genes, such as HLA DRB1*1501, as risk loci.[4–6] Moreover, many genes involved in helper T cell activation, such as IL-2RA, IL-7RA, TYK2, TNFRSF1A,

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and CD6, were discovered as susceptible loci.[4–7] These findings suggest that MS is an autoreactive helper T cell–mediated disease that leads to the pathogenic degeneration of neurons. Consistently, autoreactive $CD4^+$ T cells are functionally important for the development of experimental autoimmune encephalomyelitis (EAE), an MS model.[10]

One particularly interesting feature of MS is that neural activation induced by stress can regulate the disease status. For instance, neural stimulation caused by heat can trigger transient symptoms. In fact, about 60–80% of MS patients experience MS relapse by immersion in warm water or exposure to fire, an experience known as Uhthoff's phenomenon.[8] Furthermore, psychological stress can induce the activation of autonomic reflexes, which are affected by heat sensitivity. For example, it is known that many MS patients have a lower capacity for sweating, which affects body temperature.[8] Moreover, an association between stressful events, for example, war or the death of a loved one, with the relapse of MS is reported.[9] Thus, there exists evidence suggesting a relationship between neural activations and MS development.

EAE has proven useful to study this neural mechanism. In rodent models, the symptoms of EAE initiate with tail paralysis followed by limb paralysis. To induce EAE, CNS-specific antigens, such as myelin basic protein (MBP), proteolipid protein (PLP), and myelin oligodendrocyte glycoprotein (MOG), are immunized with emulsified adjuvant to the experimental animals. It is also possible to induce EAE symptoms by transferring CNS antigen–specific $CD4^+$ T cells to healthy animals, a method known as adoptive transfer EAE, indicating that autoreactive $CD4^+$ T cells are critical for the disease induction.[11–13]

It is believed that the CNS is an immune privilege site because of the blood–brain barrier (BBB) which restricts the traffic of substances and immune cells to the CNS. However, immune cells can be observed in the CNS of MS patients and EAE mice. In this chapter, we discuss gateways through which immune cells bypass the BBB to reach the CNS and their regulation.

BLOOD–BRAIN BARRIER AND TH17 CELLS

The BBB consists of endothelial cells that express claudins, occludins, and zonula occludens 1(ZO-1) to make a structure of highly connected endothelial cells called the tight junction.[14] Microglial cells, macrophages, pericytes, and astrocytes around the vessels also contribute to the BBB. Disruption of the BBB is associated with chronic neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease and autoimmune diseases in the CNS-like MS.[15] Many studies have revealed that inflammatory cytokines are one reason for BBB disruption. Especially, IL-17A has been reported to disrupt the BBB in vitro and in vivo.[16,17] Indeed, the symptoms of EAE are suppressed significantly in IL-17A-deficient mice.[16] The onset of the disease is delayed, the maximum clinical scores are decreased, and recovery is early in these mutant mice. Moreover, an adoptive transfer EAE model revealed that IL-17A production from type 17 helper $CD4^+$ T (Th17) cells are important for the induction of EAE.[16] Additionally, MOG-specific Th17 cells obtained from IL-17A-deficient mice cannot penetrate the BBB in the EAE model.[11] Consistent with these results, the IL-17A gene is highly expressed in MS lesions.[18] In addition, the levels of IL-17A in the cerebrospinal fluid (CSF) correlate with the extent of the lesion.[19] These results suggest that Th17 cells are critical for the disease onset. In addition to cytokine secretion, the autoantigen specificity of Th17 cells is also important, because ovalbumin-specific Th17 cells do not invade the CNS.[11,20] However, it is reported that the infusion of ovalbumin-loaded antigen-presenting cells into the CSF elicited an accumulation of ovalbumin-specific Th17 cells in the CNS of an adoptive transfer EAE model.[20] Also, antigen presentations in leptomeninges and specific blood vessels inside the CNS are important for the activation and accumulation of Th17 cells in EAE.[13,21] Together, these studies indicate that autoreactive Th17 cells against CNS antigens are critical for EAE development by weakening tight junction molecules to cause neuroinflammation.

INFLAMMATION IN THE CNS AND THE GATEWAY FOR IMMUNE CELLS

In the previous section, we discussed the role of BBB disruption and IL-17A in MS. In this section we discuss where and how immune cells, including autoreactive Th17 cells, reach the CNS. Many MS patients suffer from many prickles and/or optical problems, with various brain regions, including the cerebellum, brain stem, optic nerve, and sensory nerve of the spinal cord, affected,[22] which suggests regions in the CNS are vulnerable to inflammation. This vulnerability may come from chemokine expression that recruits autoreactive T cells. It is known that Th17 cells that express CCR6 are attracted to CCL20. In an EAE model, CCR6-deficient mice are resistant to the disease development, and Th17 cells lacking CCR6 are unable to induce EAE.[11,23] These results suggest the importance of the CCR6–CCL20 axis in neuroinflammation in the EAE system. The choroid plexus epithelium in the brain is shown to express CCL20 and is suggested to be an entry point of pathogenic Th17 cells in an actively immunized EAE model.[23] However, another report found no immune invasion through the choroid

plexus,[21] suggesting alternative gateways for autoreactive immune cells to reach the CNS. Indeed, we found that vessels in the fifth lumbar (L5) cord, but not those in the brain, as the initial entry site of pathogenic Th17 cells in a murine transfer EAE model. Active EAE models utilize complete Freund's adjuvant, which induces systemic inflammation through high serum concentrations of inflammatory cytokines and has many side reactions such as inflammatory pain induction as well as BBB leakage.[24] Therefore, the use of complete Freund's adjuvant causes brain and spinal cords damage, which obfuscates the molecular mechanism driving the immune cell infiltration. To trace the behavior of autoreactive Th17 cells in detail, we prefer an adoptive transfer model to exclude the adjuvant side effects. Using this model, we previously found a gateway where blood-circulating autoreactive Th17 cells are able to pass the BBB at the very initial phase of EAE.[11] In this phase, which is before the clinical manifestations of EAE (day 5 after the $CD4^+$ T cells transfer), Th17 cells accumulated in the L5 cord. This phenomenon is well suited for the pathogenesis of EAE because a typical disease symptom starts from the tail paralysis. Consistently, mRNA levels of CCL20 were highest in the dorsal vessels of L5 cords compared with the other levels of the spinal cord.[11] Surprisingly, the mRNA levels of CCL20 and other chemokines were upregulated in the vessels of L5 cords compared with other levels even in wild-type mice without T cell transfer. These results indicate that the L5 cords have a unique role in attracting immune cells both in healthy and disease conditions.[11]

We have reported a synergistic mechanism involving IL-6, inflammatory chemokines, and growth factor production by an IL-6 and IL-17 signaling pathway in nonimmune cells, such as endothelial cells, epithelial cells, and fibroblasts[25,26] (Fig. 5.1). This mechanism is important for the development of various disease models, including EAE, rheumatoid arthritis, and chronic graft rejection.[25,27–29] We named this synergistic effect as "inflammation amplifier."[26] Activation of the inflammation amplifier in nonimmune cells is driven by simultaneous activation of the transcription factors nuclear factor kappa B (NF-kB) and signal transducer and activator of transcription 3 (STAT3). Inflammatory chemokines, including CCL20, as well as IL-6 and growth factors are target genes of the amplifier in nonimmune cells. Evidence of amplifier activation is also observed in human clinical specimens.[29–31]

In EAE, the expressions of IL-6, chemokines, and growth factors were upregulated in the L5 dorsal vessels, suggesting local activation of the inflammation amplifier. Consistently, the activation of STAT3 was much higher in L5 dorsal vessels compared with the other lumbar cords.[11] On the other hand, the upregulation of chemokines in the L5 dorsal vessels was suppressed in inflammation amplifier-deficient mice such as mice with conditional ablation of the IL-6R subunit gp130 in type 1 collagen + nonimmune cells. These lines of evidence suggest that the creation of the gateway for immune cells requires the inflammation amplifier in specific endothelial cells[11] (Fig. 5.2).

GRAVITY-MEDIATED NEURAL ACTIVATION CREATES A GATEWAY FOR IMMUNE CELLS IN THE L5 CORD

The discovery of a gateway for autoreactive Th17 cells in L5 dorsal vessels at the early phase of EAE led us to investigate the gateway's regulation. It is known that the DRG beside the L5 cord are the largest among those of the lumbar cords, and that these L5 DRG neurons are constantly excited via the soleus muscles by the gravity of the Earth.[32,33] Therefore, we hypothesized that constant stimulation of the soleus muscles would activate sensory nerves, thus activating the inflammation amplifier at the dorsal vessels of the L5 cord to create an immune cell gateway. In order to prove this idea, we

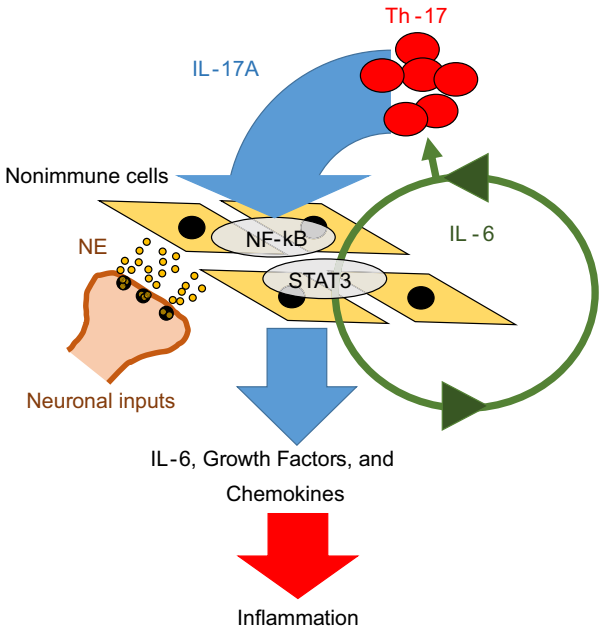

FIGURE 5.1 **The inflammation amplifier.** Synchronous activation of NF-kB and STAT3 in nonimmune cells, such as endothelial cells and fibroblasts, by various cytokines, such as IL-6 and IL-17, arise a synergistic production of IL-6, growth factors, and chemokines, which are important for inflammation induction. IL-6 produced from nonimmune cells can act in an autocrine/paracrine manner, forming a positive feedback loop that enhances STAT3 activation. Neurotransmitters such as norepinephrine (NE) also augment this effect and contribute to the induction of gateway reflexes. Local production of IL-6, growth factors, and chemokines are drastically amplified by this mechanism, causing local inflammation, a phenomenon known as the inflammation amplifier.

suspended the tails of mice so that only the front legs were able to touch the ground, freeing the hind legs of gravity forces. In this experiment, the accumulation of autoreactive Th17 cells was significantly suppressed in the L5 spinal cord, as too were clinical symptoms.[11] Instead of accumulating at the L5 cords, MOG-reactive Th17 cells were concentrated at the cervical cords, most likely because of the stronger stimulation of the forearm muscles by the gravity forces, creating a new immune cell gateway there. Consistently, mRNA levels of CCL20 were significantly reduced at the L5 dorsal venules, and neural activation at the L5 DRG was also suppressed. These results suggest that gravity-mediated neural activation from the soleus muscles plays an important role for the activation of the inflammation amplifier at the L5 dorsal vessels[11] (Fig. 5.2).

We also found evidence that sympathetic nerves regulate the gravity-mediated gateway.[11] In the tail-suspended mice, blood flow speeds were slowed at the L5 dorsal vessels, suggesting the involvement of autonomic nerves including sympathetic neurons. Intriguingly, blood flow speeds in other regions, such as brain surface vessels, femoral vessels, and the portal vein, were unchanged, suggesting the phenomenon was not systemic.[11] Moreover, administration of atenolol, a selective beta 1 adrenergic receptor antagonist or prazosin, a specific alpha 1 adrenergic receptor antagonist, significantly inhibited CCL20 mRNA expression, NF-kB activation, the accumulation of MOG-specific Th17 cells at the L5 dorsal vessels, and repressed EAE clinical scores.[11] Consistent with these results, the addition of norepinephrine, a major neurotransmitter from sympathetic neurons, enhanced chemokines and IL-6 expression in cultured endothelial cells by activating the inflammation amplifier. Thus, regional sensory-sympathetic cross talk triggered by gravitational force established a gateway for immune cells at the L5 dorsal vessels to pass through to the CNS 11 (Fig. 5.2). This interaction between neuronal signal and immune response, which is regulated by the status alteration of the vascular endothelium, is called the "gateway reflex."[34–36] We describe other examples of the gateway reflex in the following sections.

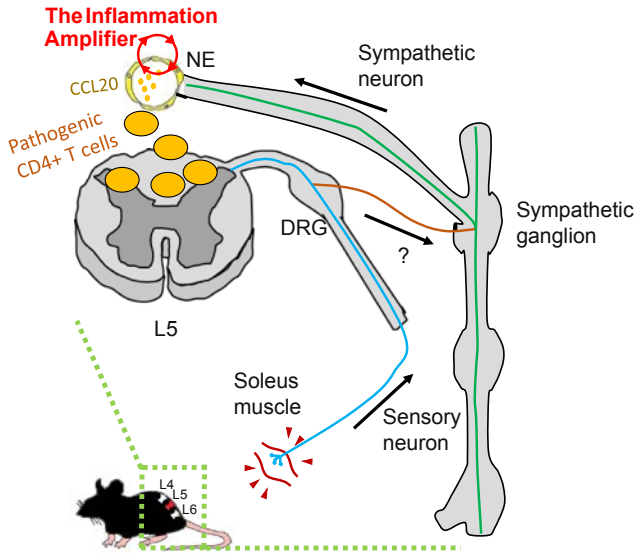

FIGURE 5.2 **Gravity gateway reflex.** Local neural activations by gravity form a gateway for immune cells at the fifth lumbar (L5) cord. Sensory nerves in the soleus muscles, located at the dorsal root ganglion (DRG) of the L5 cord, are constantly activated by gravity-mediated stimulation. The sensory activation leads to the activation of local sympathetic nerves around the dorsal vessels of the L5 cord. The activation of sympathetic nerves releases norepinephrine (NE) at the L5 dorsal vessels, which enhances the inflammation amplifier in the vascular endothelial cells there. The vascular endothelial cells thus produce chemokines such as CCL20, which attract autoreactive Th17 cells.

ELECTRIC STIMULATION-MEDIATED GATEWAY REFLEX

Specific neural activations can be induced artificially in several ways. For example, the stimulation of muscles by weak electric pulses is one method. We hypothesized that such electric stimulation could generate immune cell gateways not only at the L5 dorsal vessels but also at other spinal levels of the venules. Accordingly, we first stimulated the soleus muscles using electric pulses to mimic the effect of gravity forces, showing that chemokine expressions in the L5 dorsal vessels were restored in tail-suspended mice. We then electrically stimulated other muscles. As expected, quadriceps stimulation in mice by electric pulses, which causes priming of the sensory neurons from the L3 DRG, increased chemokine expression in the L3 dorsal vessels. Similarly, weak electric stimulation of the triceps enhanced chemokine expression in the fifth cervical to fifth thoracic cord dorsal vessels. These results indicate another example of the gateway reflex (Fig. 5.3). Importantly, specific neuronal activations by artificial electric stimuli could control the gateway reflex, indicating a potential novel therapeutic strategy against inflammatory diseases in the CNS.

PAIN-MEDIATED GATEWAY REFLEX

Most MS patients experience pain sensation during the disease onset and relapse.[37] Indeed, pain is widely associated with many diseases, but it is commonly regarded as a consequence of the disease and tissue damage. Pain is also known to activate neural responses by nociceptors such as TRPV1 expressed in sensory neurons.[38] We examined whether pain sensation might control the gateway reflex. Adoptive transfer of MOG-specific autoreactive $CD4^+$ T cells induced a transient paralytic symptom that peaked around day 14 after the

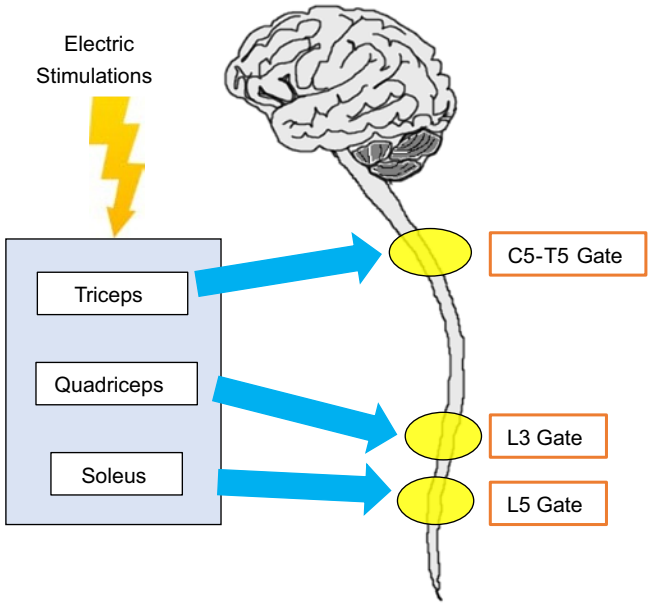

FIGURE 5.3 **Electric gateway reflex.** Electric stimulations can also induce a gateway reflex. Low levels of electric pulses to specific muscles, such as the triceps, quadriceps, and soleus muscles, cause the activation of their respective sensory and sympathetic neurons, followed by the opening of gateways. Triceps stimulation induces chemokines at the dorsal vessels of the fifth cervical (C5) to fifth thoracic (T5) spinal cord. Quadriceps stimulation induces them at the dorsal vessels of the third lumbar cord. Similar to the gravity gateway reflex, electric stimulation in the soleus muscles causes chemokine upregulation at the dorsal vessels of the L5 cord.

transfer and remitted thereafter. Once in the remission phase, the mice did not show any clinical signs of paralysis without treatment. We induced pain sensation in these EAE-recovered mice by ligating the middle branch of the trigeminal nerves because these nerves are known to be composed of sensory nerves alone.[39] Surprisingly, pain induction by the trigeminal nerve ligation induced a relapse of EAE.[13] In addition, pain induction at the time of the autoreactive T cell transfer extended the EAE disease and significantly delayed the remission. The relapse of EAE by pain induction was suppressed by pain relievers such as gabapentin and pregabalin. Pain induced by capsaicin or substance P injection also triggered the relapse of EAE. These results indicate that pain is not simply a consequence of a disease, but it actually modulates the disease condition.[13] Intriguingly, other stress-induced models, such as immobilization stress or forced swimming stress, did not induce EAE relapse even though the serum levels of epinephrine, norepinephrine, and corticosterone were elevated to levels similar to that seen with the trigeminal nerve ligation, indicating that the local nerve circuit activated by the pain sensation triggers the disease relapse rather than systemic mechanisms like hormonal stress responses.[13]

In humans, MOG-specific autoreactive $CD4^+$ T cells infiltrate the CNS from the dorsal vessels of the L5 cords during the onset of EAE due to the gravity-mediated gateway reflex.[11] The transferred $CD4^+$ T cells are then observed at other regions of the CNS, such as the brain and upper levels of the spinal cords. This observation matches the symptoms of the disease in mice, with tail paralysis occurring first and subsequently ascending paralysis followed by a remission phase, that is, EAE-recovered mice. After pain induction in the EAE-recovered mice, the disease relapse starts again from the tail paralysis, indicating that the gateway(s) of the immune cells is once more formed at the L5 cord. Unlike the initial development of the disease, however, the L5 cord of EAE-recovered mice shows different accumulation patterns of immune cells. $CD11b^+$ myeloid cells, which highly express MHC class II, were accumulated around the L5 ventral vessels 13 (Fig. 5.4), not the dorsal ones where autoreactive $CD4^+$ T cells were accumulated by the gravity gateway reflex[11] (Fig. 5.2). These $CD11b^+MHCII^{high}$ cells were derived from peripherally derived activated monocytes, but not microglial cells, and infiltrated the CNS during the first episode of EAE. $CD11b^+MHCII^{high}$ cells then accumulated at the L5 ventral venules in a chemokine CX3CL1-dependent manner after pain induction. Because phosphorylated Creb molecules increased around the ventral vessels and $CD11b^+MHCII^{high}$ cells secreted CX3CL1 after norepinephrine stimulation, we concluded that there exists an auto/paracrine loop of $CD11b^+MHCII^{high}$ cell accumulation that was regulated by the activation of local sympathetic nerves around the L5 ventral vessels. Indeed, we found that the gateway reflex caused by pain sensation induces norepinephrine expression from the sympathetic nerves around the L5 ventral vessels, resulting in the secretion of CX3CL1 from and accumulation of $CD11b^+MHCII^{high}$ cells at specific vessels.[13] Moreover, the $CD11b^+MHCII^{high}$ cells possess autoantigen-presenting ability to activate MOG-specific autoreactive $CD4^+$ T cells, allowing these T cells to secrete various cytokines to induce the inflammation amplifier at the blood vessels. When we depleted the number of $CD11b^+MHCII^{high}$ cells in the CNS, disease relapse was significantly inhibited, indicating the functional importance of these cells during disease development.[13] Depleting autoreactive $CD4^+$ T cells from EAE-recovered mice also suppressed EAE relapse, but $CD11b^+MHCII^{high}$ cells still accumulated around the L5 ventral vessels. These experiments indicated that the accumulation of $CD11b^+MHCII^{high}$ cells around the L5 ventral vessels is an event upstream of the activation of MOG-specific autoreactive $CD4^+$ T cells 13.

Similar to the gravity gateway reflex 11 (Fig. 5.2), chemical sympathectomy in mice inhibited EAE relapse after pain induction, suggesting a communication of sensory and sympathetic neurons[13] (Fig. 5.4). Pain-mediated sensory activation promoted sympathetic

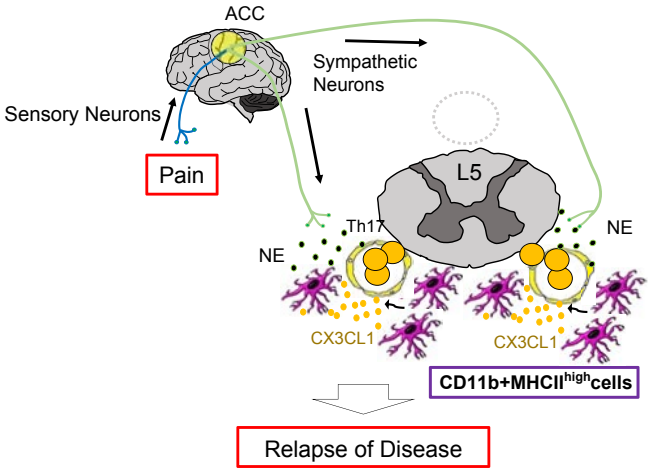

FIGURE 5.4 **Pain gateway reflex.** Pain induction causes the gateway reflex at the ventral vessels of the L5 cord, which triggers the relapse of EAE. The anterior cingulate cortex (ACC) in the brain is the site where the sensory signal changes to a sympathetic one followed by inflammation amplifier activation at the ventral vessels of the L5 cord via norepinephrine expression. Peripherally derived–CD11b$^+$MHCII^{high} cells, which had accumulated in the CNS during the first peak of EAE, express CX3CL1 after norepinephrine stimulation. CX3CL1 attracts more CD11b$^+$MHCII^{high} cells, causing the accumulation of these cells around the L5 ventral vessels. CD11b$^+$MHCII^{high} cells activate autoreactive CD4$^+$ T cells around the vessel to cause the relapse of EAE.

activation via the anterior cingulate cortex (ACC) in the somatosensory area of the brain.[40] Injecting an N-methyl-D-aspartate (NMDA) receptor antagonist at the ACC suppressed the accumulation of CD11b$^+$ MHCII^{high} cells after pain induction, while injection of an NMDA agonist induced their accumulation at the L5 ventral vessels in EAE-recovered mice without pain, suggesting the importance of the ACC for the pain-mediated relapse of EAE[13] (Fig. 5.4). These results represent a third example of the gateway reflex, one mediated by pain-mediated neural activation.

OTHER NEUROIMMUNE REFLEXES

Gateway reflexes promote inflammatory responses in the CNS. On the other hand, Tracey et al. reported that activation of the vagus nerves suppressed systemic inflammation in a murine model for septic shock. They termed this neuroimmune reflex the "inflammatory reflex."[41] Detailed experiments showed that the inflammatory reflex is activated by a microbial component, lipopolysaccharide (LPS), followed by norepinephrine release in the spleen via vagus-mediated splenic nerve activation. The resulting norepinephrine stimulates a subset of CD4$^+$ T cells to produce acetylcholine which acts on activated macrophages to suppress the expression of inflammatory cytokines such as tumor necrosis factor α (TNFα).[41] Thus, this reflex acts to inhibit excessive inflammatory reactions such as septic shock in spleen. It is also reported in mice that electrostimulation by acupuncture at the ST36 Zusanli acupoint, which is located beside the common peroneal and tibial branches of the sciatic nerve, or directly to the sciatic nerve induces vagal activation and dopamine production to inhibit septic shock.[42]

FUTURE DIRECTIONS

It is well recognized that the CNS is an immune privilege organ protected by the BBB. However, gateway reflexes can create a portal of entry for immune cells to penetrate the BBB. In this chapter we described three kinds of gateway reflexes: those induced by gravitational forces, electric stimulation, and pain sensation. These stimulations induce the activation of specific sensory and sympathetic neurons, which changes the status of specific vessels. Elucidating the precise neural pathways of gateway reflexes could offer ways to dampen unwanted inflammation in the CNS as a therapeutic means against neuroinflammatory diseases such as MS.

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CHAPTER

6

Multiple Sclerosis: Food and Lifestyle in a Neurological Autoimmune Disease

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OUTLINE

INTRODUCTION

Multiple sclerosis (MS) is a chronic, inflammatory, demyelinating, and degenerative disease that affects about 2.5 million people around the world. Women are more affected than men in a ratio of 2–3:1. MS is also a leading cause of disability among young adults (20–40 years) as the long-term prevention of deficits remains a challenge.[1] Despite the increasing variety of disease-modifying drugs (DMD) that have emerged during the last years, no curative treatment is available.[2]

Given these initial considerations, it is reasonable to believe that alternative or add-on therapies could be of some benefit. Among those, vitamin D is one of the most studied, and some limited and preliminary data suggest that it could have an impact on disease activity. In vitro studies theorize that vitamin D could halt T helper (TH) cell migration into the central nervous system (CNS) and then avoid the inflammatory process.[3] Unfortunately, many other alternative treatments have shown no significant impact on disease burden.

FOOD AND MS (DIET IN GENERAL)

It is crucial to understand that diet has no confirmed impact on MS treatment. Thus, there is no robust evidence for prescribing a particular type of diet to MS patients. However, it is also recognized that western-style diets, characterized by high salt, red meat, sugar-sweetened drinks, fried food, and low fiber intake, can increase human inflammatory response. This diet probably upregulates the metabolism of human cells toward a proinflammatory status and also leads to a dysbiotic gut microbiota that is related to a more intense immune reactivity as well. On the contrary, a diet based on vegetables,

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fish, and probiotics acts on cellular mechanisms, which downregulates the synthesis of proinflammatory molecules and maintains a healthy gut microbiota.[4]

OMEGA-3 FATTY ACIDS

One of the most significant studies on this theme was the multicenter case–control study, conducted in Australia by Hoare S and colleagues during 2003–2006.[5] They found that there was a significant decrease in clinically isolated syndrome (CIS) risk with higher intake of omega-3 polyunsaturated fats, particularly those originating from fish. On the other hand, the same authors also concluded that there was no evidence to indicate that the intake of other types of dietary fat or fat quantity in the previous 12 months was associated with an altered risk of a CIS.[5]

SALT

It is widely known that high salt conditions have a proinflammatory action. High salt intake aggravates autoimmunity by promoting T cell response in MS animal models. A study reported in 2015, conducted in Germany, showed that at least in theory, mechanisms of salt-mediated modulation of the different cell types could be critically involved in the pathophysiology of MS.[6]

VITAMIN D

Vitamin D receptors can be found in macrophages, dendritic cells, T and B lymphocytes, astrocytes, oligodendrocytes, and neurons. Thus, it is reasonable to believe that vitamin D metabolism plays a role in most of the neuroimmunological diseases, such as MS. Many preclinical data support the use of this vitamin in MS treatment. However, its definitive clinical benefit is yet to be confirmed.[7] It was reported in 2015 that 1,25 (OH) vitamin D is critical for modulating T cell response and inducing antiinflammatory T cells activity.[8] Interestingly, B cells that also take part in the inflammatory response in MS are also partially modulated by vitamin D levels.[9]

From a clinical point of view, there are also many controversies despite some nonrobust evidence of vitamin D supplementation benefits. For example, there is some evidence that a genetically lowered 25 (OH) vitamin D level is associated with increased susceptibility to MS. This conclusion comes from an interesting single nucleotide polymorphism study conducted in Canada with a large cohort. Nevertheless, the authors were not able to conclude that, conversely, vitamin D sufficiency can prevent MS onset.[10]

LIFESTYLE IN GENERAL

Undoubtedly, to comment on lifestyle and its relations with MS is a controversial issue. Firstly, because the patients' lifestyle is directly related to their particular characteristics and the degree of functional impairment. Moreover, to set lifestyle is not always an easy task.[11]

We commonly characterize such expression to the stratification of society through behavioral aspects, usually expressed as consumption patterns, routines, habits, or a way of life adapted day by day—dependent almost exclusively on the habits of patients.[12]

What interests such perspective is what we consider as harmful/suitable for these patients. Another leading question is: Why address this topic? We believe that some social standards of lifestyle are the main behavioral risk factors involved in chronic diseases and severe disabilities such as in neurological diseases. A term currently emerges as of how we must "preserve/manage" the quality of life of patients with multiple sclerosis which involves the words Acceptance; Chronic illness; Coping; Goal regulation; Multiple sclerosis; and Quality of life. One of the strategies would be to educate patients about lifestyle. Therefore, we mention some strategies that we consider crucial to this clientele.[13]

NUTRITION AND OBESITY

MS patients should be advised about the nutritional status. Patients with MS and metabolic syndrome commonly show more disabilities/impairments when compared to groups with body mass index within the normal values. Overweight affects the patterns of walking and countless fundamental and instrumental activities of daily living.[13]

Several authors consider obesity, insulin resistance, and atherosclerosis as precursors to a chronic inflammatory state of low grade. It is known that adipose tissue synthesizes complement proteins, and it is a complement activation target. The terminal complement pathway causes tissue damage associated with hyperglycemia that is characteristic of the main vascular complications of diabetes mellitus and diabetic ketoacidosis. The sum of these problems with a base disease, in this case, MS, affects the control of the disease very much and often the quality of life of this population.[4,14,15]

SMOKING

Both active and passive smoking have been considered risk factors for immune-mediated and inflammatory diseases. It is currently known that either form of smoke exposure is related to the pathogenesis of MS

patients according to the dose and time of use/exposure.[15] From the perspective of public health, the impact of active/passive smoking on the risk of MS is considerable. In this sense, numerous preventive measures to reduce exposure to tobacco smoke are therefore essential. Patients and family members should be guided regarding the risks of smoking.[16]

There is evidence that smoking is also associated with the acceleration of the disease process in MS. In conclusion, MS patients should be advised to quit smoking, not only to minimize the risk of comorbidities but also to avoid the worsening of disabilities/impairments caused by the disease. To sum up, smoking worsens the prognosis of patients with MS.[8,16,17]

PHYSICAL ACTIVITY AND FATIGUE

When guiding patients with MS for performing therapeutic exercises, we commonly describe the line between disuse atrophy/overtraining. Of course, certain physical activities are essential for better management of muscle strength, for balance, for carrying out transfers, and for ambulation. The literature suggests the individualized approach, submaximal and adapted to the particularities of each condition, with reasonable treatment goals and often reanalyzed, to avoid overtraining and muscle fatigue.[18]

We must accept that a reduction in the level/intensity/frequency of therapeutic exercises would supposedly make an activity ineffective; however, too many stimuli in an already weakened system overloads it, further damaging its function. There is no (and of course there may not be any) protocol focused on groups of patients with MS or standardized assessments. This fact applies merely to individuality, motor impairment standard, and associated comorbidities of patients with MS. Programs should be developed based on clinical findings of patients and according to the natural history of the disease addressed.[19,20]

The exchange of knowledge between professionals, the use of support and protection equipment, as well as psychological support should be part of the rehabilitation proposal. The main goal of rehabilitation is to minimize the deficiencies through adjustments; educate the patient and family; prescribe appropriate exercises; prevent complications related to immobility; and eliminate or prevent pain. All these objectives together contribute to a better quality of life for these clients.[19,20]

Fatigue is a very common symptom of MS. Theoretically, fatigue may be related to neuromodulation by soluble products of the autoimmune process or by disruption of central nervous system pathways necessary for sustained activity, but little empirical evidence supports these possibilities. Fatigue is defined as a state with reduced capacity for work following a period of mental or physical activity. In casual use, however, patients often use the term "fatigue" to describe a much broader range of symptoms. We should always guide patients for signs/symptoms of fatigue (central/peripheral) and seek to carry out activities in submaximal limits.[19,21]

ALCOHOL

The deleterious effects of the use of alcohol on the central nervous system (CNS) are well known, although not all have satisfactory explanation. Symptoms can affect any neuraxial level including the brain, peripheral nerves, and muscles. Considering that the use of alcohol can cause myopathy, neuropathy, cerebellar function disorders, and dementia, we imagine such sums to MS patients, which we now call "overlapping."[22]

The use of alcohol, in agreement with previous studies, can be considered as a modifiable risk factor in the development of MS. Still, research suggests inconsistent results. A study by Hedström AK (2014)[23] showed a dose-dependent inverse association between alcohol consumption and the risk of statistically significant MS development in both sexes. An issue that curiously draws our attention is that the harmful effects of smoking were more pronounced among abstemious.

Still, alcohol consumption, according to the conclusion of the aforementioned research study, shows an inverse association, dose-dependent, with MS. Besides, alcohol is associated with the attenuation of the smoking effect. The results may have relevance to clinical practice because they do not give support so as to counsel patients with MS to abstain completely from alcohol.[23]

After two years without new research linking alcohol consumption and MS, a meta-analysis by Zhu T (2015)[24] suggested that there is no evidence that alcohol consumption is associated with an increased risk of developing MS. It may even suggest a potential protective effect of alcohol consumption on the incidence of MS. However, this tendency may not be obvious and should be validated by further research. We believe that the previously mentioned results are carefully assessed. Of course, we encourage MS patients to reduce/cease some alcoholic beverages, since the number of neurological diseases that alcohol might cause is well known. Such diseases, when associated with MS, can considerably worsen the clinical condition of these patients.[18]

COFFEE

The caffeine intake was also not significantly associated with risk of MS in most current literature studies. Consumption of alcoholic beverages, coffee, and fish

was inversely associated with progression of disability in relapsing onset MS, but not in progressive onset MS. These findings allow supporting the hypothesis that different mechanisms might underlie progression of disability in relapsing and progressive onset MS. Unfortunately, more randomized controlled studies are made necessary to characterize the benefits of coffee and drinks composed of caffeine in patients with MS.[25,26]

CANNABINOIDS (Cs)

The research on cannabinoids (Cs) and their effects began to gain legitimacy with the identification of their chemical structure, the possibility of obtaining its individual components, and how they might function in the body. In addition to its active ingredient, delta-9-tetrahydrocannabinol (D9–THC), Cs contain other 65 substances called phytocannabinoids (FCs).[27]

Many studies have focused on *Cannabis sativa* (CS) due to its analgesic potential and its ability to alleviate symptoms related to disorders of the central nervous system. Several randomized controlled studies have shown that Cs can manage spasticity and pain in patients with MS, which currently possess evidence level 1 or 2.[28]

Unfortunately, the doses and frequency are not unique. As cognition and reasoning processing speed can be affected, there is a potential concern with the use of these derivatives. Professionals who have experience with such products still need to evaluate the risk/benefit ratio when considering the use of Cs.[29]

ACUPUNCTURE

The search for integration of body and mind become so intense nowadays, as shown, on a world scale, by the flood of literature on alternative therapies, seeking to provide a better quality of life. The use of acupuncture and other alternative techniques in patients with MS, despite having significant proportion in in the literature, still requires double-blind, randomized controlled studies with more methodological robustness.[30]

CONCLUSION

In summary, notwithstanding the number of emergent therapies, MS continues to be an important cause of disability among young adults. It is reasonable to believe that diet and lifestyle may have an impact on disease progression. Unfortunately, there still is a lack of scientific evidence to recommend interventions based on most of these two aspects of daily living for MS treatment.

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CHAPTER

7

Narrative and the Multiple Sclerosis Body

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OUTLINE

As I wandered about in my living room in the cold Colorado air in the early morning hours of January 10, 2009, I was surprised by the sight of two waning moons outside my window. I closed one eye and still saw two moons. The closure of the other eye also produced the image of two moons. I took this multiplication of moons as a sign that it was time to go back to bed. In the morning, I was assaulted by a chaotic cascade of black and white squares from the morning crossword. I regard this period of double vision as the creation of the story of my dance with multiple sclerosis (MS). I did not know whether this dance would lead to the coming home of body or that through pain and confusion my life would deepen around concepts like gratitude, love, and self-compassion. Finally, I did not know that I would come to understand Frederick Nietzsche's idea that "pain is affirmed as a great teacher" and that "All identity … is implicitly expressed through the body and its relation to trauma and suffering.[1]"

We are in the midst of a dramatic change in perspectives on illness and health that sees the body as more of a living entity where good food, fun, purpose, meaning, community, and love are seen as holding enormous curative elements.[2] Exciting brain research with Tibetan Buddhists have drawn together neurological understandings and ancient Buddhist texts to deepen scientific understanding of the brain and to affirm ancient Buddhist ideas of self.[3] The rift between qualitative social science and quantitative and mechanical perception of the body in science has softened. The Narrative Medicine department at Columbia Medical School is a powerful example of this change.

Narrative medicine understands that illness unfolds in stories and that a competent medical practitioner must not only be trained in the physiology of the body and appropriate treatments, but must also be trained in narrative competence. Rita Charon writes, "If narratives are stories that have a teller, a listener, a time course, a plot, and a point; then narrative knowledge is what we naturally use to make sense of them."[4] Making sense of stories involves an understanding of how time shifts within a story, how each story is singular yet also intertwines with the stories of others, and finally how causality is experienced within the life of the main character. Narrative competence means absorbing, reflecting, and finally being able to act on story appropriately and effectively.[5]

This chapter utilizes metaphor and narrative in relation to the immune system and my particular story of MS. Metaphor and narrative offer a different perspective on autoimmune diseases. Disease is an event that calls us into our bodies and changes our perception of

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everything. Human beings live in story. We live in story to such an extent that in cases of split-brain syndrome, we will invent reasons for our actions that are clearly not accurate.[6] Story provides us with direction and a moral compass. If narrative is the map, then metaphor adds a surprising analogy of depth to the narrative. The first part of this chapter explores the impact of denial and disappearance of the body and MS. The second part explores metaphor and the immune system, and the third part relates the biological concept of mast fruiting to human health and culture.

EXPERIENCE DENIED AND THE DISAPPEARANCE OF THE BODY

One of the causalities of modern society has been awareness of the body. The body and the bodily senses have become an appendage to our rational minds that are to be managed and controlled. The old adage "children are to be seen and not heard" more aptly applies to the human body than to children today. We live in bodies that are to be tolerated and not to be valued. We appear to have lost the acute and consistent awareness of touch, sight, sound, smell, and feeling in everyday life. As philosopher David Abrams writes, "one of the countless signs that our thinking minds have grown estranged from the intelligence of our sensing bodies, is that today a great many people seem to believe that shadows are flat…the actual shadow does not reside primarily on the ground; it is a voluminous being of thickness and depth, a mostly unseen presence that dwells in the air between my body and that ground.[7]"

Pain has become a sign of weakness and therefore shame and guilt. Eva Johansson et al. write, "Part of the problem in pain management is that scientific medicine has reduced the experience of pain to 'an elaborate broadcasting system of signals', rather than seeing it as molded and shaped by the individual and their particular social cultural context.[8]" The medicalization of pain, these authors argue, lead to the mind–body split and to what I would call the ultimate disappearance and denial of the body. The denial and subsequent disappearance of the experience of the body is also gendered. Those of us with a multitude of mysterious symptoms walk through life feeling that we are irritable whiners and complainers that are suggestive of Sigmund Freud's hysterics. Women's bodies in fact were the first to become disappeared, as our bodies actually belonged to men: fathers and husbands in civilized societies and our minds did not really exist at all. Even as women's bodies began to be legally returned to them, the embodied experience disappeared for all of us. The shame we feel is one of the elements that Rita Charon recognizes as dividing us from health care practitioners[9] and I would add that this shame divides us from ourselves.

My first symptoms of MS in my early 20s were cluster headaches and thyroid problems. My doctors' visits would leave me feeling that my health problems were either not real or were vaguely my fault. The technology and knowledge related to autoimmune diseases did not exist in the 80s and headaches have never been easy to diagnose or treat. It is only recently, since early 2010s, that researchers are studying the link between cluster headaches and early signs of MS.[10] I was told that headaches were related to stress and would be given handfuls of narcotics to "break the pain cycle." I felt like a drama queen and a pill popper even as I struggled to understand what was happening to me. My aunts, who have also struggled with undiagnosed autoimmune syndromes, lived under similar suspicions of "it all being in their heads." It is because of my aunts' belief in their stories and mine that my bodily experience never disappeared completely under a shadow of shame and guilt.

My parents met and married in Chile when they served in the Peace Corps in 1963. My twin sister and I were born shortly thereafter in Wyoming. When we were nine my parents took a trip to Chile without us. It was considered too dangerous for us to go as they were returning to Chile after the military coup of Augusto Pinochet and many people were "disappearing." Hereafter, my sister and I grew up with the concept of "The Disappeared" or the idea that people who had been going about their business in El Salvador, Guatemala, Chile, Argentina, Uruguay, Mexico, Peru, and Colombia could one day disappear into prisons, torture chambers, or mass graves. The phenomena of "the disappeared" is not only that loved ones never get to bury their husbands, wives, daughters, and sons but also that it is never recognized that a disappearance has happened at all. The disappeared are buried under layers of silence; and the silence remains. We see this in Germany where Nazism is not discussed; in Ireland where there is very little overt memory of the potato famine; and the experience of the disappeared is the proverbial elephant under the table in many in Latin America. A similar phenomenon exists with the embodied experience of pain and autoimmune symptoms.

The disappearance and denial of pain in the female body begins with adolescence and poor body image in this culture. Studies estimate that currently 80% of women are dissatisfied with the appearance of their bodies[11]. Due to the shame associated with our bodies, the culture does not deal with self-loathing and young women grow up learning to associate with their bodies as little as possible.

I grew up in a rural corner of southwest Colorado. We lived on what was an abandoned homestead that my parents worked to refurbish without "real" electricity or running water. We gardened and farmed with draft horses and traveled extensively throughout Latin

America. Despite this unusual, delightful, and adventuresome childhood that challenged mainstream ideas of what was normal, I grew up like most girls in the United States hating my body. Around 6th grade, I decided I was fat and spent the next decade engaging in various diets that only increased my self-loathing.

In college I learned to quit dieting and eat normally but I did not learn to live in my body. My academic pursuits encouraged me to pursue the "life of the mind" and various health problems including mysterious thyroid issues, hypoglycemia, and numerous long-term cluster headaches diminished my confidence in my body and in the health-care system that was supposed to fix my body. It appeared that the needs of my body and my mind were at cross-purposes. I worked to sharpen and school my mind as much as possible and ignore my body as much as headaches and chronic pain would allow.

The common treatment for issues of low self-esteem like body checking and body avoidance is still based on a 6-step program of self-discipline rather than practices of self-compassion. It was only through working with a chiropractor post MS diagnosis that I was taught to "feel what I feel" and subsequent meditation practices taught me to experience my body even if my body was in pain.

After I started teaching, I developed chronic lung infections that did not respond to antibiotics. I then discovered acupuncture and herbal remedies that cured my infections. This success lead me to aggressively treat sore throats and colds through large amounts of vitamin C, Echinacea, golden seal, and various Chinese herbs. I sought to fix my body through alternative medicines but the goal was the same: to silence my body. At one point, I would develop a sore throat every Friday, which would send me into a spiral of worry and a frantic desire to cure myself through herbs. I wanted to take something so badly that it felt like an addiction. I regarded my body as a barrier to my ability to work and teach and I refused to surrender. It was war. It was only after my MS diagnosis that I began to listen to sore throats. Sometimes today, occasionally, I can even experience the pain of sore throats without moving to action. I also learned through the Rocky Mountain MS society that many immunity boosters are *harmful* to people with MS. As Arthur Frank states, "the body is not mute, but it is inarticulate; it does not use speech, yet it begets it. The speech that the body begets includes illness stories; the problem of hearing these stories is to hear the body speaking them. People certainly talk about their bodies in illness stories; what is harder to hear in the story is the body creating the person.[12]"

To live in a world where we silence our narratives and deny our pain is to live in a world of stress; and we are only beginning to understand the physiological impact of stress. Studies show that stress releases cortisol that triggers the amygdala and causes us to be perpetually stuck in fight, flight, or freeze mode[13]. It appears that this triggered state confuses the immune system and causes many other diseases[14]. We also know that youth who dislike their bodies show elevated levels of cortisol that may be related to the development of autoimmune diseases[15]. Veterans of the wars in Afghanistan and Iraq with posttraumatic stress disorder (PTSD) appear to have an above-average chance of autoimmune disorder[16] and patients of MS are counseled to reduce stress to help prevent future relapses. Yet, the question still haunts us: how do we deal with stress?

Jon Kabat-Zinn's guided meditation on loving kindness was my first experience with meditation. Following the advice of a friend, I used this meditation to deal with chronic insomnia. Jon Kabat-Zinn is a retired professor of medicine and is well known for the Stress Reduction Clinic and The Center for Mindfulness in Medicine, Health Care, and Society at the University Of Massachusetts School Of Medicine. I was surprised to find that I had no idea how to forgive myself or that I never felt safe until I followed Kabat-Zinn's instructions. It was as a result of this daily guided meditation that I began to overcome the anxiety related to insomnia and eventually insomnia itself. This experience taught me a powerful lesson on allowing myself to feel. Yet initially this practice was embarrassing as if I was somehow a new-age dork for practicing loving kindness toward myself every day.

The process of *Truth and Reconciliation* has become a powerful metaphor for learning to hear my pain. *Truth and Reconciliation* has been used to seek healing for some of the world's greatest social evils from the Nazis in Germany to Apartheid in South Africa to genocide in Rwanda and Guatemala. Elements of the process include: "(1) recalling what happened in order to recognize the need for healing (not the need for vengeance); (2) search for acknowledgment that past incidences have led to harm; (3) creating a climate that helps maintain a healing atmosphere for the victim.[17]" For me, compassionate and knowledgeable doctors and nurses validated the story of a litany of symptoms that had been formerly dismissed by the medical system.

In sum, non-Western health modalities have helped me envision other ways of healing and being with this disease. Throughout this process I have been able to work with neurologists to be on the appropriate medication to slow and stop the progression of MS. Understanding MS has not only provided powerful metaphors to allow me to understand my body but it has also helped me understand more about the world sociologically. As Rita Charon writes, "Non narrative knowledge attempts to illuminate the universal by transcending the particular; narrative knowledge, by looking closely at the individual human beings grappling with the conditions of life, attempts to illuminate the universals of the human condition by revealing the particular.[18]"

We are on the verge of allowing metaphor and narrative to exist alongside Western medicine. Stress is both a physiological and a psychological condition that cannot be reduced only to life style. Traditions in meditation, yoga, and so on are shown to have huge curative effects. Yet we need to continue to search for the means and methods to integrate the validation of one's experiences and narrative into mainstream culture. This integration is urgently needed especially for young women facing all kinds of emotional and physical traumas.

IMAGO

Imago is the Latin word for image. In biology it is the word for the perfect complete adult insect after metamorphosis. Imago in psychology, is an unconscious idealized image of someone, perhaps a parent. I use Imago to visualize the physiological processes of the body. Metaphor and narrative allow for contradiction and paradox in ways that the natural laws of science may not. The disease of MS appears paradoxical as the immune system, which is supposed to protect the body, destroys neurons in the brain and spinal cord which give the body movement, thought, speech, and the active part of life. *Narrative chaos*, says Arthur Frank, is that time in life when the map of ourselves and our future is temporarily lost because of illness or disease.[19] The idea of the self-destroying the self is even a more powerful image of disease than most.

The term autoimmunity was not coined until 1957 because it was outside of medicine's imagination to comprehend the idea of the self-attacking the self.[20] After German physician Robert Koch confirmed that a *Bacillus* was responsible for anthrax, microbe hunters were revealing the cause of one disease after another.[21] The idea of microbes as being responsible for diseases was also true for autoimmune diseases. MS was commonly attributed to syphilis in the 15th century and the early part of the 20th century.[22]

Anderson's and MacKay's book *Intolerant Bodies* is part of a Johns Hopkins series on the biography of disease. The perspective of biography goes beyond the scientific understanding of disease to explore the identity of disease itself. Charles Rosenberg in his foreword writes, "The very term biography implies a coherent identity and a narrative, a discernible movement through time."[23] Because autoimmunity is a disease of the self against the self, it calls into question what is the self and how does self-connect with identity. The virologist, MacFarlane Burnet, who made major breakthroughs in understanding the immune system in the 1960s and 1970s, spent time considering the philosophical idea of self. Medicine and philosophy are still comparing notes on the question of the self. Philosopher Evan Thompson points to a deepening understanding of the self by looking at the scientific understanding of cells and ancient Buddhist concepts of self.[3] Science understands the cell as an independent being that practices self-making. The Buddhist idea of self is one of co-becoming through the five aggregates of form (registering), feeling (appraising), cognition (stereotyping), inclination (readying), and consciousness (attending). Thus science is coming closer to understanding components of the body as "sentient selves" whereas Buddhism teaches us that the overall self is less an entity than we commonly believe.[24]

Arthur Frank says, "The body sets in motion the need for new stories when its diseases disrupt the old stories."[25] I am an empath. I can walk into a room and know how everyone is feeling. I am such an empath that if my daughter, husband, or sister says they have a sore throat or a headache, I immediately acquire one. I am now able to remind myself "oh wait, that is not my headache." This empathetic ability is related to MS and is one of the problems I have with who I will term *the fixers*. A common experience of those of us who have received a diagnosis of MS is that *our* diagnosis frees the world to recommend cures for our disease. This advice comes from a loving place but illustrates Arthur Frank's idea that our culture is most comfortable with the "restitutive narratives" around health[26] or health issues that can be fixed. So yes, I have heard of Dr. Paolo Zamboni's vascular treatments of MS. I have watched Terry Wahl's video on the Paleo diet curing her MS. I have also been told of MS sufferers entering sweat lodges with a limp and coming out cured, of patients being cured by naturopaths, and of others curing MS through daily consumption of colostrum. I have been told that autoimmune diseases do not exist and that we have to instead clear pollution from our hearts.

Yet in order to be cured of MS, I would have to change myself. I could no longer be an empath. Moreover, I believe that my empathic nature is not only in my mind but also in my body. I am currently experiencing pain in my arms in the evening and night. The rheumatologist told me that this latest malady is a stress-induced autoimmune response. I tell her that I have not felt stressed but then I tell her of a long list of stressful events in my life including dying relatives, aging parents, daughter with autoimmune issues, and the first flare-up with MS since I was diagnosed. It is not uncommon, I discover, for stress to skip the emotional response and manifest directly in the body. Psychoneuroimmunology is starting to think that the immune system can also affect our psychology, that it is not just a one-way street for our perceptions and our senses to trigger our immune systems. Perhaps our immune system also influences our emotions.

So I am an empath; and empaths have troubles with boundaries. It is only since I have been diagnosed that I have come to realize that not everyone is an empath.

Others have very good boundaries which leads to different health problems. Because I see myself as an empath, I am very grateful to be on disease modifying that help protect my boundaries. I was told that Rebif (interferon beta 1-a) tagged and therefore prevented immune cells from gaining access to my brain. When Rebif began to affect my thyroid, I switched to Aubagio (teriflunomide) and I forgot to ask how it works. Maybe that is why it was ineffective in preventing new lesions so I switched to infusions of Tysabri (natalizumab). I understand that Tysabri helps the blood–brain barrier (BBB) discern against immune cells and specifically targets and destroys the immune cells that are attacking the myelin sheath. I initially hated being on pharmaceuticals and numerous supplements including Vitamin D. It is not how I imagined myself. Now I imagine that these drugs and supplements help me be a healthier empath.

Boundaries

The conversation with my body begins with the BBB.[27] "A border which is too porous is not really a border" I tell my BBB. "A border which is a wall creates isolation, disconnection, and the extinction of the jaguar" my BBB answers. "Ouch" I know that my BBB is referring to the US–Mexico border wall that is fragmenting jaguar habitat. I have read that the endothelial cells are the wallpaper of the BBB and they are the most discerning of all cells. Yet it appears in MS that these endothelial cells actually recruit immune cells to jump the BBB making it more porous than it should be. This feels like empathy to me. It feels like when I get too involved in other people's business and everything seems all squishy and muddled and no one knows whose business is whose. I try to imagine porosity with discernment.

Impulsive Defense

Next I talk to my Natural Killer (NK) cells. These cells are always ready for action and do not need to go through a million bureaucratic hoops to decide who is the enemy. The NK cells are ready for action. I understand the need for impulsive action sometimes. It is the NK cells that attack without thought. When I was septic from the plague, I needed NK cells to protect me while the doctors worked to save me. Yet at the same time, there are moments when impulsiveness is not needed. I am also an activist. Sometimes, unfortunately, I attack without reflection.

Reflective Defense

My conversation with my B cells and helper T cells is perhaps the most complex. As an activist and an empath, I am very good at organizing people, which helps my teaching. I can reflect and then direct action quite well. I understand the role of these immune cells and I do not quite understand what is going wrong. I sense that neither do they. The B cells are the key to the functioning of the immune system. They have to find pathogens and enlist the support of helper T cells. It is only with the "consent" of the T cells that the B cell is activated to destroy pathogens. B and helper T cells appear to both discern and cooperate. These cells have been especially trained for this process. They have had to pass test after test while still in the bone marrow making sure that they attack only the non-self. If B cells attack the self while still in the bone marrow they are reabsorbed. And even after this process, B cells still have to recruit helper T cells; in addition, immune cells appear to have memory that is passed through generations of cells. Therefore, the process of jumping the BBB and attacking the myelin sheath is stored in the memory of these elegant discerning and cooperative cells. They are hurting my brain and my spinal cord. I envision that they feel betrayed by the very medication that makes it impossible for them to attack my brain. It seems that my B cells, helper T cells, and I are not in agreement as to what the ultimate goal is.

The Good-of-the-Whole Regulation

Most mysterious and elegant of the immune cells to me are the regulatory T cells. The regulatory T cells seem to have the function to destroy the self when the self is no longer working for the good of the whole. This function seems miraculous to me and makes me wonder about the world I live in. I do not see any system that looks out for the good of the whole. Some of the biggest mass criminals in the world like Mao, Hitler, and Stalin have implemented brutal systems and killed millions for the "good of the whole." Today institutions like the Catholic Church or political parties seem more interested in the continuation of their institutions then in health of the faith or democracy. I see nothing today that guides us toward a greater good without attachment to survival of ego or institution. It appears we live without regulatory T cells. Regulatory T cells seem to be in short supply in those of us with MS and cancer. The regulatory T cells destroy cancer cells or rogue B cells. Perhaps there is a social and individual urgency to reimagine regulatory T cells.

MS has opened up a world of imagination and metaphor. This disease has allowed me to ponder the existence of myself in ways I never anticipated. Arthur Frank writes that "a wounded story teller is a moral witness, re enchanting a disenchanted world … Illness stories provide glimpses of perfection."[28] Illness provides us a chance to imagine the elegant delicate dance of the immune system and the body. We can also imagine the integration of the body into the human story. And this

story is not finished. I have much more to learn about the immune system and in this chapter, I have not even discussed the narratives emerging from science of fear, flight and fight, and the activities of the amygdala and the hippocampus. I have also not mentioned the miraculous plasticity of the brain and how exercise, balance, and learning a language changes the brain and appears to prevent disabilities.

In sum, Rita Charon writes that for doctors to provide clinical support they need to imaginatively enter the world of the patient.[18] I would add that for patients to be of medical support to our own bodies, we need to enter our own bodies imaginatively. Imago allows us to listen to our bodies.

MAST FRUITING AND CO-BECOMING

Biologist Robin Wall Kimmerer tells the story of her grandfather taking off his pants in order to collect the thousands of pecans that he and his brother had come upon as children. They carried their pants stuffed with pecans home to their mother in Oklahoma's Indian Territory among his tribe of the Potawatomi. The reason for this plethora of nuts is what is now known as the phenomena of mast fruiting among wild pecans. Kimmerer writes,

> If one tree fruits, then all (trees) fruit—there are no soloists. Not one grove, but the whole grove; not one grove in the forest, but every grove; all across the county and all across the state. The trees act not as individuals but somehow as a collective. Exactly how they do this we don't yet know. But what we see is the power of unity. What happens to one happens to us all. We can starve together or feast together. All flourishing is mutual.[29]

A similar phenomenon has been observed in Giant Bamboo which fruits every 135 years and this fruiting will happen simultaneously not just across states but also across oceans and continents.[30]

The language of beginning often fails us. This failure is rooted in the logical need to develop a linear explanation based on the origins of one entity being grounded in a causal chain with that of another. Without this logic we are left with the circular logic of "which came first; the chicken or the egg." Humans are collectively facing the greatest environmental change/crisis that it appears we have ever faced as a species. This crisis implies a paradigmatic change and a dramatic change in our narrative. When old paradigms fail, according to French scholar Michel de Certeau, we often have no words to describe what is happening or what is needed. Just as we have no social concept of regulatory T cells, we also have no imagination for mass fruiting. Kelly Joyce[31] explores the "traffic" between the social sciences and science and medicine. Cultural ideas may inform the focus of medicine or breakthroughs in technology or medical understandings may change the narrative of the culture. It appears that the idea of autoimmunity is both changing the cultural understanding of the body and vice versa. The last portion of this chapter looks at how we can understand the little-understood idea of mast fruiting within the context of the immune system and perhaps look to create new language for understanding human reality.

Evan Thompson explores the ancient Buddhist idea of the self as a process rather than the Cartesian notion of the self as an entity. He writes,

> "In complex systems theory (of the cell), the term used to describe this kind of biochemical self-production is autopoiesis … An autopoietic system is a biochemical system that produces its own molecular components including a boundary that defines what's inside verses what's outside the system … Should something interrupt this self-specifying system, the cellular components will gradually diffuse back into the molecular soup and will no longer form a distinct whole …[32]"

Within this constant activity of co-becoming at the cellular level, scientists have identified a process known as chemotaxis. Bacteria appear to have specialized receptor molecules that will allow them to identify what is harmful and what is beneficial in their environment. "Living," writes Thompson, "is not just sense making; it's sense-making in precarious conditions.[33]"

However, it is not just this process of co-becoming or finding sense that is of importance here; it is also that these processes exist as a glue or a support system which holds the group of cells together. "Remove these processes from the support group they form and they waste away,[33]" writes Thompson.

The process of mast fruiting or a molecular support system is an example of collectivity that the ontological basis of this culture finds difficult to understand. Yet we must embrace the idea that there is something that collectively holds us together and allows us to act as one even when we are many. This mystery applies to our immune system and our collective health.

Some agents of the collective within the immune system and the body appear to be cytokines or interleukins, neuropeptides, and the endocrine system. It appears that cytokines are a protein that are released within the body that can have an impact on the peripheral nervous system. The peripheral nervous system then appears to communicate and perhaps *change* the central nervous system.[34] Immunologists have identified many different cytokines that appear to have an involvement with MS and are also being examined in the fight against cancer.[35] Neuropeptides are chains of amino acids that can activate other neurons in the brain or blood stream and cause changes in behavior or emotions. The endocrine system is also involved in modulating the immune system and communicating between different systems.[36]

These findings are only a beginning of the complexity and communication that happens between the mind and the body. Yet all of this information also causes one to reject the notion that there is somehow a chain of command. Instead, it appears that there is a process of co-becoming or mast fruiting where all these distinct elements appear at once. Can breakthrough in quantum mechanics, string theory, and time relativity relate to the body? Is this all happening at once and if so how does that happen? It is here that we are without language or concepts to understand a fundamental part of living which is that of connectivity.

In 2014 my husband and I walked the northern route of the Camino de Santiago, the 1000-year-old pilgrimage in Northern Spain. Everyone who has walked this now popular pilgrimage will have something to say on the experience of collectivity. I felt as if I were connected with the pilgrims who had walked 1000 years ago. I felt connected with others on the trail, I felt connected with the landscape and the economic troubles of Spain. I was walking in a great collective story. It is this collectivity which the narrative of MS can offer.

We will probably never know what and if there is a mechanism that is unleashing such an astounding number of interactions and becoming in the body, but I cannot help but wonder about the radical proposition of love. Can this unnamed often-quoted force of love be a fundamental force for this process of co-becoming? Does self-love change immune responses and neuropathology? Does love from others create safety and help the BBB discern who should be in and who should be out? Does love for the world we live in change the way we co-become? Does love provide a glue that holds us together and the grease which keeps us moving and turning? Is love the narrative?

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SECTION II

VITAMINS AND MINERALS IN MULTIPLE SCLEROSIS CAUSATION AND THERAPY

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CHAPTER

8

Risk Factors for Low Bone Mineral Density in Multiple Sclerosis

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OUTLINE

INTRODUCTION

Multiple sclerosis (MS) is a persistent inflammatory disease of the brain and the spinal cord. This chronic condition not only affects the central nervous system, but also contributes to a number of comorbidities and secondary conditions. Low bone mineral density (BMD) is considered as one of the secondary conditions among patients with MS.[1] These patients seem to be at higher risk for low BMD when compared with normal populations, and approximately one-third of the patients with MS experience osteoporosis. The most important consequence of low BMD/osteoporosis in this patient group is fracture, which leads to high morbidity and/or mortality.[2–4]

In light of the aforementioned knowledge, in order to prevent such a decrease in BMD and to avoid fractures, it is of paramount importance to be aware of the potential risk factors of low BMD among patients with MS. In this chapter, risk factors for low BMD in patients with MS are reviewed.

BMD (g/cm^2) represents the amount of minerals in grams within a square centimeter of a bone segment. Measurement of BMD per unit area is performed by dual-energy X-ray absorptiometry. Measures of BMD can be interpreted in T-scores or Z-scores by comparing them with two different normative data.[5] T-score is based upon the comparison of an individual's BMD to that of 30-year-old, sex- and ethnicity-matched, healthy subjects. On the other hand, Z-score represents the number of standard deviations that BMD lies away from the normative data belonging to the age-, sex-, and ethnicity-matched healthy subjects.[1,6,7] In men aged ≥50 years and in postmenopausal women, T-scores are used in order to evaluate BMD. Mean BMD of the femoral neck, total hip, or lumbar spine that is 2.5 standard deviations or more below the mean value for reference young adults indicates "osteoporosis," while T-score between −1.0 and −2.5 refers to "osteopenia." However, T-scores are not applied for the evaluation of BMD in men below the age of 50 years or in premenopausal women. Rather, the International Society for Clinical Densitometry

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recommends using Z-scores for these populations. Z-score ≤ −2.0 is defined as "low BMD for chronological age" or "below the expected range of age."[7,8]

Low BMD is a common feature of MS. Decrease in BMD might appear as a direct or an indirect consequence of MS. In other words, MS, itself, might contribute to a decrease in BMD or BMD might be indirectly affected by the disease-related secondary conditions, such as disability, exposure to glucocorticoids, and so on.[9] Additionally, the factors that determine the peak bone mass among the general population, such as heredity and nutrition, might also be applied for the bone loss in MS.[10]

Bone impairment in MS patients appears to be multifactorial, rather than being dependent on a unitary etiology.[11] The risk factors for low BMD in MS will be reviewed under the subtitles including: demographic and lifestyle variables, reduced mobility, hypovitaminosis D, medications, and direct effect of the disease course.

DEMOGRAPHIC AND LIFESTYLE VARIABLES

There are several demographic and lifestyle variables that play a role on the development of osteoporosis in the general population, including increased age, female gender, decreased body mass index, smoking, and alcohol intake.[12] Most of these variables were shown to serve as risk factors for low BMD among patients with MS, as well.[5,13–15]

Age

Increased age is a contributing factor for reduced BMD.[16] Age-related bone loss was confirmed also among patients with MS.[17] Reduction in BMD by age appears both at the femoral and the lumbar sites of the body.[13] Age can contribute to reduced BMD through several mechanisms. The most recognized of these is the age-dependent decrease in sex hormones. The other theoretical explanation might be age-related reduction in mobility. In line with this theory, a significant association between age and Expanded Disability Status Scale (EDSS) score was reported.[13] On the other hand, the more a person gets older, the more the disease duration gets longer. Expectedly, it means that the subject would be exposed to the disease-associated factors that diminish BMD.

Gender

Female sex is known to be a risk factor for osteoporosis in general population. Is that so for patients with MS? The results of the existing studies on this issue seem opposite to this fact. Ayatollahi et al.[13] found no difference between females and males in terms of lumbar and femoral BMD measures among 51 patients with definite relapsing–remitting MS. However, 88% of the study population was female, which is a clear limitation for a comparative analysis between the two genders.[13] Weinstock-Guttman et al.[18] focused on male MS patients and compared their BMD measures to women either with or without MS. The percentage of reduced BMD in males with MS, non-MS females, and women with MS was 80%, 45.1%, and 81.7%, respectively. Male and female patients with MS revealed similar rates for reduced BMD, which are higher than the proportion of low BMD in non-MS women. The authors, therefore, pointed out the increased risk of reduced BMD not only in women but also in men with MS.[18] Cosman et al.[19], in their prospective follow-up study, evaluated the annual BMD loss among MS patients. Regarding the femoral neck BMD, annual-bone loss was similar in men (7.3%) and postmenopausal women (6%) with MS, which is higher than that in premenopausal women (3%) with MS. With regard to the lumbar spine, bone loss per year was higher among women but similar in men, when compared to that in healthy individuals.[19] Taken as a whole, research so far has demonstrated similar bone loss among male and female patients with MS. Further comparative studies with larger sample sizes are needed to elucidate this issue.

Menarche/Menopause, Breastfeeding, and Parity

The evidence in terms of the influence of menarche, parity, and lactation history on bone health among female MS patients is limited.[20] Sioka et al.[20] studied the impact of these variables on BMD in 46 premenopausal ambulatory female MS patients. BMD did not differ between women with and without parity. However, women with menarche age ≥13 years revealed 7–9% lower BMD scores at all measured sites (lumbar spine, total hip, and femoral neck) than those with age at menarche <13 years.[20] This result highlights the protective effect of longer exposure to estrogen from low BMD. According to the same study, history of breastfeeding seemed to adversely affect BMD. Menopause is another component of bone impairment. Time from menopause was shown to be strongly associated with cumulative hazard of fractures in female MS patients.[21] Moreover, urine N-terminal telopeptide of type 1 collagen level increases among MS patients in menopausal transition, who are breastfeeding, and who had a recent delivery.[22]

Body Mass Index

Increased body weight/high body mass index plays a protective role on osteoporosis.[23] Higher body mass index is associated with higher BMD, especially in the

femoral region.[18] The positive correlation between body mass index and BMD might be explained in several ways. Firstly, body weight leads to an increased load on bone tissue, which in turn contributes to acceleration in bone formation. Other than the mechanical impact, there are also hormonal and/or metabolic effects of obesity on bone health.[13,17,18] Fat tissue, where peripheral estrogen production takes place, accounts for the hormonal effect.[17] Metabolic impact of obesity is considered being carried out through adipokines. In line with this consideration, omentin-1, a novel adipokine expressed particularly from visceral adipose tissue, was found to be positively correlated with femoral BMD in MS patients.[24] Even after the adjustment for age, 25-hydroxyvitamin D (25-OH-D) level, and body mass index, the correlation of omentin-1 with BMD still remained. However, no correlation was detected between BMD and vaspin, which is another adipokine.[24] These findings highly indicate the interaction between adipose and bone tissues through omentin-1, among subjects with MS.

Smoking

Smoking is one of the well-known modifiable risk factors related with osteoporosis.[16,25,26] It has both direct and indirect effects over bone cells. Smoking serves its indirect effects via altering the levels of gonadal, calciotropic, or adrenocortical hormones, including estradiol, 25-OH-D, 1,25-OH2-D, cortisol, and dehydroepiandrosterone.[26] On the other hand, smoking was shown to have a role on the other bone-related factors including receptor activator of nuclear factor kappa-B (RANK), RANK ligand (RANKL), and osteoprotegerin (OPG) system.[27,28] Apart from the aforementioned pathways, smoking might be expected to contribute to low BMD by deteriorating the disease course, in an MS patient. Smoking leads to a significant reduction in $CD4^{+}CD25+FoxP3$ regulatory T cells, and thus worsens the prognosis of MS.[29] Nevertheless, current studies on bone health in MS patients have not revealed a direct relationship between smoking and low BMD.[2,18,30–32] Moreover, Moen et al.,[22] in their population-based study, identified that smoking had no impact on urinary N-terminal telopeptide of type 1 collagen level, which is a marker of bone resorption. However, these negative results might be attributed to the relatively small number of smokers included in the studies.[18,22,31] The impact of smoking on bone health among patients with MS needs to be further investigated.

Alcohol Intake

Excessive alcohol intake (3 or more units per day) emerged as a risk factor for low BMD and it took its place among the 12 determinants of WHO fracture risk assessment tool (FRAX).[33,34] However, studies on patients with MS have not shown this kind of relation.[18,31,32] No clear impact of alcohol consumption more often than 1/week on BMD was determined.[31] Excessive alcohol intake was not found to be related with reduced BMD either.[18] Nevertheless, since data regarding the impact of alcohol on bone health among patients with MS is limited, future studies focusing on this subject is necessary in order to clarify this issue.

REDUCED MOBILITY

Weight-bearing physical activity is a must for bone health.[35] Mechanical loading on bones induces bone formation and integrity.[36] On the other hand, reduced mobility, which means decreased mechanical stress on bone, initially leads to bone resorption and in the long-term can lead to the deterioration of bone formation.[9] Patients with MS generally experience a reduction in mobility during the disease course due to various causes. Contributing factors of decreased mobility among patients with MS include impaired muscle strength, loss of balance, and visual deficits. Reduced mobility with relation to any of these causes was identified as a contributing factor for the acceleration of bone loss, particularly at the femoral site, among patients with MS.[13,18,21,37–39] Impairment in mobility was shown to be associated with low bone mass, particularly in the femoral region.[18,38,39] The degree and duration of neurological impairment is of great importance.[9] In most of the studies on MS, researchers used the EDSS developed by Kurtzke[40] in order to quantify the degree of neurological impairment in MS patients. Higher EDSS scores, which represent reduced mobility, were found to be negatively correlated with BMD.[18,19,38,39,41,42] EDSS starts to possess risk for bone loss, when the score is over 3.[43] In addition, EDSS score ≤ 5.5 (defined as ambulation without assistance) emerges as an important determinant in terms of diagnosing normal or low BMD.[18] Plasma type 1 collagen cross-linked C-telopeptide, which is an important predictor of bone resorption, shows an arise among MS patients on low-dose glucocorticoids with EDSS score above 5.5, while no biomarker change is observed among those with good physical condition (EDSS = 1–5.5).[44] Increased EDSS scores are associated with reduced muscle mass,[37] which has emerged as a predictor for BMD.[45] In a study by Ayatollahi et al.,[13] higher EDSS scores were related with lower BMD both at the femoral and the lumbar sites. However, when the patients were categorized into three groups according to their disabilities (mild, moderate, and severe disability levels were represented by EDSS score of 0–3.5, 4–5.5, and 6–8, respectively), BMD values did not differ among groups.[13]

Past history regarding physical activity is also of importance in terms of bone health in an MS patient.[32] In their population-based study, Steffensen et al.[32] examined the relation of bone mass with past and current exposures to physical activity among 80 fully ambulatory subjects with MS. Walking distance and possibly physical activity during growing up were related with age-, body mass index-, and sex-adjusted BMD values at the lumbar site.[32]

Contrary to the previously mentioned findings, a number of studies showed that the reduction in BMD started while the patients were ambulatory.[31,32,46] Moen et al.[31] reported that low BMD is prevalent among patients who were newly diagnosed with MS or clinically isolated syndrome, although the median value for their EDSS score was 1.0.[31] This finding confirms the judgment that there are other determinants, apart from reduced mobility, which contribute to the early onset of bone loss in patients with MS.[47]

HYPOVITAMINOSIS D

Vitamin D is a key determinant for bone metabolism. It regulates the intestinal calcium absorption, thus the level of parathyroid hormone, which is an important component of calcium hemostasis.[19] With its anti-inflammatory features, vitamin D also seems to have an impact on the disease course in patients with MS. Higher 25-OH-D level was detected to be an independent factor that decreases the risk of demyelinating events[48] and is also related with lower relapse risk.[49] An increase of each 10 nmol/l in 25-OH-D reduces the risk of relapse by 12%.[49] In accordance with the consideration that vitamin D regulates clinical disease activity, 25-OH-D levels tend to decrease during relapses than those in the remission period.[50,51]

Vitamin D deficiency/insufficiency is frequently seen among patients with MS,[39,42,52] due to several reasons including sun avoidance, low dietary intake, disability-associated limitation in outdoor activities, and exposure to glucocorticoids.[39] Hypovitaminosis D not only serves as a risk factor for central nervous system demyelination, but also is associated with the EDSS score of the patient, in other words with the neurological impairment and disability.[53] Patients with EDSS score above 3 revealed significantly lower levels of 25-OH-D, when compared to healthy controls. However, this kind of relation was lacking in MS patients with lower disabilities.[53] This paramount impact of hypovitaminosis D on the disease course serves as an additional risk factor for low BMD among MS patients with hypovitaminosis D.

A number of studies so far showed a relationship between hypovitaminosis D and low BMD in patients with MS.[52,54] However, some revealed no association between serum 25-OH-D levels and BMD scores.[32,39,55]

Although the results of the previous studies are contentious, as an important factor for demyelination, relapse risk, and bone health, hypovitaminosis D deserves serious attention among patients with MS.

MEDICATIONS

Glucocorticoids

Long-term oral glucocorticoid therapy is a risk factor for bone impairment[56] and fractures.[57] On the other hand, the effect of repeated high-dose glucocorticoid pulses is conflicting.[9] Short-term steroid treatment leads to a decrement in bone formation, along with an acceleration in bone resorption.[58] High dose of intravenous methylprednisolone for 10 days was shown to cause a rapid decrease in serum osteocalcin and aminoterminal propeptide of type 1 collagen levels on the second day of therapy. In addition, urinary calcium/creatinin ratio[59] and carboxyterminal telopeptide of type 1 collagen level show progressive increase, following the therapy.[58] Although these rapid alterations occur in bone metabolism, BMD appears not to be adversely affected by high-dose, short-term glucocorticoid infusion.[58] These findings highlight the transient effect of glucocorticoid regimen, followed by a reparative phase after discontinuation of the treatment.[17,58,59]

Several mechanisms lie behind glucocorticoid-induced bone loss. A combination of changes including reduction in intestinal calcium absorption, increase in renal calcium excretion, and secondary hyperparathyroidism may be involved in glucocorticoids' systemic actions.[14,37,58] However, direct effect of glucocorticoids on bone cells seems more prominent. The steroids increase the apoptosis of osteoblasts, while decreasing their differentiation[37], which can be documented by reduced concentration of osteocalcin and propeptides of type 1 procollagen.[44] On the other hand, rapid bone impairment following short-term intravenous glucocorticoid therapy is attributed to the extension of octeoclasts' lifespan.[60] Glucocorticoids also lead to a duration-dependent depletion in fat-free mass, and thus to the catabolism of the skeletal muscle.[37]

Overall, there is limited evidence that glucocorticoids lead to low BMD[21,55] or fractures in patients with MS.[61] Most of the studies in MS patients have shown no deleterious effect of glucocorticoid pulses or cumulative corticosteroid dose on BMD.[13,17,30,32,38,52,54,62,63] Zorzon and colleagues[30] evaluated not only the impact of high-dose glucocorticoid therapy at relapses, but also the long-term effect of continuous pulse administration of methylprednisolone on bone loss. Although the

frequency of osteopenia differed between MS patients and controls, neither the regular pulsed treatment nor the high-dose methylprednisolone administration during relapses had any impact on lumbar and femoral BMD.[30] Huang et al.[43], in their meta-analysis, reported that steroid therapy was associated with an increased risk of bone loss when the total dose exceeds 15 gr. In terms of fragility, a number of researchers reported that neither the cumulative steroid dose nor the short-term methylprednisolone therapy during the previous year serve as confounders for fractures among MS patients.[4,21]

At last, contrary to the continuous regimen, intermittent corticosteroid therapy seems to have no/less adverse effect on BMD.[15] The lack of association between steroid therapy and BMD might be attributed to glucocorticoids' therapeutic effects on the inflammatory process of the disease, as well as on the patients' ambulatory status.[39,54]

Antidepressants and Anticonvulsants

Due to the increased risk of developing epilepsy in MS,[64] anticonvulsant use among patients is a common issue. Similarly, psychiatric comorbidities, thus the use of antidepressants are also frequent in this population.[65] The widespread use of these medications might put MS patients at risk for low BMD.[11]

Antidepressants, particularly those with a high affinity for 5-hydroxytriptamine reuptake transporter (5-HTT), such as selective seratonin reuptake inhibitors (SSRIs), have the potential to alter bone metabolism.[66,67] Exposure to antidepressants during the previous 6 months was shown to increase fracture risk almost twofold in patients with MS.[4] This finding is attributed to the impact of antidepressant drugs on fall frequency,[68] along with their effect on BMD through serotonin, which has an important role in bone metabolism.[69,70]

There is a recognized relationship between reduced BMD and anticonvulsant medications, as well.[71,72] The enzyme-inducing drugs, such as phenytoin, sodium valproate, carbamazepine, and phenobarbital, constitute higher risk for bone impairment than the noninducing anticonvulsants.[73] The antiepileptic drugs have also the potential to increase fracture risk in a duration-dependent manner.[74] Different mechanisms were identified regarding the impact of anticonvulsant use on BMD. The most recognized theory is the indirect effect of antiepileptic drugs over vitamin D level. They might perform their indirect effects by inducing cytochrome P450 enzyme system.[73] However, a study on MS patients showed no difference in 25-OH-D level between users and nonusers of anticonvulsants.[24] The anticonvulsants have also the potential to accelerate bone turnover through a direct influence on bone cells.[74,75] Long-term phenytoin exposure was shown to reduce BMD in the tibia of growing rats, along with a decrease in osteocalcin level.[76] Nevertheless, the same effect was not seen in rats taking daily 30 mg/kg menatetrenone, a vitamin K analogue. This finding indicates that phenytoin inhibits bone formation concomitant with reduced vitamin K levels.[76] On the other hand, carbamazepine was determined to increase sex hormone–binding globulin concentrations both in men and women.[77] Other suggested mechanisms regarding the anticonvulsant-associated bone impairment are homocysteine and leptin-dependent pathways.[73] Increased homocysteine levels, along with decreased serum folate were detected in patients treated with phenytoin, phenobarbital, and primidone.[78] This reflects the role of inducer antiepileptics on homocysteine metabolism.[78]

Despite the previously described findings and the conclusions regarding the anticonvulsant- and antidepressant-induced bone loss, research evaluating the impact of these drugs on BMD among patients with MS needs extension.

Interferon

The effect of interferon therapy on BMD is another controversial issue in MS. Interferon treatment was suggested to have a protective effect from bone loss through the inhibition of osteoclastogenesis. Interferon-beta was shown to operate by interfering with RANKL-induced expression of c-fos, which is of importance in terms of osteoclast proliferation and formation.[79] Shuhaibar et al.[80] studied the impact of immunomodulatory therapy on BMD in 37 MS patients. Although 80% of the study group received intermittent corticosteroid treatment along with the immunomodulatory therapy (interferon-beta-1a, interferon-beta-1b, or glatiramer), mean Z-scores at the lumbar spine and the femoral neck were 0.53 and 0.72, respectively, which are both greater than zero.[80] Weinstock-Guttman et al.[81] found that bone homeostasis–mediating markers such as RANKL and osteoprotegerin were affected by the intramuscular injection of 30 μg interferon-beta-1a, among relapsing–remitting MS patients. Therefore, they speculate that BMD might also be modulated by interferon-beta treatment.[81]

Contrary to these findings, some studies in MS patients showed no correlation between BMD and being on interferon treatment.[13,32,82] Altintaş et al.[62] reported that neither BMD nor osteopontin levels were differed by being on interferon treatment. Furthermore, Pérez Castrillón et al.[83] found decreased BMD scores in male patients treated with interferon than those who had not received interferon therapy. Among women, BMD results were similar in patients who received interferon and those who were treated with corticosteroids.[83]

DIRECT EFFECT OF THE DISEASE COURSE

MS, the demyelinating disease of the central nervous system, develops with continued inflammation. MS and osteoporosis share a common immunopathogenic background in some way through T cells and similar proinflammatory cytokines. There are a number of molecules that play a role both in the pathogenesis of MS and in the development of osteoporosis, including IL-1, IL-6, IL-11, tumor necrosis factor-alpha, osteopontin, and RANKL.[31,38,84] Shared cytokines are not only involved in the disease course of MS, but also constitute risk for low BMD. Proinflammatory cytokines cause an imbalance in osteoclastogenesis, with a dominancy of activation over inhibition, thus leading to bone resorption in patients with MS.[36] Higher frequency of low BMD among ambulatory MS patients also highlights the role of disease course itself. Terzi et al.[54] studied ambulatory premenopausal female patients with relapsing–remitting MS. Since the study sample was homogenous in terms of ambulatory status, the BMD changes were attributed to the inflammatory disease course.[54]

On the other hand, there is evidence that central nervous system plays a potential role on bone hemostasis via bone-regulating mediators, such as leptin, serotonin, neuromedin U, and neuropeptide Y.[84–86] Binding its receptor in hypothalamus, leptin regulates bone remodeling/metabolism through sympathetic nervous system.[86,87] Neuromedin U modulates this interaction of leptin and sympathetic system with bone cells.[84] Supporting the view that central nervous system operates bone metabolism, Batista et al.[85] found a significant relation between cognitive impairment and bone loss at the femoral site in patients with MS. The authors commented that neuroinflammatory process, which contributes to cognitive impairment, is also a trigger for bone-regulating mechanisms.[85]

In part due to the previously mentioned mechanisms, longer disease duration was shown to be significantly associated with lower BMD scores in MS.[13,38,41] In a meta-analysis by Huang et al.[43], BMD on the femoral and lumbar sites was determined to reduce when the disease duration exceeded 7 years. Longer disease duration means higher exposure to both MS-related inflammatory changes and accompanying disabilities, which causes much more bone loss.

Along with the disease duration, number of attacks is also associated with BMD.[41,54] Therefore, it can be speculated that MS type might have a role on the deterioration of bone health. Supporting this finding, secondary progressive MS patients tend to have more bone loss than their relapsing–remitting counterparts.[9] This finding, in some extent, can be attributed to the data that patients with progressive MS forms are more prone to reduced mobility.[54] Tüzün et al.[38] also reported lower BMD values at all sites (lumbar, femoral neck, and total femur) among secondary progressive MS patients when compared to the subjects with relapsing–remitting MS. However, as their mean disease duration is longer than that of patients with relapsing–remitting MS, this finding cannot be ascribed to disease course alone.

At last, low BMD among patients with MS is multifactorial in nature. Apart from the common risk factors for osteoporosis in the general population, there are also disease-related factors that contribute to reduced BMD in MS. Awareness regarding these factors and BMD screening are of importance in order to tailor preventative measures for bone loss and fractures.

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CHAPTER

9

Role of Vitamin D in Multiple Sclerosis Pathogenesis and Therapy

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OUTLINE

INTRODUCTION

Multiple sclerosis (MS) is a progressive and poorly treatable inflammatory demyelinating disease of the central nervous system. It is a clinically heterogeneous condition with a complex etiology involving both environmental and genetic factors. Possible environmental factors contributing to MS pathogenesis include Epstein–Barr (EB) virus infection, tobacco smoke, and vaccinations.[1] Epidemiological studies suggest increased MS prevalence with higher latitude. Sun exposure, particularly to ultraviolet (UV) light, is necessary for vitamin D production, and exposure levels are usually lower at higher latitudes. The association between latitude and MS prevalence supports the vitamin D hypothesis of MS, which posits that lower vitamin D levels are a major risk factor for MS. Indeed, many studies have demonstrated significantly lower serum vitamin D levels in MS patients than in healthy controls,[2–5] and a large prospective study found an association between high circulating levels of vitamin D and lower MS risk.[6]

Vitamin D has essential and well-described functions in bone formation and mineral homeostasis. Physiological levels of vitamin D enhance osteoclast differentiation and ensuing calcium resorption from bone, absorption of calcium in the small intestine, renal calcium reabsorption, and mineralization of the bone collagen matrix.[7] Vitamin D has numerous additional biological functions, including modulation of cell proliferation, differentiation, and apoptosis, as well as various signaling functions in the nervous and immune systems.[8] The immunomodulatory functions of vitamin D in particular have attracted attention for possible involvement in immune-related diseases. For instance, vitamin D and its analogues suppress experimental autoimmune encephalomyelitis (EAE), an animal model of MS (reviewed in Ref. 9).

This chapter briefly reviews the metabolism and immunological functions of vitamin D and discusses possible contributions of vitamin D to MS pathogenesis and therapy.

METABOLISM OF VITAMIN D

Most vitamin D in the human body (between 80% and 90% of basic requirements) is derived from skin exposure to sunlight,[10] whereas a smaller fraction is derived from

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food such as fish and milk, fortified edibles, and vitamin supplements. These additional sources can compensate for vitamin D insufficiency. Skin exposure to UV-B (280–315 nm), which is influenced by season, latitude, and sunblock, converts 7-dehydrocholesterol to previtamin D (Fig. 9.1). Previtamin D isomerizes to vitamin D, which is biologically inactive, but is rapidly converted to 25-hydroxyvitamin D (25(OH)D) by 25-hydroxylase. Most potential 25-hydroxylases are expressed primarily in the liver, and all are members of the cytochrome P450 family (CYP2C11, CYP2D25, CYP27A1, CYP3A4, CYP2R1, and CYP2J2/3).[11] Subsequently, 25(OH)D is hydroxylated to the more bioactive form 1,25(OH)2D by 1-α-hydroxylase encoded by *CYP27B1* on chromosome 12q13.1-3. The proximal tubule of the kidney expresses high levels of 1-α-hydroxylase and, thus, is a major site for synthesis of circulating 1,25(OH)2D. However, other tissues also express 1-α-hydroxylase, including the central nervous system, prostate, and colon.[10,12] In addition, immune cells such as $CD4^+$ T cells, macrophages, and dendritic cells (DCs) can also generate 1,25(OH)2D, which may act as a local autocrine or paracrine signaling factor.[13]

Although 1,25(OH)2D is the most active form among vitamin D metabolites, 25(OH)D is considered a better marker for estimating vitamin D status because its serum half-life is relatively long (about 3 weeks) compared to 1,25(OH)2D (only 4–6 h). The 25-hydroxylation step is unregulated, and thus, the rate increases with substrate availability.[14] Under physiological conditions, however, serum 25(OH)D concentrations are a 1000-fold higher than serum 1,25(OH)2D concentrations[15] because 1,25(OH)2D production is regulated by a negative feedback loop involving both 1,25(OH)2D-mediated inhibition of 1-α-hydroxylase and stimulation of the 24-hydroxylase encoded by *CYP24A*, which converts 1,25(OH)2D to the inactive form 1,24,25(OH)3D. This mechanism allows for the tight control of circulating 1,25(OH)2D levels and prevents excessive vitamin D activity and signaling.

The majority of circulating 25(OH)D and 1,25(OH)2D is tightly bound to vitamin D–binding protein (DBP). It is known that DBP level and genotype affect 25(OH)D levels[16,17] and 1,25(OH)2D bioavailability.[18] After entering a target cell, 1,25(OH)2D dissociates from DBP and diffuses across the nuclear membrane. Most of the known biological effects of 1,25(OH)2D require the vitamin D receptor (VDR), a member of the superfamily of nuclear hormone receptors. The 1,25(OH)2D–VDR complex forms a heterodimer with the retinoid-X receptor (RXR) (Fig. 9.1). This 1,25(OH)2D–VDR–RXR complex binds to vitamin D response elements (VDREs) in target genes, thereby influencing transcription. Among these target genes is that encoding VDR; thus, vitamin D autoregulates transcription modulation via VDR.[19] By altering transcription, 1,25(OH)2D regulates various physiological processes directly and indirectly through cross talk between signaling cascades.[8]

Both environmental and genetic factors contribute to 1,25(OH)2D status. Important environmental factors include UVB, dietary intake, age, infection, airborne pollution, and cigarette smoke.[19,20] Among genetic factors, melanin pigmentation of the skin is one of the most important influences on vitamin D levels.[20] Melanin absorbs UV-B light, thereby inhibiting the generation of previtamin D from 7-dehydrocholesterol, and individuals with more darkly pigmented skin are at a higher risk of vitamin D deficiency.[21] On the contrary, the lower melanin content of lighter skin allows for more UV-B penetration and greater vitamin D production.[19] In fact, serum 25(OH)D levels in blacks are substantially lower than in Caucasians,[6] and serum 25(OH)D levels are significantly lower in Hispanics with MS than in non-Hispanic whites with MS.[22] Genome-wide association studies (GWASs) have also identified polymorphisms in or near genes involved in cholesterol synthesis (*DHCR7*), 25(OH)D and 1,25(OH)2D hydroxylation (*CYP2R1* and *CYP24A1*, respectively), and vitamin D transport (*GC*) that are significantly associated with circulating 25(OH)D levels.[16,19,23] Alternatively, there is little evidence for an association between *VDR* polymorphisms and 25(OH)D levels.

IMMUNOLOGICAL FUNCTIONS OF VITAMIN D AND EFFECTS ON EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

Immunological functions of vitamin D have been extensively investigated in vivo and in vitro. Several immune cell types—including T cells, B cells, monocytes, and macrophages—express VDR, allowing vitamin D to regulate the transcription of target genes reviewed in Ref. 24 and exert multiple immunomodulatory effects on both the innate and adaptive immune systems. For instance, 1,25(OH)2D repressed the production of interferon gamma (IFNγ), interleukin (IL)-2, and IL-6[25] and increased the production of IL-4, IL-5, and IL-10.[25–28] Further, 1,25(OH)2D decreased the production of IL-17, IL-21, and IL-23—cytokines related to T-helper (Th)17, a T cell type that may be a major contributor to MS pathogenesis.[7,25,27] Further, 1,25(OH)2D can increase $CD4^+CD25^+FoxP3^+$ regulatory T (Treg) cells.[28] These effects on cytokine production and T cell profile promote the switch from the more inflammatory Th1/Th17 response to the less inflammatory Th2/Treg response,[15] which could potentially improve immunological conditions and suppress MS progression. Further, 1,25(OH)2D has been shown to modulate humoral immunity by

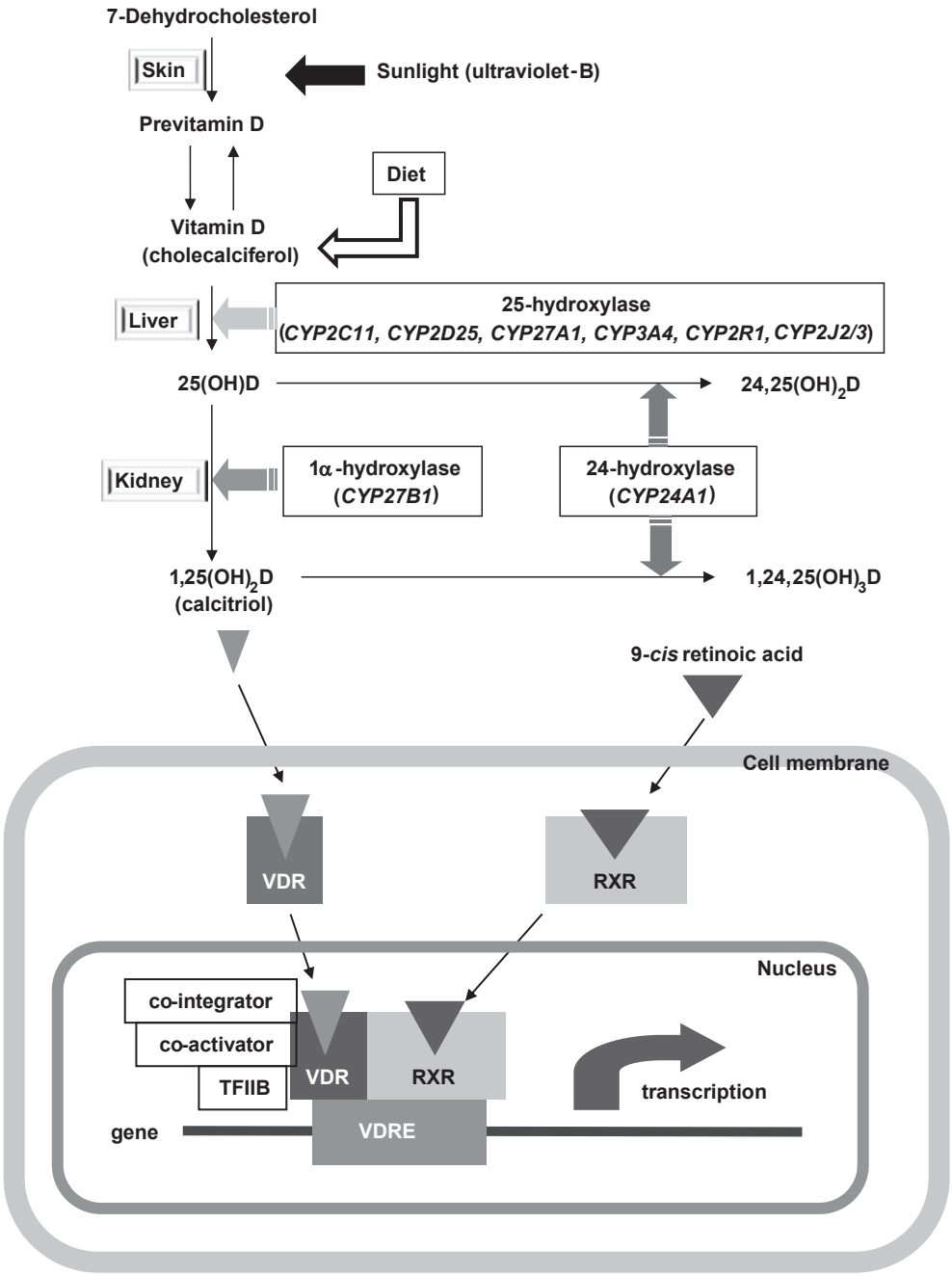

FIGURE 9.1 Vitamin D metabolism and gene regulation through vitamin D receptor binding. 7-Dehydrocholesterol is converted to previtamin D by skin exposure to ultraviolet-B. Vitamin D3 is then synthesized from previtamin D before being converted to 25-hydroxyvitamin D (25(OH)D) by 25-hydroxylase in the liver. A second hydroxylation by 1-α-hydroxylase in proximal convoluted tubule cells of the kidney converts 25(OH)D to $1,25(OH)_2D$, the biologically active metabolite. The $1,25(OH)_2D$ and vitamin D receptor (VDR) form a complex with the retinoid X receptor (RXR), and this resulting complex binds to vitamin D response elements (VDREs) on target genes, resulting in regulation of gene expression. *VDRE*, vitamin D response element; *RXR*, retinoid X receptor; *VDR*, vitamin D receptor; and *TFIIB*, transcription factor II B. *This figure was adapted from Niino M, Miyazaki Y, Fukazawa T, Kikuchi S. Vitamin D and multiple sclerosis in Asians and Caucasians: environmental and genetic aspects.* Clin Exp Neuroimmunol *2013;4(Suppl. 1):59–67.*

inhibiting B cell proliferation, generating class-switched memory B cells, inducing B cell apoptosis, and decreasing plasma cell conversion and antibody production.[29,30] Vitamin D also inhibits DC differentiation and maturation, thereby preserving the immature DC phenotype as evidenced by decreased expression of MHC class II molecules, costimulatory molecules, and IL-12.[7] Inhibition of DC differentiation and maturation is particularly important in the context of autoimmunity and the abrogation of self-tolerance.[7]

As discussed in the section Metabolism of Vitamin D, immune cells including T cells, B cells, and antigen presenting cells are capable of synthesizing 1,25(OH)2D, which can then act as an autocrine or paracrine signal[7] to modulate expression levels of genes related to immune regulation.[31] These immunomodulatory functions of vitamin D suggest possible efficacy for disruption of MS pathogenesis, and in fact, many studies have reported amelioration of EAE by treatment with vitamin D in mouse and rat models (reviewed in Ref. 9). However, high-dose vitamin D is usually required to reduce EAE, and such high-dose supplementation may produce severe adverse events such as hypercalcemia and renal calcification. To mitigate or decrease side effects associated with high-dose vitamin D supplementation, synthetic structural analogues of 1,25(OH)2D have been developed and tested in the EAE model, whereas other studies have examined possible synergistic effects of 1,25(OH)2D with other immunosuppressive agents such as cyclosporin A and rapamycin. These studies have generally demonstrated positive effects on EAE symptoms with reduced adverse side effects (reviewed in Ref. 9). Moreover, vitamin D mitigated EAE whether administered before induction or after symptom expression, (reviewed in Ref. 9) suggesting that vitamin D-based treatments reduce MS symptoms after relapse as well as relapse risk. Besides immunomodulatory effects, studies in mice suggest that vitamin D supplementation can promote remyelination and reduce demyelination.[32,33]

VITAMIN D AND MULTIPLE SCLEROSIS

Previous epidemiological studies on MS prevalence with latitude have suggested that lower vitamin D levels are a risk factor for MS. Smoking and obesity, two additional MS risk factors, have also been associated with lower vitamin D levels.[34,35] A retrospective study demonstrated that lower serum vitamin D levels in patients with clinically isolated syndrome (the first isolated episode of demyelination associated with brief symptom appearance) could be a risk factor for developing MS.[36] Lower vitamin D levels were also associated with greater disease activity and more severe disability,[37] whereas higher vitamin D levels were associated with lower disease activity, including reduced risk of clinical relapse and smaller or less numerous lesions on MRI.[31,38–40] One study also reported higher levels of circulating DBP in patients with MS,[41] which would reduce active levels, although this observation was not confirmed in other studies.[5,42] In addition to immune modulation, vitamin D has a direct neuroprotective effect through neurotrophin production and release, stabilization of intracellular calcium, and prevention of oxidative damage.[43] Smolders et al. found elevated *VDR* and *CYP27B1* mRNA expression levels in active MS lesions, suggesting increased sensitivity to vitamin D in MS-affected CNS tissues and a possible endogenous role for local vitamin D metabolism in the suppression of active MS lesions independent of immune homeostasis.[44]

Genetic association studies in MS patients have also identified several genes involved in vitamin D homeostasis. A large GWAS from Australia and New Zealand identified *CYP27B1* (encoding a potential 25-hydroxylase) as a candidate MS gene,[45] and another GWAS supported this association.[46] It was reported that rare loss-of-function variants in *CYP27B1* increased MS risk,[47] although this finding was not replicated.[48] The catabolic *CYP24A1* has also been suggested as a candidate MS gene.[46] Many studies have been conducted to assess associations with genetic polymorphisms of *VDR*, but results have not been consistent. One meta-analysis concluded that the *ApaI*, *BsmI*, *FokI*, and *TaqI* polymorphisms of *VDR* were not associated with MS risk[49] and another concluded that the *FokI* and *TaqI* polymorphisms of *VDR* were not associated with MS risk.[50] However, a recent updated meta-analysis concluded that the AA *ApaI* and FF *FokI* genotypes were associated with increased MS risk, while *TaqI* and *BsmI* polymorphisms were not.[51] The human leukocyte antigen allele *HLA-DRB1*1501*, which has been the most consistently associated with MS susceptibility, showed lack of interaction with *VDR FokI* and *TaqI* in increasing MS risk.[50] Thus, these polymorphisms may have independent effects on the immune system that both lead to enhanced susceptibility to MS. Further studies are needed to confirm the associations of these *VDR* polymorphisms with MS. Another GWAS reported that SNPs at the 16p13 locus containing *CLEC16A* increased MS risk,[46,52,53] and strong upregulation of CLEC16A was reported in the white matter of MS patients.[54] Further, 1,25(OH)2D exposure reduced CLEC16A expression in monocyte-derived DCs.[54] Although the functions of CLEC16A are still not well defined, it is a known regulator of the HLA class II pathway and is associated with multiple immune-related diseases (including type II diabetes and arthritis as well as MS). Collectively, these results underscore the potential relevance of CLEC16A to MS pathogenesis and to the therapeutic mechanisms of vitamin D.

Circulating vitamin D levels may also be influenced by allelic variation in vitamin D-related genes. Several studies have suggested that mutations or variants in *CYP27B1* affect circulating levels of 1,25(OH)2D[55,56] and 25(OH)D.[57] Two GWASs of MS patients also reported significant associations between serum levels of 25(OH)D and SNPs rs7041 and rs2282679 in the gene encoding DBP (*GC*) and SNP rs10741657 in *CYP2R1*.[16,23] Significant associations between 25(OH)D and these SNPs were also found in a cross-sectional study of MS patients.[58] Further, with increasing 25(OH)D levels, resultant regulation of a

large gene–gene interaction system was associated with decreased MS activity.[31]

As mentioned, *HLA-DRB1*1501* is the most important known genetic risk factor for MS. A VDRE motif was identified in the proximal *HLA-DRB1* promoter region, allowing vitamin D to influence its expression.[59] Vitamin D increased the expression of *HLA-DRB1*1501*, and this effect was not observed in cells with other *DRB1* haplotypes.[59] In Caucasian populations, the *HLA-DRB1*1501*-associated VDRE motif is widely represented among *HLA-DRB1* alleles with the exception of the *HLA-DRB1*04*, *-DRB1*07*, and *-DRB1*09* alleles.[60] Further, the risk of MS conferred by *HLA-DRB1*1501* was moderately modulated by VDR variants.[61]

Several studies have suggested that vitamin D levels influence the efficacy of disease-modifying drugs (DMDs), possibly through effects on IFN.[40,62,63] Higher 25(OH)D levels conferred a significant protective benefit against disease activity and progression in MS patients treated with IFNβ.[40,62,64,65] However, another study reported no association between 25(OH)D levels and disease activity after initiation of IFNβ.[63] A prospective longitudinal study found greater production of vitamin D from sun exposure in patients receiving IFNβ therapy.[62] In contrast to IFNβ, beneficial effects of vitamin D were not confirmed in MS patients treated with fingolimod.[65] Among MS patients treated with glatiramer acetate, there was significant improvement in new gadolinium-enhanced lesions with higher 25(OH)D levels.[65] Taken together, effects of vitamin D adjunct therapy on the activity and progression of MS may depend on DMD class.

As discussed earlier, one possible reason for the higher prevalence of MS with latitude is lower vitamin D synthesis by UV-B. However, one meta-analysis of cross-sectional studies, including our data in a Japanese population, found no significant influence of latitude on 25(OH)D levels,[5,20] suggesting that this hypothesis needs to be reconsidered. Recent studies have demonstrated that UV radiation suppressed EAE independent of vitamin D[66,67] and that UV radiation is related to MS pathogenesis independent of vitamin D. A large epidemiological study suggested that sun exposure and vitamin D could have independent effects on MS risk.[68]

POSSIBLE THERAPEUTIC APPLICATIONS OF VITAMIN D FOR MULTIPLE SCLEROSIS

Animal studies demonstrating vitamin D efficacy in animal models of MS as well as epidemiological studies and GWASs linking vitamin D insufficiently to disease risk suggest that supplementation may decrease disease activity or slow progression. However, clinical trials of vitamin D supplementation (Table 9.1) so far have yielded disappointing results. Possible reasons include small sample sizes, insufficient doses and durations of vitamin D administration, nonrandomization, and patient heterogeneity. Alternatively, several clinical trials have demonstrated hopeful results based on immunological effects. For example, daily supplementation with 2000 IU vitamin D for 3 months in healthy subjects significantly influenced expression levels of 291 genes involved in apoptosis, immune function, transcriptional regulation, epigenetic modification, response to stress, cell cycle progression, and differentiation of white blood cells.[69] Thus, vitamin D supplementation could alter immune function to reduce MS risk. Further, a 2015 randomized, double-blind, placebo-controlled trial of the vitamin D analogue alfacalcidol for patients with MS demonstrated decreased fatigue and improve quality of life (QOL).[70] This study also showed significant reduction of relapse number and an increase in the number of relapse-free patients.[70]

How does high-dose vitamin D treatment affect immune functions in patients with MS? One study found that high-dose vitamin D supplementation increased serum levels of IL-10 in patients with MS;[71] however, another study did not find such effects.[72] Supplementation by high-dose vitamin D also increased serum latency activated peptide (LAP) of TGF-β, which forms an inactive complex with TGF-β, in MS patients.[73] Further, high-dose vitamin D supplementation also reduced IL-17 production by $CD4^+$ cells and decreased the proportion of effector memory $CD4^+$ T cells, with a concomitant increase in central memory $CD4^+$ T cells and naive $CD4^+$ T cells in patients with MS.[74] To date, there have been only a few studies on the immunological effects of high-dose vitamin D supplementation in MS patients, and further investigations are needed.

A 2016 study on vitamin D supplementation found that MS patients exhibited a smaller increase in serum 25(OH)D levels than healthy controls at the same dose.[75] Further, the functional activity of vitamin D may differ between MS patients and healthy controls. In an in vitro assay, vitamin D differentially modulated LPS-induced IL-10 and IL-12/23 release by peripheral blood mononuclear cells (PBMCs) from MS patients compared to PBMCs from healthy controls.[76] The direction of the effects suggests that vitamin D has a weaker antiinflammatory effect in MS patients. Although the reasons for these differences in vitamin D response are currently unclear, they may relate to the aforementioned genetic variation in *VDR*, *GC*, and/or *CYP2R1* alleles. Furthermore, the lower efficacy of supplemental vitamin D to increase sum 25(OH)D and the lower bioactivity for immune modulation may account for negative clinical trial results.

TABLE 9.1 Clinical Trials of Vitamin D for the Treatment of Multiple Sclerosis

Intervention of vitamin D Maximum dose	Duration of treatment	n (treatment/control)	Design of trial	Clinical results	References
Cholecalciferol					
280,000 IU/week	28 weeks	12 (12/0)	U	53% decreased mean Gd + lesions in MRI from baseline No differences in relapse rates and disease progression between baseline and the end of study	81
40,000 IU/day (in control group, up to 4000 IU/day of cholecalciferol permitted if desired)	52 weeks	49 (25/24)	O, R, C	41% decrease of annualized relapse rates in treatment group More stable/improved EDSS in treatment group vs. controls	82
20,000 IU/day	12 weeks	15 (15/0)	U	Decreased fatigue (n = 8), relapse (n = 1)	83
300,000 IU/month	6 months	62 (28/34)	DB, R, C	No difference in EDSS and Gd + lesions between treated group and controls	84
20,000 IU/week	96 weeks	68 (35/33)	DB, R, C	No difference in percentage change in bone mineral density between treated group and controls No difference in ARR, EDSS, MSFC components, grip strength, and fatigue between 2 groups	85, 86
20,000 IU/week	1 year	66 (34/32)	DB, R, C	A significantly lower number of Gd + lesions in treated group vs. controls A tendency to reduced disability accumulation and to improved timed tandem walk in treated group vs. controls	87
10,400 IU/day in high-dose group vs. 800 IU/day in low-dose group	6 months	40 (19 in high-dose group/21 in low-dose group)	DB, R, C	Significant increase of 25(OH)D in high-dose group vs. low-dose group One relapse in each treatment arm	74
Calcitriol					
2.5 μg/day	48 weeks	15 (15/0)	U	4 patients; experienced clinical relapses 4 patients; worsened in EDSS	88
0.5 μg/day	12 months	50 (25/25)	DB, R, C	No difference in effects on the EDSS score or relapse rates between 2 groups	89
Alfacalcidol					
1 μg/day	6 months	158 (80/78)	DB, R, C	Decreased fatigue and improved QOL Reduced number of relapses and higher proportion of relapse-free patients	70
Ergocalciferol					
6000 IU twice daily +1000 IU in high-dose group vs. 1000 IU daily in low-dose group	6 months	23 (11 in high-dose group/12 in low-dose group)	DB, R, C	A higher exit EDSS and a higher proportion exhibiting relapse in high-dose group vs. low-dose group No difference in MRI-based outcome measures between 2 groups	90

EDSS, Expanded disability status scale; *ARR*, annualized relapse rate; *MSFC*, multiple sclerosis functional composite; *U*, uncontrolled; *DB*, double blind; *R*, randomized; *C*, controlled; *O*, open labeled.
Modified and updated from Niino M, Miyazaki Y. Genetic polymorphisms related to vitamin D and the therapeutic potential of vitamin D in multiple sclerosis. Can J Physiol Pharmacol *2015;93:319–325.*

One of the greatest concerns of high-dose vitamin D therapy is adverse events, such as hypercalcemia and renal stones. A 25(OH)D level ≥375 nmol/L is considered toxic due to the risk of hypercalcemia and renal stones, although for such 25(OH)D levels, daily consumption of ≥50,000 IUs is needed.[77] Based on clinical research, doses of 10,000 IU of vitamin D per day do not appear to cause toxicity.[78]

Several clinical trials of vitamin D in MS, some testing vitamin D as an add-on therapy with DMDs, are ongoing (https://clinicaltrials.gov/). Hopefully, forthcoming results will shed light on the therapeutic potential of vitamin D for MS. Even if clear clinical efficacy of vitamin D for MS is demonstrated in large clinical trials, critical issues remain to be resolved, such as the optimal dose for specific patients, the adverse effects of long-term use, and which metabolites or analogues are most efficacious. Further, basal 25(OH)D levels differ among ethnicities, so genetic background may be another important factor influencing therapeutic efficacy.

CONCLUSIONS

Vitamin D insufficiency has been suggested as a possible factor in MS pathogenesis. Indeed, genetic studies have reported links between MS susceptibility and several vitamin D-related genes. Further, numerous environmental factors linked to MS incidence may also alter vitamin D levels. The susceptibility to these environmental risk factors may be modulated by genetic variation and epigenetic mechanisms, such as DNA methylation, histone modification, and ncRNA.[19] Epigenetic regulation is of growing interest in complex diseases, including MS, and epigenetics is expected to explain part of MS heritability.[79] We suggest that the association between vitamin D and MS should be reconsidered. The therapeutic potential of vitamin D is an old idea, but further studies are still needed to definitively determine its efficacy and provide mechanistic explanations for putative benefits against MS.

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CHAPTER

10

Multiple Sclerosis in Women: Vitamin D and Estrogen Synergy for Autoimmune T-Cell Regulation and Demyelinating Disease Prevention

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OUTLINE

INTRODUCTION

In 1756, Miss Elizabeth Foster of Berwickshire, Scotland, suffered from a "paralytic disorder, which sometimes affected the arm, sometimes the leg, of the left side" according to Scottish physician Sir Robert Whytt.[1] He wrote, "in this condition she remained till the spring 1756, when unexpectedly she grew much better; but not so far as to get quite rid of her paralytic complaints." She was "apprehensive of a more violent attack; which accordingly soon happened." She became paralyzed on her left side and "in this state she continued throughout last winter with the addition of some new complaints." Miss Foster appears to be the first person whose affliction

with relapsing–remitting multiple sclerosis (RRMS) was recorded in the medical literature.[1]

Miss Foster's case illustrates RRMS well. She partially recovered from "a violent nervous fever" at age 18, before her neurological attacks came to Whytt's attention at age 33.[1] RRMS patients experience a variable course of neurological impairment, followed by remissions with some recovery of neurological function.[2] Primary progressive MS (PPMS) patients experience unrelenting accumulation of disability.[3] The MS diagnosis requires an observation of neurological impairments disseminating in time and space.[4] This was true for Miss Foster, whose speech and vision faltered after she developed impaired motor function. Her father suffered and died from "the palsy," and her neurological symptoms worsened in the winter and abated somewhat in the spring, illustrating the genetic and environmental components that contribute to MS.

MS is incurable. It gradually deprives patients of movement, sensory perception, cognition, emotional well-being, independence, and livelihood. Once rare, MS is becoming increasingly common. A 1956 Scottish survey recorded one MS case per 1818 citizens.[5] A 2009 survey recorded one female MS case per 170 women in the Scottish Orkney Isles.[6] This is an extreme example of an alarming trend observed in many, but not all countries.[7–20] It affects mainly women but also postpubertal girls,[15,21–23] and applies to RRMS but not PPMS.[6,7,16,18,24,25] The global scope, magnitude, and rapidity of this female-biased increase in RRMS incidence imply that it has nongenetic etiological roots and that many RRMS cases could be prevented by modifying the nongenetic drivers of the trend appropriately in the MS risk acquisition timeline.[26]

Our purpose in writing this review is to examine these nongenetic etiological roots, propose a novel estrogen and vitamin D synergy hypothesis that may account for a major proportion of the female-biased increase, and suggest intervention strategies that may reverse it. Known sex-based differences in MS are outlined, and the evidence for rising female MS incidence is summarized, particularly the unexpected epidemiological details that require explanation. The major nongenetic exposures implicated in MS are presented and the plausibility of hypotheses invoking these exposures to explain the trend are examined using Bradford–Hill criteria.[27] Evidence for declining global vitamin D status is reviewed, and the concept that this trend may be undermining estrogen and vitamin D synergy for immune tolerance in women is introduced. Finally, the work of an international task force convened to strategically address vitamin D and MS prevention is summarized. This task force judged that the evidence for low vitamin D_3 status as a causal contributor to MS disease, particularly in women, is sufficiently strong to warrant action to correct this deficiency. Implementing their recommendations may spare millions of young adults from the debilitating neurological disease that befell Miss Elizabeth Foster in 1756.

GENES, ENVIRONMENT, AND AUTOIMMUNE T LYMPHOCYTES IN MS

A complex interplay of genetic, environmental, and hormonal factors fuel the pathological processes that cause MS.[28] The MS brain shows focal demyelinated lesions harboring infiltrating immune cells and activated glial cells, observations that suggest an autoimmune-mediated attack on the axon–myelin unit.[29] Axonal injury and neuronal cell loss have also been observed independently of demyelination.[30] The relative importance of the autoimmune-mediated versus the immune-independent component is debated, but this may be a false dilemma. A common underlying biological abnormality may link these two seemingly independent pathological processes. There is no debate that axonal injury and neuronal cell loss are the basis for progressive neurological disability in MS.

Family and ethnicity studies have highlighted the genetic component in MS susceptibility.[31] MS risk increases about 2–6% among first-degree biological relatives of an MS case,[32] some families have more than one affected member,[31] and about 30% of monozygotic (MZ) twin pairs are both affected.[33,34] Some ethnic populations show MS susceptibility, notably people of Northern European ancestry,[35] whereas others like Hispanics and Asians seem to be relatively MS resistant.[36] Intensive genetic studies have cataloged >100 candidate MS susceptibility genes, many relating to deregulated immunological processes.[37] Curiously, RRMS and PPMS share genetic risk factors despite their different natural histories.[34]

Epistatic interactions between MHC susceptibility and resistance genes dominate MS genetic risk.[38,39] There are parent-of-origin and *trans*-generational risk effects that center on the major histocompatibility (MHC) class II genes and implicate epigenetic effects in MS risk, particularly in women.[32] The *HLA-DRB1* genes, especially *HLA-DRB1*15*, exert the strongest genetic influence on MS risk, with an odds ratio (OR) of 5.4.[38,40] These genes encode the MHC class II molecules that present peptide antigens to $CD4^+$ T lymphocytes for immune recognition.[41,42] However, MS susceptibility cannot be predicted even by MHC genotype,[34,43] indicating MS is not a purely in-born disease where all individuals who carry disease genes develop the disease.

The relative importance of the genetic versus the environmental component to MS risk is debated, but this may also be a false dilemma, since an interaction between the two is required.[44] The nongenetic exposures are dominant in MS risk, as evidenced by the variation in prevalence with latitude and ambient UV radiation,[45,46] the modification of risk by migration,[47–53] the seasonal variation in patient birth and disease onset months,[54–63] and

the nutritional impacts on the disease.[64–67] Development of MS is not universal in individuals who are exposed to these nongenetic risk factors, so MS is not a purely acquired disease like nutritional rickets,[68,69] where all severely vitamin D–deficient children develop the disease. Nongenetic exposures acting on the relatively few individuals (<1%) who harbor a genetic predisposition are believed to set in motion the biological processes that are the root cause of MS. Since one cannot arrange to be born in a favorable season in a low-risk geographical region to genetically resistant parents, MS prevention must come from modifying nongenetic exposures implicated in MS risk.

Unequivocal evidence has demonstrated a role for pathogenic $CD4^+$ T lymphocytes specific for myelin peptides presented by MHC class II molecules in demyelination and neuronal damage in MS[29,70] and the animal model of MS.[71] Myelin-specific T cells were observed in the bloodstream of individual MS patients,[70,72] and crossing the blood–brain barrier to form nascent MS lesions.[73] Systems biology approaches have confirmed the involvement of antigen presentation, the immune synapse, T-cell activation, and T-cell deregulation in MS pathology.[37,39,74–77] In summary, complex interactions between MHC class II susceptibility and resistance genes, and between risk genes and nongenetic exposures initiate the deregulated interactions between antigen-presenting cells (APC) and $CD4^+$ T cells that lie at the heart of MS molecular etiology. Precisely when, where, why, and how the deregulated APC-$CD4^+$ T-cell interactions occur is unclear.

SEX-BASED DIFFERENCES IN MS AND THE ROLE OF ESTROGEN

Sex-based differences in MS have been studied intensively because of their potential to yield etiological insights.[78] Recent reviews have summarized data on sex hormones and sex chromosomes in MS disease.[79–84] To better understand mechanisms underpinning the rapid global rise in young female MS cases, we emphasize genetic, hormonal, and pregnancy influences on female MS risk.

Genes

Genetic susceptibility to MS affects females and males differently.[85] There is a maternal parent-of-origin effect. In blended families, maternal half-siblings were MS disease concordant about twice as often as paternal half-siblings.[32,86] These maternal parent-of-origin effects were also seen in Caucasian–North American Aboriginal admixture matings.[87] There is also higher MS risk gene penetrance in females. In a genetically isolated population, kinship between mothers of patients was 3.8-fold higher than kinship between fathers indicating MS risk genes were more likely to yield an MS phenotype in females than males.[88] Also, the female:male (F:M) ratio was greater for *HLA-DRB1*15* individuals than for non-*HLA-DRB1*15* carriers, and *HLA-DRB1*15* daughters of *HLA-DRB1*15* mothers had a higher risk of developing MS than *HLA-DRB1*15* sons of *HLA-DRB1*15* fathers.[32] Scrutiny of the X-chromosome has not revealed an MS susceptibility locus acting alone or in concert with *HLA* genes to influence female risk.[89] However, in systemic lupus erythematosus, DNA hypomethylation of X-linked genes was implicated in female risk.[90] To our knowledge such a mechanism has not been ruled out in MS.

The evidence mentioned in the preceding paragraph suggests that events taking place in utero or very early in postnatal life under the influence of maternal factors contribute to MS risk in *HLA-DRB1*15* female offspring. Installation of epigenetic marks like DNA methylation that would directly or indirectly influence *HLA-DRB1*15* gene expression and MS risk in a female-biased manner would be one possible mechanism.[32,60,91] Indeed, striking differential methylation with a peak signal at a CpG island in *HLA-DRB1* was observed in $CD4^+$ T-cell DNA from MS cases compared to controls.[92] In summary, the maternal parent-of-origin effects implicate events in utero or very early postnatal life in MS risk, particularly in *HLA-DRB1*15* female offspring, leading to higher risk gene penetrance in females. The molecular details are unknown, but an environmentally determined maternal influence on epigenetic marking in fetal and neonatal $CD4^+$ T cells is a candidate mechanism. Unanswered questions concern the *HLA-DRB1*-related biological processes that may be under epigenetic regulation.

Puberty

Significant neuroendocrine and hormonal changes that support reproductive capability occur during puberty.[93] The pubertal transition affects MS risk differently in females and males. Canadian pediatric MS patients (onset <16 years of age) were 75% females, and 75% had RRMS. Before puberty (onset age ≤12 years), the F:M incidence ratio was 1.6, whereas after puberty (onset age ≥13 years) the F:M ratio was 3.7.[94] The authors noted this change at puberty "is so highly significant that it must be related… to the acquisition of the disease" in females. Further, the data indicate "a possible endocrine explanation for the unbalanced sex ratio"…"probably acting on the thymus gland or on T-lymphocyte subsets." Thus, these investigators hypothesized that female hormonal changes at puberty acting on the thymus or on T lymphocytes increased female susceptibility to RRMS.

Their observations have been confirmed. In Italian pediatric cohorts, the F:M ratio was about 1.4 before

puberty and about 4.7 thereafter.[95,96] In a German pediatric cohort, the F:M ratio was about 1.3 before puberty and about 2.9 thereafter.[97] In an Iranian pediatric cohort with mean onset age 14 years, the F:M ratio was 4.5.[21,98] Age of menarche also correlated directly with age of MS onset[99] and inversely with MS risk (hazard ratio 0.64).[100,101] Moreover, female mice became more susceptible to an MS model disease after the pubertal transition compared to age-matched, prepubertal controls.[101] The RRMS phenotype predominated by as much as 97% in all of the human cohorts. Together, the data strongly suggest that puberty's hormonal changes increase female RRMS risk.[94]

Pregnancy

Some data suggest pregnancy triggers protective biological processes that decrease MS risk, while other data do not support this view. The pregnancy data have been reviewed.[79,81,102] An early Swedish study found 48% of female MS patients were childless at MS disease onset compared to an expected 33%.[103] Furthermore, the ratio of nulliparous to parous women was 1.5 for those with MS onset at age 20 years and >3 for those with MS onset at age 50 years. Australian[104] and Danish[105] studies were consistent with these findings. The average age at first birth in the Australian cohort was 31.9 years and in the Danish cohort was 26.2 years. Among Australian women, 37% were childless at the time of the first demyelinating episode compared to 22% expected based on fecundity data. Similarly, more Danish women with MS were childless or had fewer births in the 5 years preceding clinical onset (OR = 0.54 for one child; OR = 0.68 for >1 child) than the number predicted by fecundity data. Male fecundity had no impact on male MS risk in these studies. These investigators hypothesized that female hormonal changes during pregnancy decreased female susceptibility to RRMS.[103–105]

Other data are not consistent. Danish investigators evaluated number of pregnancies and live-born children in 4.4 million men and women.[106] The relative risk of MS varied inversely with number of children in both sexes, and MS risk in women was unrelated to pregnancy history, arguing against a biological role of pregnancy in MS risk. Swedish investigators also found that men and women who became parents within 5 years prior to the index year had a substantially reduced risk of developing MS.[107] Iranian investigators evaluated parity and found the hazard ratio of MS was 4.5-fold in women who had one child compared to childless women.[108] These data argue in favor of reverse causality, specifically that undiagnosed MS may influence fecundity. Yet to be ruled out in the parity debate is the possibility that some estrogen antagonists used in female contraceptives elevate MS risk.

Estrogenic Compounds and Their Synthesis

Estrogen synthesis changes dramatically with puberty and pregnancy.[109] Before puberty, extra-gonadal tissues produce estrogen for local (paracrine and intracrine) but not systemic (endocrine) hormone signaling. At puberty, the ovaries produce estradiol (E2), the most potent physiological estrogen, and release it systemically. During the menstrual cycle, circulating E2 levels rise to a preovulatory peak of about 1.5 nmol/L, decline to about 0.6 nmol/L during the luteal phase and to about 0.2 nmol/L in the follicular phase. Brain neurons also produce E2 for paracrine hormone signaling in support of sexual differentiation and reproductive behavior. In pregnancy, the placenta produces estriol (E3), the least potent physiological estrogen[110]; the E2 rises to about 44 nmol/L and E3 to about 55 nmol/L. Thus, the estrogenic compound, its level, the site of synthesis, and the systemic release for endocrine signaling vary greatly before and after puberty and during pregnancy.

Two estrogen receptors (ERs), ERα and ERβ, mediate the estrogen effects mainly as estrogen-dependent transcriptional regulators.[109,111] Upon ligand binding, they translocate to the nucleus, bind to estrogen-responsive elements (EREs) in DNA, and recruit factors to stimulate or repress transcription. ERα and ERβ show distinct expression patterns. Both receptors are expressed in the brain, but ERα dominates in the hypothalamus (neuroendocrine function) and ERβ dominates in the hippocampus (memory).[109] Most innate and adaptive immune system cells express ERα, while ERβ expression is more limited.[111] ERα expression is most relevant to immune regulation because it dominates in $CD4^+$ T lymphocytes.

Animal Modeling

Experimental autoimmune encephalomyelitis (EAE) in rodents has proven valuable for probing sex hormone–mediated protective mechanisms in demyelinating disease.[112,113] The pathogenic and protective $CD4^+$ T cell–mediated mechanisms are well understood in EAE.[114] Three rodent EAE models have been used. In the prevention model, genetic, hormonal, and nutritional manipulations are introduced prior to immunizing rodents with myelin antigens and assessing the impact of the variables on the immunological and neurological aspects of the disease. In the treatment model, EAE disease is induced by myelin antigen immunization or by adoptive transfer of myelin antigen-specific $CD4^+$ T cells before introducing hormonal and nutritional manipulations and assessing their impact. The prevention and treatment models involve discrete immunological and neurological mechanisms. Hence, it is imperative to apply the appropriate model to the specific research question. We focus on results derived from the EAE

prevention model to understand why MS risk is rising in young women.

Caution is needed when extrapolating animal modeling results to humans because EAE is an induced demyelinating disease with a defined autoimmune origin, whereas MS is a spontaneous disease of uncertain etiology. EAE cannot faithfully model microbial contributions to MS. However, EAE reliably models immunological features, specifically myelin-specific T-cell trafficking through the blood–brain barrier to initiate focal demyelinating lesions, activation of macrophages and microglia, production of inflammatory mediators and reactive oxygen species, and B-lymphocyte production of oligoclonal immunoglobulins in the central nervous system (CNS). EAE also reliably models neurological damage, specifically focal perivascular lesions in white and gray matter, the brain stem, and the optic nerves, gliosis, myelin sheath destruction, partial remyelination, and axonal damage and neurological dysfunction.

Estrogen Mechanisms

EAE has been used to model the role of estrogens in female susceptibility to MS.[115–118] We focus on the anti-inflammatory effects, acknowledging that E2 also has profound neuroprotective effects (preservation of myelin, axons, and synapses).[82,117,119] We also emphasize effects on $CD4^+$ T cells, acknowledging that E2 also affects B cells, dendritic cells, microglia, and astrocytes.[118,120,121] Testosterone's protective effects in neurodegenerative disease have been reviewed and are not discussed here.[117,122,123]

To study estrus- or diestrus-level E2 effects on demyelinating disease risk, ovariectomized (OVX) adult females were compared to sham-operated (SHAM) controls[124] or to OVX females given estrus- or diestrus-level E2 implants before EAE disease induction. The E2-replete females had delayed EAE onset and reduced severity compared to E2-depleted females.[125–128] The E2 limited inflammatory cell accumulation and demyelination[125] as well as cytokine, chemokine, and chemokine receptor expression in the CNS.[126] The E2-depleted females had higher pathogenic $CD4^+$ T-cell numbers than the E2-replete females, suggesting reduced pathogenic cell influx or increased cell clearance.[128]

Another model system explored puberty's effects.[101] OVX surgery prevented the pubertal transition in immature females. These females were later compared to intact females for EAE susceptibility. EAE incidence and severity were higher in the sexually mature females than prepubertal females. Higher co-stimulatory molecule expression and APC function plus stronger myelin-specific Th1 and Th17 cell responses correlated with more severe EAE in the sexually mature females. Many biological changes accompany sexual maturation, hence these changes may not result directly from E2 exposure. The data are consistent with previous reports that E2 promoted dendritic cell maturation and APC function, and with other data demonstrating more robust immune responses in sexually mature females.[111,129]

To study pregnancy-level estrogen effects on demyelinating disease risk, investigators have compared nonpregnant female mice given placebo or pregnancy-level E2 implants before EAE disease induction.[125] Pregnancy-level E2 completely suppressed demyelinating disease development in nonpregnant female rodents.[118,124,125,130–135] The pregnancy-level E2 dramatically reduced CNS inflammation and demyelination[125] as well as cytokine, chemokine, and chemokine receptor mRNA in the CNS.[126] Females with pregnancy-level E2 had a complete absence of infiltrating immune cells in the CNS, whereas these cells were numerous in the placebo group.[135]

Accompanying disease suppression, Th1 cell and Th17 cell proliferation and cytokine synthesis were suppressed in the periphery[135,136] and *Ifng*, *T-bet*, *Il17*, *ROR-γt* transcripts indicative of Th1 and Th17 cells were suppressed in the CNS.[133] Importantly, E2 also enhanced the development of T-regulatory (Treg) cells that enforce immunological self-tolerance. Pregnancy-level E2 or E3 promoted the development of myelin antigen-specific $CD4^+$ T cells producing high levels of IL-10,[130,133] the signature cytokine of the type 1–regulatory T cells (Tr1).[137] MS patients are known to have a defect in Tr1 cells.[138–141] The pregnancy-level E2 also increased the expression of FoxP3, the lineage-determining transcription factor of $CD4^+$ Treg cells.[133,142–144] Importantly, $CD4^+$ T cell–specific *Esr1* gene targeting eliminated all beneficial E2 actions in EAE.[131,132,135] Therefore, E2 and ERα regulation of $CD4^+$ T-cell function is the core protective mechanism.

In summary, puberty increases female RRMS risk in part due to E2-stimulated increases in APC maturation and function and strengthening of $CD4^+$ T-cell responses. In contrast, pregnancy-level E2 and E3 signaling through ERα in T cells dampens pro-inflammatory $CD4^+$ T-cell responses and improves $CD4^+$ Treg cell responses. Unanswered questions concern the precise mechanistic details. Since estrogens can epigenetically regulate gene expression by recruiting DNA methyltransferases to CpG-rich regions,[145] and lineage-specifying transcription factors acting in the context of epigenetically marked DNA control $CD4^+$ T-cell subsets,[146,147] an attractive hypothesis is estrogen regulation of epigenetic marks related to $CD4^+$ T cell lineage–specifying transcription factor networks.

RISING FEMALE MS INCIDENCE

Globally, MS affected women and men equally until around 1950, when a small female bias was first recorded.[45] An age-cohort analysis of MS in Northumberland and

Durham, England, provided the first evidence of rising MS prevalence.[148] The MS prevalence was $10.7/10^5$ population for the 1904–1913 year-of-birth (YOB) cohort and $48.8/10^5$ for the 1924–1928 YOB cohort, a 4.6-fold increase in about two decades.

An increase in MS prevalence also became evident in Scandinavia. Norwegian[149] and Finnish[150] investigators monitored the F:M ratio to control for temporal changes in demographics and methodologies. The F:M ratio increased 3.56% per year between 1957 and 1982 in Hordaland County, Norway, 5.76%/year between 1950 and 1991 in More and Romsdal counties, Norway,[7] and 6.47% per year between 1964 and 1978 in Vaasa, Finland.[150] Importantly, female RRMS cases increased, but PPMS cases did not.[7] Male-prevalence rates were stable, so the data indicate female RRMS cases rose.

Canadian data echoed this trend. In Alberta, Canada, the MS incidence rate increased from $1.9/10^5$ population in 1959 to $7.3/10^5$ population in 1989 and to $44.2/10^5$ in 2002, a 23-fold increase in about 40 years.[9,151] Plotting the Canadian F:M prevalence ratio by YOB controlled for changes in time to diagnosis and age of disease onset.[8] The F:M ratio increased from 1.9 in the 1931–1935 YOB cohort to >3.21 in the 1976–1980 YOB cohort. Male-prevalence rates were stable, and survival rates did not differ between the sexes, so the data indicate female MS cases rose. Female RRMS cases increased but PPMS cases did not.[24]

Recent global surveys have measured MS incidence trends (Fig. 10.1). A composite F:M incidence ratio for Australia, Europe, Scandinavia, the UK, and the USA increased from 1.4 in 1955 to 2.3 in 2000.[11] A composite F:M incidence ratio for North America and Western Europe increased by a factor of 1.014 annually beginning around 1950.[12] A composite F:M incidence ratio for Canada, Europe, Denmark, Australia, and New Zealand[16] increased fractionally between 1930 and 1989. Stratifying the data by residence latitude, it was clear that the slope of the F:M ratio by YOB plot increased dramatically for individuals born after 1970 in northern countries (Belgium, Canada, Germany, Denmark, and the Netherlands), but the slope was relatively flat for individuals residing in southern countries. Significantly, the age-adjusted MS incidence rate varied strongly and inversely with residence latitude only in women.

Additional reports have confirmed these trends in Canada,[8,152–155] Norway,[18] Sweden,[19] Denmark,[12] Scotland,[6] Japan,[17] Tasmania,[13] and New Zealand[20] (Fig. 10.1). The trends reflect rising female RRMS incidence.[6,7,16,18,24,25] The world's highest MS prevalence rates have been recorded in Nova Scotia ($267/10^5$)[154] and Alberta ($358/10^5$),[152] Canada, and Scotland,[6] where Sir Robert Whytt recorded the first MS case. The MS prevalence rate in the Scottish Orkney Isles is $402/10^5$ population.[6]

Iranian data collected since 2000 may prove crucial to understanding the drivers of the female MS incidence trend.[14,15,23,25,108,156–160] The Iranian population is stable,[157] clusters genetically with Europeans,[161] and harbors known MS-susceptibility genes.[162] Isfahan Province was previously thought to have a low MS risk,[163] but a 2005 survey revealed a surprisingly high MS prevalence, $35.5/10^5$ (F:M 3.6).[164] This finding prompted collection of F:M prevalence ratio data.[14] The F:M ratio for MS prevalence gradually increased from 1.33 for the 1936–1940 YOB cohort to 3.72 for the 1981–1985 YOB cohort. This

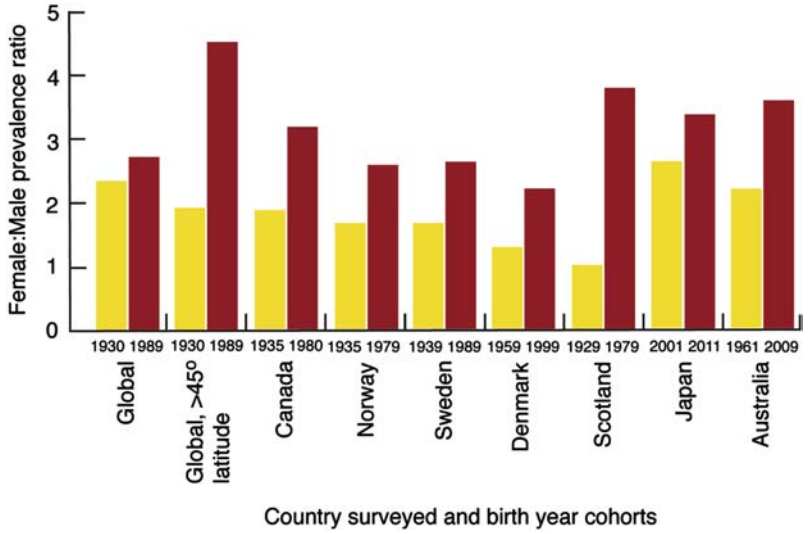

FIGURE 10.1 Global rise in the F:M prevalence ratio by year of birth. The data are from surveys worldwide[16] and within Canada,[8] Norway,[18] Sweden,[19] Denmark,[12] Scotland,[6] Japan,[17] and Australia.[13]

graph showed a joinpoint where the ratio jumped to 7.64 for the 1986–1990 YOB cohort (Fig. 10.2A). MS incidence also jumped from 3.64/10^5 in 2007 to 9.1/10^5 population in 2009. The mean age at onset was 28.2 ± 9 years, so those diagnosed between 2007 and 2009 would have been born roughly between 1979 and 1981. Pediatric MS cases also rose sharply between 1997 and 2003.[21,23] The pediatric F:M ratio was 4.5, and 78% of the cases were diagnosed in adolescents age 14–16 years (Fig. 10.2B). Data from Tehran echo these trends.[15] Adult MS incidence data, plotted by year recorded (1989 and 2005), showed a sharp increase in females from 1.01 to 7.11/10^5 and a gradual increase in males from 0.38 to 2.2/10^5 so the F:M incidence ratio remained stable at about 3 (Fig. 10.2C). Pediatric female incidence increased 10-fold between 1991 and 2007 (Fig. 10.2D).[159] These sharp increases exclude methodological changes, genetic factors, and environmental factors of small effect size as plausible explanations.

Iranian immigrants to Norway (60°N latitude), Sweden (55–69°N latitude), and British Columbia (54°N latitude), Canada, incurred a significant increase in MS risk compared to their risk in Iran (32°N latitude). MS incidence among first-generation Iranian immigrants to Norway exceeded that in Iran by a factor of 2–3.[50,51] Similarly, MS prevalence among Iranian immigrants to Sweden[52] and to British Columbia, Canada,[53] exceeded that in Iran by factors of 2–3 and at least 4, respectively. A strong female bias was evident in all reports. Thus, Norway, Sweden, and Canada share a latitude-linked

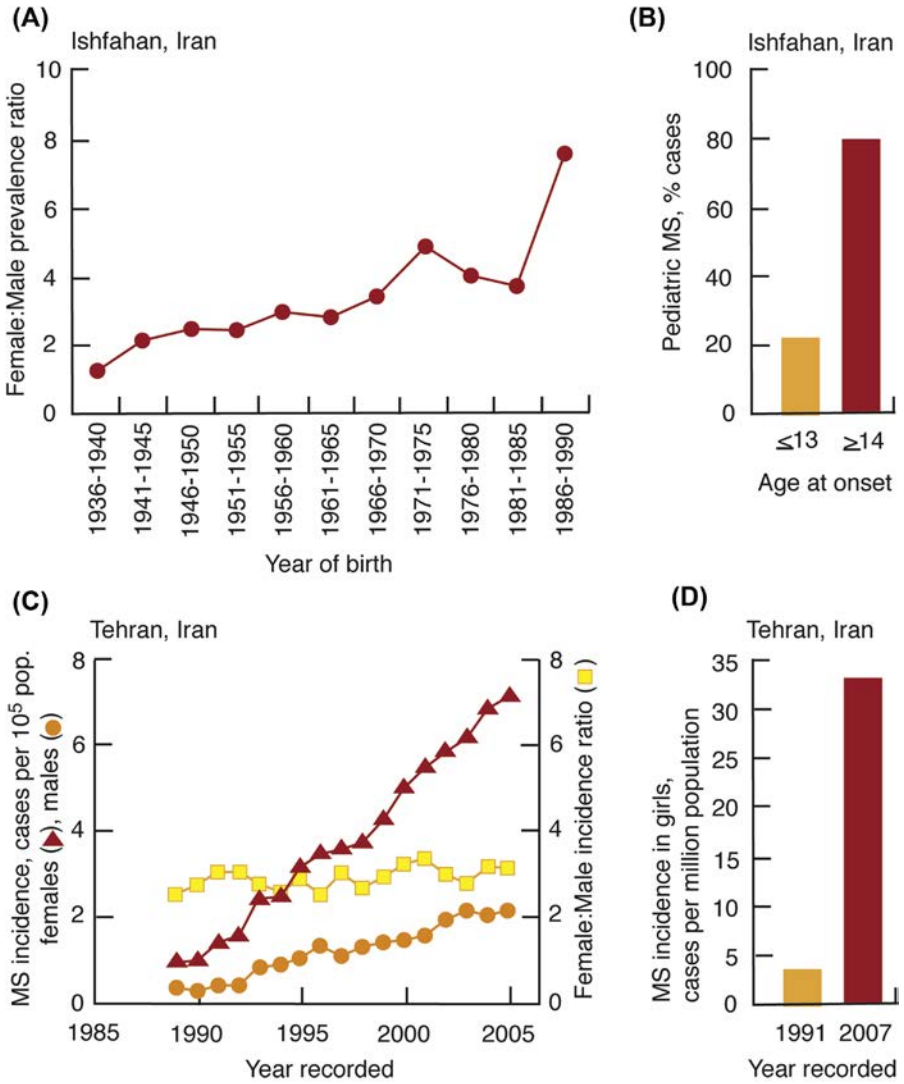

FIGURE 10.2 Rapidly rising MS prevalence and incidence in Iran. (A) The F:M prevalence ratio in Ishfahan, Iran, as a function of year of birth.[14] (B) Pediatric MS cases recorded between October 1997 and February 2003 in Ishfahan, Iran, by age at onset in girls (82% of cases) and boys (18% of cases).[21] (C) The female and male MS incidence rates and the F:M incidence rate ratio in Tehran, Iran, as a function of the year the data were recorded.[15] (D) The MS incidence rates in girls, mean age 14.1 years, in Tehran, Iran, as a function of the year the data were recorded.[159]

factor that elevates MS risk at least threefold in Iranian women.

To summarize, unequivocal epidemiological data support the conclusion that global female MS incidence is rising. The magnitude of the rise is large, >10-fold in the Scottish Orkney Isles and in Iran, and >23-fold in Alberta, Canada. The trend has been observed in North America, Scandinavia, the UK, Europe, and the Middle East, elevating MS risk in different people, places, and circumstances. The trend began with the birth cohort of about 1930–1935 (possibly earlier), and appears to have accelerated in cohorts born after 1970. It is female biased, affecting girls as young as 14 in some countries. Curiously, the trend applies to RRMS but not PPMS, is most evident in high-latitude regions, and affects first-generation immigrants to these regions. Hypotheses seeking to explain the global trend must contend with these epidemiological facts.

NONGENETIC EXPOSURES IN FEMALE MS RISK

The etiological roots of this global trend cannot be genetic. Instead, nongenetic exposures and hormonal factors must be enabling higher penetrance of MS risk genes in young women. The environmental factors implicated in MS risk are low sunlight exposure and vitamin D status,[165] EBV infection,[166] and smoking.[167,168] These major risk factors together with *HLA-DRB1*15* accounted for about 64% of MS risk, with the impact of multiple factors being additive.[169] Reviews on MS and low vitamin D status,[28,170,171] EBV infection,[172–175] and smoking[176–178] provide detailed summaries of the evidence.

We summarize each nongenetic exposure's effect size, association with latitude, impact on females versus males, influence on RRMS versus PPMS, and age when the exposure is relevant to MS risk. We compare these data to the epidemiological facts looking for a nongenetic exposure that increased sufficiently to drive a >threefold increase in female RRMS incidence between about 1950 and 2010, particularly at high latitudes, in both the pediatric and adult populations.

Smoking

Smoking is modestly associated with MS risk.[179,180] Ever-smokers had about 1.3–1.6 higher risk of developing MS than never-smokers in case–control and longitudinal studies.[167,176,181–184] There was an excess risk for males.[177,185] Smoking did not correlate with a specific disease course, RRMS or PPMS.[180] Smoking correlated with MS risk independently of the age at smoking debut,[186] but stratifying data by age revealed that smoking begins contributing to MS risk at 20–26 years of age.[182,187] Maternal active or passive smoke exposure was not associated with MS risk in the offspring.[177,187] In summary, smoking shows a weak correlation with MS risk (OR ~1.5), no association with latitude, no differential impact on females versus males, no preferential association with RRMS, and most relevance to MS risk during young adulthood.

Epstein–Barr Virus

EBV, a common gamma herpes virus that infects B lymphocytes, is the causative agent for infectious mononucleosis (IM), a disease of late adolescence and young adulthood.[188] Several lines of evidence have associated EBV infection with MS risk.[172–174,189–191] Antibodies specific for EBV nuclear antigen (EBNA) were detected in virtually 100% of pediatric and adult MS patients compared to about 95% of the general population.[192]

The relative risk of developing MS was about 2.6–3.9 for individuals with antibodies to EBNA compared to seronegative individuals.[189,193,194] Individuals with a history of IM had a 2.17-fold higher risk of developing MS than individuals without this history.[195] The proportion of EBNA-seropositive individuals correlated with latitude independently of MS status (OR = 1.06).[191] Moreover, IM was reported more frequently in Norway than Italy, although its association with MS was similar in the two regions (Italy, OR = 1.72; Norway OR = 2.12).[196] In both cohorts, IM was more frequent in spring than fall. Examination of relative MS risk by IM sex ratio did not reveal a sex bias, indicating IM-affected female and male risk equally.[195] A comprehensive data review in 2015 linked exposure to EBV with an increased risk of RRMS, but the linkage to PPMS was unclear.[180]

EBV Mechanisms

EBV infection appears to modify B-lymphocyte antigen presentation to $CD4^+$ T cells. The EBV EBNA-3 protein binds the VDR, blocking its activation of target genes.[197] EBV also produces a viral interleukin-10 (IL-10) mimetic[198,199] capable of disrupting the cellular IL-10 signaling that is essential for Tr1 cells.[171,200] EBV also produces viral peptide antigens that mimic myelin peptides and activate myelin-specific T cells.[70,201,202] Thus, EBV infection of B lymphocytes may be distorting $CD4^+$ T-cell activation toward a pro-inflammatory phenotype. In summary, EBV infection correlated with a 2- to 4-fold increased risk of MS and showed a weak association with latitude. However, EBV had no differential impact on females versus males, no preferential association with RRMS, and greatest relevance to MS risk in late adolescence and young adulthood.

Vitamin D

Vitamin D_3 is synthesized in skin exposed to ultraviolet (UV) sunlight (Fig. 10.3).[203] Few foods naturally contain this hormone precursor, hence UV exposure is the main source. Vitamin D_3 synthesis varies inversely with latitude, rises in spring, and declines in fall.[204] Confusion regarding the animal form, vitamin D_3, and the plant form, vitamin D_2, confounds the scientific literature. These compounds are chemically and biologically distinct.[205] The D without a subscript denotes both forms. A liver 25-hydroxylase modifies the 25-position of vitamin D_3 and vitamin D_2 and releases the 25-hydroxyvitamin D (25-(OH)D) metabolites into the circulation. Most assays do not distinguish 25-(OH)D_3 from 25-(OH)D_2. However, 25-(OH)D_3 has a longer half-life and yields a more active hormonal form than 25-(OH)D_2.[205]

Calcitriol (1,25-dihydroxyvitamin D_3) is the biologically active vitamin D_3 hormone (Fig. 10.3).[203] The *CYP27B1* gene encodes the 25-hydroxyvitamin D_3-1α-hydroxylase that catalyzes the rate-limiting step in calcitriol synthesis. *CYP27B1* is expressed in the skin, kidney, activated

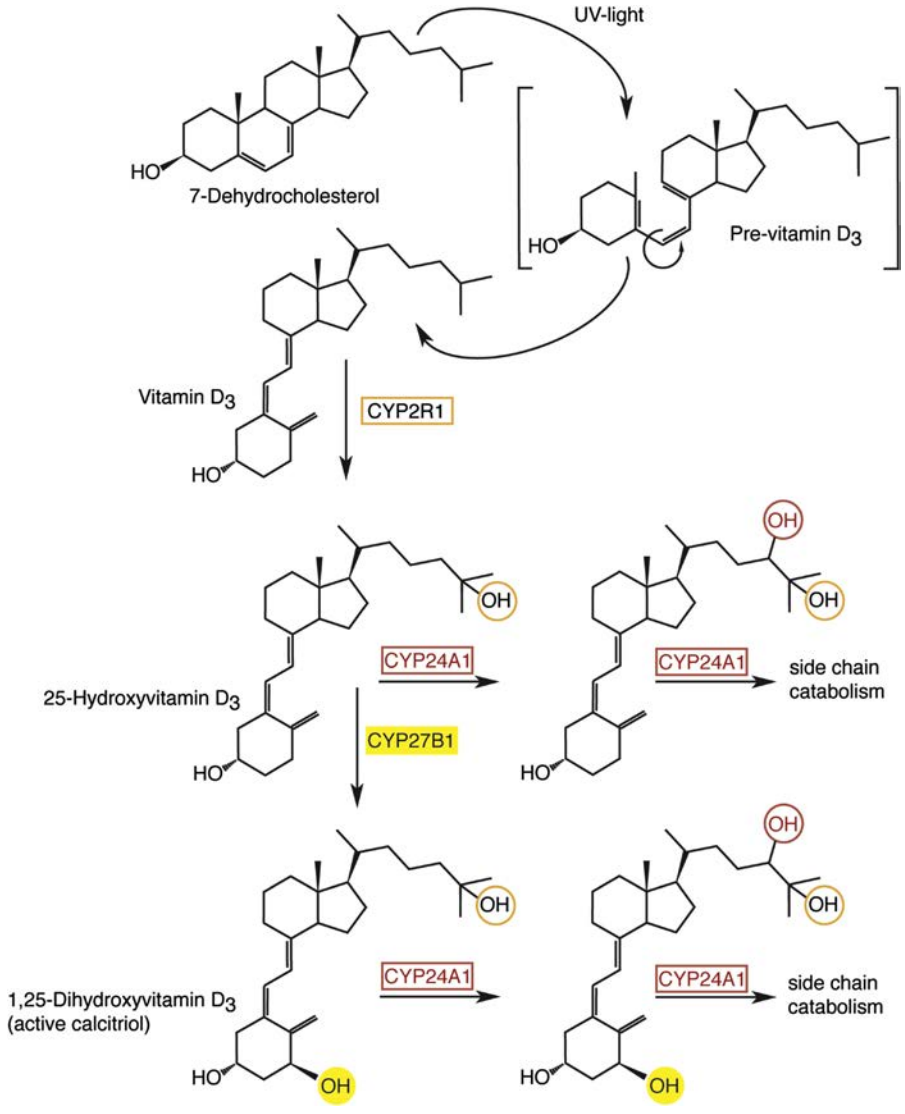

FIGURE 10.3 Vitamin D activation and inactivation pathways. Exposure of skin to solar UVB radiation (290–315 nm) causes the photolysis of 7-dehydrocholestrol to form previtamin D_3, which isomerizes into vitamin D_3.[203] High latitude, winter season, dark pigmentation, clothing coverage, sunscreen use, and advancing age decrease cutaneous vitamin D_3 production. The *CYP2R1* gene encodes the vitamin D_3-25-hydroxylase that metabolizes vitamin D_3 into 25-hydroxyvitamin D_3, the biologically inactive metabolite that is used as a biomarker of vitamin D_3 status. The *CYP27B1* gene encodes the 25-hydroxyvitamin D-1α-hydroxylase that metabolizes 25-hydroxyvitamin D_3 into 1α,25-dihydroxyvitamin D_3 (calcitriol), a biologically active seco-steroid hormone. The *CYP24A1* gene encodes an enzyme that catalyzes multiple hydroxylation reactions of the side chains of both 25-hydroxyvitamin D_3 and 1α,25-dihydroxyvitamin D_3, ultimately producing the biliary metabolite calcitroic acid. The *CYP27B1* and *CYP24A1* genes are subject to complex regulation including calcitriol-mediated feedback inhibition of *CYP27B1* and induction of *CYP24A1*.

myeloid lineage cells, the CNS, the gut, and other tissues. The *CYP24A1* gene encodes the 24-hydroxylase that degrades both 25-(OH)D_3 and calcitriol (Fig. 10.3). Calcitriol exerts its biological activity through the nuclear vitamin D receptor (VDR).[209] The VDR functions as a calcitriol-activated transcriptional regulator. Severe vitamin D_3 deficiency in early life causes nutritional rickets.[68,69] Inheritance of *CYP27B1*-null alleles causes a heritable form of rickets.[210]

Circulating 25-(OH)D varies with latitude, season, skin pigmentation, occupational sunlight exposure, age, clothing, diet, and genotype (Fig. 10.4A).[204,206,207] Sun avoidance may lead to lower 25-(OH)D levels in females than males (Fig. 10.4B).[208] There is no certain relationship between a single 25-(OH)D measurement and the level that may have been present at a time relevant to MS risk acquisition. Therefore, studies using aggregated 25-(OH)D data to demonstrate correlation or lack thereof with MS risk must be interpreted cautiously.

Low UV light exposure, particularly in the winter, correlated with MS risk.[45] Evidence correlating UV light exposure with MS risk has been reviewed.[28,211] UV irradiance varies 400-fold with latitude,[212] correlating inversely with about a 400-fold variation in MS prevalence in populations with disparate ancestries, distinct dietary and smoking habits, and dissimilar exposures to infectious and commensal organisms.[46] UV light exposure in childhood and adolescence is closely associated

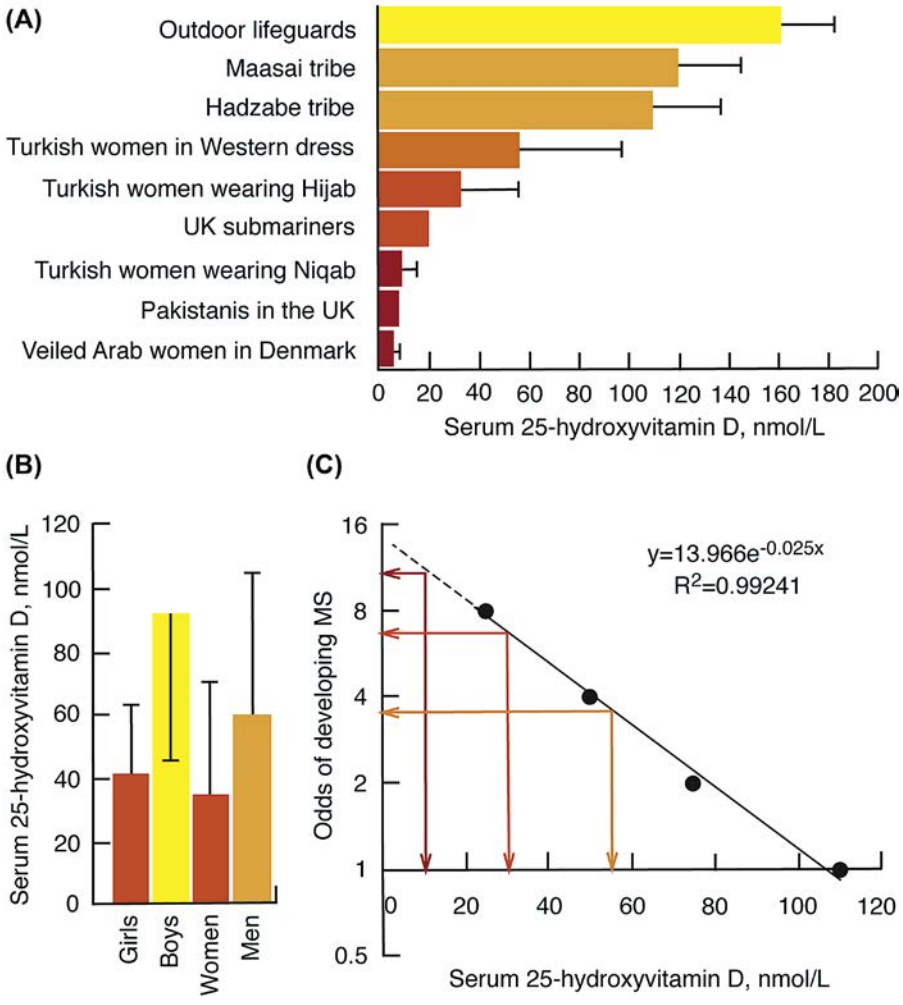

FIGURE 10.4 Variation in circulating 25-hydroxyvitamin D levels and theoretical relative odds of developing MS. (A) Circulating 25-hydroxyvitamin D levels in populations exposed to abundant sunlight like lifeguards[233] and Tanzanians living ancestral lifestyles[230] compared to populations with little or no sunlight exposure like veiled Turkish women,[232] UK submariners, Pakistanis in the UK,[233] and veiled Arab women living in Denmark.[234] (B) Circulating 25-hydroxyvitamin D levels as a function of gender in Iranian adolescents[208] and adults.[325] (C) Graphical representation of the theoretical relative odds of developing MS as a function of circulating 25-hydroxyvitamin D levels. The graph is based on data from a Mendelian randomization study in populations who cluster genetically with Europeans.[229] The arrows represent the theoretical impact of variation in circulating 25-hydroxyvitamin D levels attributable to wearing Western dress (gold), traditional Hijab dress with face and hands exposed to sunlight (orange), or traditional Niqab dress with no skin exposed to sunlight (red) on the relative odds of developing MS, assuming the women cluster genetically with Europeans.

with MS risk in adulthood.[66,213–216] Vitamin D_3 and calcitriol are UV light's hypothesized biological signal transducers due to their ability to selectively regulate T cell–mediated autoimmune responses.[165]

Abundant and diverse evidence now supports this hypothesis.[28,171] Only the most compelling evidence is presented here. Rare *CYP27B1* gene loss-of-function mutations correlated with a significantly increased risk of MS.[217–220] In multi-incident MS families, 35 of 35 cases inherited one defective *CYP27B1* allele, an inheritance pattern with 1 in a billion odds of a chance occurrence.[218] *CYP27B1* mutations are highly penetrant but exceedingly rare, so they mark calcitriol synthesis as a key determinant of MS risk, but do not contribute genetic risk in the vast majority of MS cases. Also, a vitamin D-response element (VDRE) was discovered in the *HLA-DRB1*15* gene promoter suggesting that vitamin D status may influence the expression of this gene.[221]

Observational studies have correlated low vitamin D status with later risk of MS independently of personal UV light exposure.[222–225] This relationship was stronger in females than males.[226] Two studies have reported a vitamin D influence during gestation. The children of pregnant women who had modest vitamin D intake had about a 40% lower risk of MS compared to children of women who had low maternal vitamin D intake.[227] Conversely, the children of pregnant women who were vitamin D-deficient had a 2-fold higher risk of MS compared to children of women who were vitamin D sufficient.[228]

Determining unequivocally whether vitamin D_3 influences MS risk and estimating the effect size has proven difficult due to the challenges posed by conducting a randomized controlled trial (RCT) for a relatively rare disease with a decades-long incubation period. A Mendelian randomization study met these challenges and delivered stunning results.[229] The investigators identified four single nucleotide polymorphisms (SNPs) associated with reduced 25-(OH)D_3 using SUNLIGHT study data (n = 33,996) and confirmed the effect of the four SNPs on 25-(OH)D_3 in a second data set (n = 2347). Then 14,498 MS cases and 24,091 healthy controls were sorted into groups according to the four SNPs and the distribution of MS cases was determined. The lower the genetically determined 25-(OH)D_3 level the higher was the frequency of MS cases. "Each genetically determined one-standard-deviation decrease in log-transformed 25(OH)D level conferred a twofold increase in the odds of MS (95% CI: 1.7–2.5; $p = 7.7 \times 10^{-12}$)." It would be extremely interesting to learn if there were sex-based differences in the odds of MS.

We plotted the Mendelian randomization study data, assumed a log–linear relationship and fitted an equation to the plot (Fig. 10.4C). The equation allows one to calculate the theoretical relative odds of developing MS as a function of 25-(OH)D level in this population. Severe hypovitaminosis D (25-(OH)D < 10 nmol/L) would increase MS risk >11-fold compared to 111 nmol/L, the level in the individuals with minimal MS risk. Notably, 111 nmol/L is very close to the 115 nmol/L level observed in contemporary Africans leading ancestral lifestyles (Fig. 10.4A).[230] The 115 nmol/L range likely reflects a physiological level that was optimized during human evolution.[231]

The equation also allows one to calculate the theoretical relative odds of developing MS in populations with reduced 25-(OH)D due to occupational, residential, or clothing choices that limit sunlight exposure, assuming equal exposures to other genetic and nongenetic risk factors (Fig. 10.4A). For example, other things being equal, the theoretical relative odds of developing MS would be >11-fold higher in Turkish women wearing traditional Niqab dress,[232] Pakistani immigrants to the UK,[233] and veiled Arab women living in Denmark[234] compared to the individuals with minimal MS risk. At the other end of the 25-(OH)D spectrum, the theoretical relative odds of outdoor lifeguards in Florida developing MS would be negligible.[233]

In summary, low vitamin D status appears to be the strongest nongenetic exposure contributing to MS risk in genetically susceptible individuals. This exposure alone may account for a >11-fold variation in MS risk. Exposure to low vitamin D status increased with latitude, increased MS risk in a female-biased manner, was strongly associated with RRMS versus PPMS,[235–243] and appeared to contribute to MS risk in gestation, early neonatal life, childhood, and adolescence. Some evidence suggests that low vitamin D status may interact with *HLA-DRB1*15* in gestation and early neonatal life,[55,221] with estrogens at puberty (see following section), and with EBV infection postpuberty[169,244] to determine the emergence of RRMS in females.

Vitamin D Mechanisms

To study the impact of vitamin D_3 on demyelinating disease risk, vitamin D_3-deficient mice have been compared to vitamin D_3-replete mice for signs of EAE after myelin antigen immunization.[127,245,246] In these systems, vitamin D_3-replete females had delayed EAE onset, reduced peak severity, lower cumulative disease severity, less CNS pathology, and less demyelination compared to vitamin D_3-deficient females. The vitamin D_3–replete and vitamin D_3–deficient males did not differ for any EAE parameter. The females had reduced *CYP24A1* gene expression and higher calcitriol in the CNS than males, correlating with significantly less severe EAE disease. Importantly, although vitamin D_3 supplementation inhibited EAE induction in a female-biased manner, it had no effect on established disease.

In contrast, calcitriol inhibited EAE induction and established EAE disease in females and males.

Mechanisms of vitamin D_3 and calcitriol action in the EAE model have been reviewed.[171] The protective mechanisms are entirely dependent on the *Il10* and *Vdr* genes in CD4+ Th1 cells.[247,248] There are some inconsistencies, but there is general agreement that diminished CD4+ Th1 and Th17 cell and increased IL-10-producing CD4+ Tr1 and CD4+Helios+FoxP3+ Treg cell accumulation in the CNS are observed. Mechanisms reported for reduced pathogenic Th1 and Th17 cell accumulation were increased sensitivity to apoptosis, suppressed cytokine gene transcription, and post-transcriptional inhibition of cytokine production. Mechanisms reported for increased CD4+ Tr1 cell and CD4+Helios+FoxP3+ Treg cell accumulation included suppression of IFN-γ and IL-2 production and enhancement of IL-10 production to promote Tr1 cells, and enhanced *Ikzf2* and *Foxp3* gene transcription to promote Helios+FoxP3+ Treg cells.

We hypothesize that vitamin D status influences thymic selection. High-level *Vdr* gene transcription was observed in medullary thymic epithelial cells (mTECs) (Fig. 10.5A).[249] The mTECs express the autoimmune regulator gene, *Aire*, upon signaling through receptor activator of NF-kappaB (RANK).[250–253] AIRE (autoimmune regulator) allows promiscuous expression of tissue-restricted proteins like myelin in mTEC, so these cells display restricted self-peptides for autoreactive T-cell deletion[254] or diversion into the Treg cell lineage.[255] Calcitriol increased both RANK and RANK-ligand (RANKL) expression by VDR-dependent transcriptional mechanisms.[256,257] The *Tnfsf11* gene encodes RANK ligand. There are two VDREs in the *Tnfsf11* promoter that are 90% conserved between mouse and human.[257] Vitamin D_3 might also influence thymic selection through *Ikzf2* gene expression.[134] Helios was a unique marker on strongly autoreactive CD4+ thymocytes undergoing negative selection.[258,259] We hypothesize that low vitamin D status decreases RANKL–RANK signaling, *Aire* induction, and mTEC display of tissue-restricted myelin peptides, such that myelin peptide-specific thymocytes are not purged in the thymus or converted to Treg cells, and instead seed the periphery with potentially encephalitogenic CD4+ T cells. New data showing variation in thymic output by season of birth are consistent with this hypothesis.[260] Testing this hypothesis is an MS research priority.

There are many reports of calcitriol action on human T cells from MS patients in vitro, but very few studies of the effects of vitamin D_3 supplementation on T cell–mediated immune responses in MS patients in vivo. One in vivo study found that as serum 25-(OH) D_3 levels rose to >100 nmol/L, proliferative responses of PBMC to myelin antigens declined significantly.[261] Another study confirmed and extended this finding,

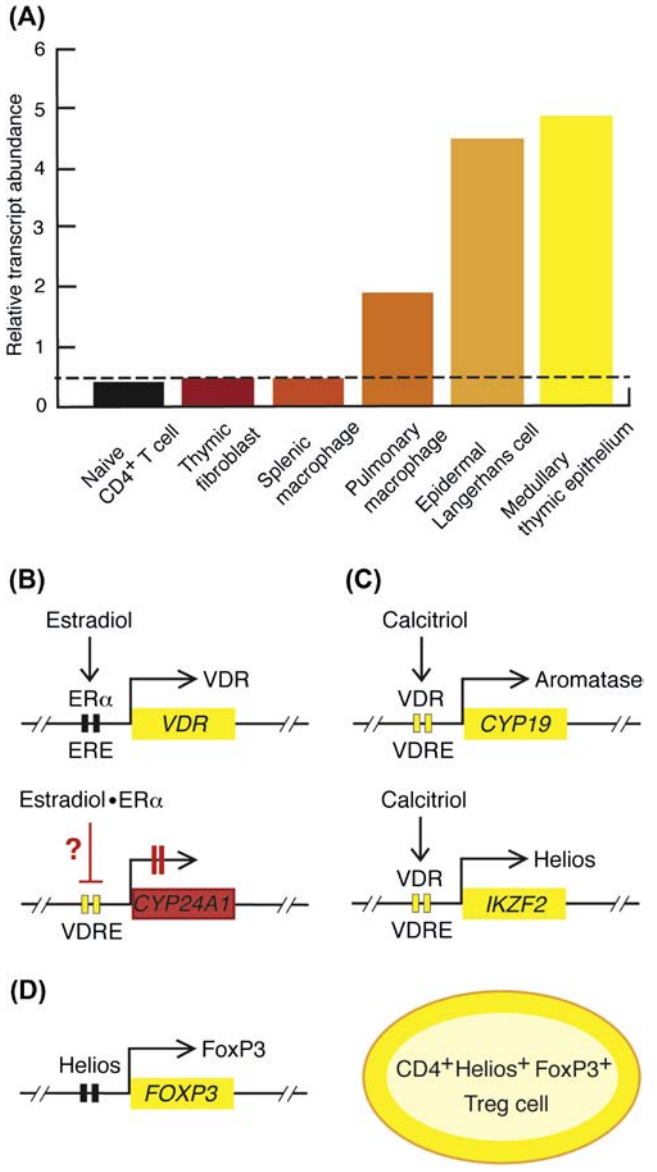

FIGURE 10.5 Possible calcitriol actions in the thymus and in activated CD4+ T cells to promote immune tolerance. (A) High-level *Vdr* gene transcription occurs in the medullary thymic epithelium. The data are from the ImmGen database (https://www.immgen.org/).[249] Flow-sorted cells were obtained from C57BL/6 mice. The RNA transcript analysis was performed using Affymetrix microarrays. The *dashed line* represents the limit of detection based on expression levels in cells that are known to lack *Vdr* gene expression. (B) E2 increases *Vdr* gene expression and decreases *CYP24A1* gene expression. These actions prolong calcitriol signaling and increase calcitriol responsiveness. (C) Calcitriol may increase *Cyp*19 gene expression to promote E2 synthesis from testosterone. Calcitriol increases *Ikzf*2 gene expression to promote differentiation to a Treg phenotype. (D) Helios promotes *FoxP*3 gene expression to induce and stabilize the Treg phenotype.

demonstrating enhanced IL-10 production by CD4+ PBMC in the vitamin D_3-supplemented group.[262] The IL-10 findings have been replicated.[263–265] Decreased IFN-γ production and improved Tr1 cell suppressor function by CD4+ PBMC were also observed in vitamin

D_3-supplemented MS patients,[263] but no significant change in IL-17 production was observed.[266] However, a study performed in healthy human subjects during the UV-restricted winter months found as serum 25-(OH)D_3 levels rose to 159 nmol/L, the proportions of Th1 and Th17 cells in the peripheral blood decreased.[267] Additional vitamin D_3 supplementation studies are ongoing and results are expected soon.

VITAMIN D AND ESTROGEN SYNERGY IN T-CELL SELF-TOLERANCE

It has proven difficult to explain the rising female bias in RRMS incidence rates. The fact that sexually mature females produce more robust immune responses than males is cited as the cause of the female bias observed in MS.[78–80,83,268] However, one might expect the more robust female immune response to cause more aggressive MS disease in females than males, but the opposite is true.[79] This explanation is consistent with the increase in the F:M ratio after puberty, but inconsistent with the known protective functions of E2 in MS and demyelinating disease models. Moreover, this explanation cannot account for the rapid rise in female RRMS but not PPMS cases.

New research in the EAE model has revealed a female-specific cooperative interaction between vitamin D_3 and E2 in $CD4^+$ T cells to drive differentiation of the $Helios^+FoxP3^+$ Treg cells that maintain self-tolerance (Fig. 10.5). Loss of this self-tolerance mechanism as population vitamin D status declines globally may be contributing to the increasing female RRMS incidence rates. In the EAE model, female OVX surgery eliminated and diestrus-level E2 replacement restored the female-specific vitamin D_3–mediated protective effects, as described earlier.[127,245] Unlike pregnancy level E2, the diestrus-level E2 did not inhibit EAE in OVX mice independently of supplementary vitamin D_3. Serum calcitriol levels did not differ between the vitamin D_3-replete OVX and SHAM females. However, spinal cord *CYP24A1* transcript levels were lower and calcitriol levels were 7.5-fold higher in the SHAM females and OVX females with E2 replacement than in the OVX females without E2 replacement. These data provided the first evidence that gonadal E2 synthesis reduced spinal cord *CYP24A1* transcript levels and stimulated de novo calcitriol synthesis and accumulation in the CNS to support calcitriol–mediated anti-inflammatory and neuroprotective functions.

EAE research also demonstrated a vitamin D_3–mediated twofold enhancement of gonadal E2 synthesis and an E2-mediated about 32-fold enhancement of *Vdr* transcripts in the inflamed CNS.[127] These observations prompted the hypothesis that E2 might attenuate demyelinating disease through a VDR-dependent mechanism. Since myelin-reactive $CD4^+$ T cells were identified as the targets of both calcitriol[247] and E2[128,135] in the EAE model, the hypothesis was addressed by examining E2-mediated inhibition of EAE in female mice with T cell–specific *Vdr* gene targeting.[134] In myelin-reactive $CD4^+$ T cells in vitro, E2 silenced *CYP24A1* gene transcription and increased *Vdr* gene transcription >5-fold, which would prolong calcitriol's half-life and increase the T cell's calcitriol responsiveness. The E2 increased the number of $CD4^+Helios^+FoxP3^+$ Treg cells in the CNS by twofold, and decreased the encephalitogenic $CD4^+$ T cells in the CNS by 80%. All of these E2 actions depended on a functional *Vdr* gene in the $CD4^+$ T cells.

Linking these several mechanisms together yields a cooperative amplification loop (Fig. 10.5). The calcitriol enhances E2 biosynthesis probably by VDR-mediated up-regulation of *Cyp19* gene expression, although this has not been demonstrated in T cells. The E2 silences *CYP24A1* to prolong calcitriol's half-life and increases *Vdr* gene transcription to enhance the T cell's calcitriol responsiveness. Finally, the cooperative interaction between the two hormone systems decreases encephalitogenic $CD4^+$ T cells and increases $CD4^+Helios^+FoxP3^+$ Treg cells, possibly through a hormone-mediated, bistable switch mechanism.[171]

There is some evidence that a cooperative E2 and vitamin D_3 amplification loop could exist in women, although it has not been specifically demonstrated in any cell type. Changing estrogen levels during the menstrual cycle[269,270] and in pregnancy[271] preceded and correlated with fluctuations in serum calcitriol. Also, therapeutic E2 administration increased serum calcitriol in young[272] and postmenopausal women.[273–276] These data support a cause–effect relationship between E2 and calcitriol synthesis. Calcitriol increased transcription of the human *CYP19* gene encoding aromatase, the rate-limiting enzyme in E2 synthesis.[277,278] These data support a cause–effect relationship between calcitriol and E2 synthesis. E2 enhanced *VDR* gene transcription in a variety of human tissues and cells through EREs in the human *VDR* gene promoter.[279–283] These data support a cause–effect relationship between E2 and *VDR* gene expression. To our knowledge there are no data on possible E2- or calcitriol-mediated enhancement of *Ikzf2* gene transcription and Helios protein expression in the human. Helios is a transcription factor that enhances *Foxp3* gene transcription, and FoxP3 is the lineage-determining transcription factor of Treg cells.[284–286]

The functional synergy between vitamin D_3 and E2 in $CD4^+$ T cells may have evolved in female mammals to induce $CD4^+$ Treg cells capable of protecting a fetus from a maternal T cell–mediated immune attack directed to paternal antigens.[127,134,171,287] High-level *CYP27B1* gene expression and calcitriol synthesis in human and

rodent maternal decidua and fetal trophoblasts supports this possibility.[210,288,289] Seasonally fluctuating 25-(OH)D levels in combination with microbial antigens that mimic myelin antigens and a peripheral T-cell repertoire that has not been purged of cross-reactive T cells could drive MS relapses and remissions by alternately promoting and suppressing the cross-reactive T-cell responses. Consistent with this possibility, RRMS attacks vary seasonally peaking in late winter and early spring and reaching a nadir in late summer and early fall.[155,223,238,240,290–292]

HYPOTHESES FOR RISING FEMALE MS INCIDENCE

Several hypotheses have been advanced to explain rising female MS incidence (Table 10.1). They include increased smoking,[176] a rising rate of late EBV exposure,[211] or the global decline in population vitamin D status.[28] We also included the estrogen–vitamin D synergy hypothesis because it provides a plausible mechanism for the trend's female bias.[127,134]

Bradford–Hill criteria have proven valuable in the effort to determine whether an environmental factor is causally linked to a disease.[27] Therefore, we adapted them to evaluate the hypotheses (Table 10.2). We acknowledge that there are other valid approaches. Although a single hypothesis is unlikely to explain all of the epidemiological facts and nongenetic exposures almost certainly interact, we sought to identify a dominant modifiable factor to exploit in MS-prevention efforts.

We selected three case studies, Canada,[152] Scotland,[6] and Iran,[14,15,25,156–160] for examination in detail. We chose Canada because it is a high-latitude country where the rising female RRMS incidence trend is long-standing, steep, and well documented. Moreover, two Canadian Provinces, Nova Scotia ($267/10^5$) and Alberta ($358/10^5$), represent the extreme high end for MS incidence and prevalence. We chose Scotland because it is the high-latitude country with the highest MS prevalence rate in the world, and to honor Scottish physician Sir Robert Whytt who contributed the first MS case study to the medical literature.[1] Finally, we chose Iran because it is a low-latitude country where the rising female RRMS incidence trend is very recent and steep. We anticipate that close scrutiny of these three extreme examples has the highest probability of providing unambiguous insight into a dominant underlying causal factor.

Smoking

Changes in smoking behavior have probably not contributed substantially to rising female MS risk.[176] The smoking effect size was weak (OR = ~1.5), there was no association between this risk factor and latitude, and smoking's contributions to MS risk appeared to peak in the third decade of life. Smoking showed no consistent differential impact on females versus males, nor a preferential association with RRMS versus PPMS. Iran

TABLE 10.1 Hypotheses to Explain the Female-Biased Increase in MS Incidence

Factor	Observation and OR[a]	Hypothesis	References
Smoking	Smoking correlates with MS risk; the estimated OR was 1.5 for ever- compared to never-smokers	Rising smoking rates in females relative to males may have increased female MS risk	53, 167, 168, 176, 178
Epstein–Barr virus infection	EBV infection, particularly infectious mononucleosis (IM), correlates with MS risk; the estimated OR was 2.6 for EBV infection compared to no EBV infection; the estimated relative risk was 2.2 for history of IM compared to no IM history	Rising rates of late EBV exposure in females relative to males may have increased female MS risk	192, 195, 293, 294
Low vitamin D	Low vitamin D status correlates with MS risk, particularly female MS risk; the estimated relative risk was 2.6 for individuals with 25(OH)D < 63 nmol/L compared to 25(OH)D > 99 nmol/L; the estimated OR increased by 2.0 for each SD decrease in the genetically determined 25(OH)D level	The global trend toward lower population vitamin D status due to decreases in UVB light exposure and vitamin D ingestion particularly in females may have increased female MS risk	28, 211, 226, 229, 245
Estrogen and vitamin D synergy	Puberty correlates with increased female MS risk; low vitamin D status correlates particularly with female MS risk; animal modeling demonstrated a cooperative amplification loop involving estrogen and the vitamin D hormone interacting synergistically within $CD4^+$ T cells to promote $Helios^+FoxP3^+$ Treg cell development	Estradiol and the vitamin D hormone act interdependently to regulate $CD4^+$ T cell mediated immune responses in females; the global trends toward lower population vitamin D status, later maternal age, and lower offspring number may be disrupting this interdependent hormonal regulation of self-tolerance and increasing female MS risk	100, 101, 127, 134, 245, 295

[a]*OR*, Odds ratio.

TABLE 10.2 Bradford–Hill Criteria and Hypothesis Evaluation[a]

Criterion	Description	Smoking	EBV	Low Vitamin D	E2 and Vitamin D Synergy
Strength	The factor influences MS risk sufficiently strongly that an increase in exposure could significantly increase MS incidence		☐	☐	☐
Consistency	The factor influences MS risk reproducibly in different people, places, and circumstances	☐	☐	☐	☐
Specificity	The factor influences MS risk in a female-biased manner			☐	☐
Temporality	Exposure to the factor precedes increased MS risk; the lag is appropriate to a cause–effect relationship		☐	☐	☐
Biological gradient	There is a dose–response relationship; as exposure to the factor increases, MS risk increases	☐		☐	☐
Plausibility	There exists a plausible biological mechanism consistent with current knowledge to explain how increased exposure may increase MS risk		☐	☐	☐
Coherence	The cause–effect explanation regarding increased exposure to the factor and increased MS risk coheres to generally known facts of the natural history and iology of MS disease		☐	☐	☐
Experiment	Interventions that decrease or increase, exposure to the factor decrease or increase MS risk, respectively			☐	☐
Analogy	Increased exposure to the factor correlates with an increase in the risk of other related diseases			☐	☐

[a]*Adapted from Bradford–Hill.*[27]

serves as an illustrative case study. Although smoking is an MS risk factor in Iran,[296,297] particularly among men,[184] fewer than 11% of Iranian women smoke.[298] With the possible exception of Iranian immigrants in British Columbia,[53] smoking seems an unlikely explanation for the Iranian MS trend data particularly the pediatric cases (Fig. 10.2). Swedish investigators estimated that 41% of *HLA-DRB1*15* MS cases were attributable to smoking.[178,186] Since female gender increased the penetrance of the *HLA-DRB1*15* risk gene in their study population, they concluded that smoking habits may be contributing to the female-biased MS incidence trend. Norwegian researchers drew similar conclusions.[299] However, Australian investigators found no interaction between smoking and *HLA-DRB1*15*.[169] We agree that smoking may be increasing the female MS risk gene penetrance in some populations and regions, but it is unlikely to be a dominant factor from a global perspective.[176]

Epstein–Barr Virus

We found no evidence that rates of EBV infection have increased. The EBV effect size was moderate (OR = ~2–4), there was a weak association between this risk factor and latitude, and its contributions to MS risk appeared to peak in the second decade of life when IM is common. There was no preferential association of EBV with female versus male MS risk or with RRMS versus PPMS risk. Scotland and Iran serve as illustrative case studies. The annual rate of positive IM tests peaked at 17 years in Scottish females and 19 years in males, but IM incidence rates decreased from $174/10^5$ in 1997 to $67/10^5$ in 2012.[300] Seropositivity for EBV was not associated with MS in Iran.[296,301] We conclude that rising rates of EBV exposure have probably not contributed significantly and independently to rising female MS risk.

Low Vitamin D Status

Vitamin D deficiency is reemerging as a global health problem,[302–304] and the hypothesis that this change could be a dominant contributor to increased female RRMS risk satisfied all Bradford–Hill criteria. An estimated 1 billion people worldwide now have vitamin D insufficiency (25-(OH)D < 75 nmol/L) or deficiency (25-(OH) D < 50 nmol/L).[302] Trend data from the NHANES survey show that vitamin D deficiency doubled in the American population in a single decade.[303] In the European population the prevalence of vitamin D deficiency has climbed to 40.4%, with 3- to 71-fold higher prevalence in some darkly pigmented ethnic subgroups.[304] As clear

evidence of this trend, rickets is reemerging as a global health problem even in regions with high ambient solar radiation.[68,69,305,306]

In Scotland, urbanization and diminished oily fish consumption have caused an epidemic of vitamin D deficiency.[307,308] The mean 25-(OH)D level in the Scottish population has dropped to 37.5 nmol/L, less than the cut-off for vitamin D deficiency, and 47% of the population is severely deficient (25-(OH)D < 25 nmol/L).[309,310] Nutritional rickets has reemerged especially among Middle Eastern and South Asian immigrants to Scotland. The number of rickets cases recorded doubled between 2002 and 2008.[311] Several rickets cases have been identified incidentally by radiological criteria.[312] The 2008 Scottish National Dental Inspection Programme found that 42% of 5-year-old children had carious, extracted, or filled deciduous teeth.[313] Poor childhood dental health is also a sign of vitamin D deficiency.[314,315] Poor childhood dental health correlated strongly with MS risk.[316]

Canadian surveys report that 70% and 97% of Canadians have vitamin D insufficiency and many have profound deficiency.[317] The prevalence rates of vitamin D deficiency and insufficiency were relatively high (exceeding 30–35%) in pregnancy and the post-partum period.[318] Vitamin D-deficiency rickets has also reemerged in Canadian children with an overall annual incidence of $2.9/10^5$.[319] Rickets incidence rates were highest among children residing in the north. Canadian Aboriginal children were particularly at risk; between 1999 and 2013 their incidence of rickets increased 2.3-fold for every 4° increase in latitude.[320]

In the Persian Gulf states (Bahrain, Iran, Iraq, Kuwait, Oman, Qatar, Saudi Arabia, and United Arab Emirates), vitamin D inadequacy has become prevalent due to a transformation of lifestyle based on technology, sedentary activity, lack of sunlight, and unhealthy dietary patterns.[321,322] Iranian surveys reported in 2004 found vitamin D deficiency in 79.6% of the subjects,[323] particularly pregnant women and their newborns,[324] young girls (Fig. 10.4B),[208,325–327] and women wearing traditional Islamic clothing as required by law.[328] Among Islamic women, vitamin D deficiency was evident in 60% of those with hands and face exposed and 100% of those with hands and face covered (Fig. 10.4A).[232] A "red alert" was published in 2012 concerning a 91.7% prevalence of vitamin D deficiency in school-age children in Tehran.[329] Rickets has also emerged as a health problem in Iranian girls.[330,331] The trend line joinpoint (Fig. 10.2A) and the jumps in pediatric (Fig. 10.2B and D) and adult female cases (Fig. 10.2B) all point to 1979–1981 as a period of cultural changes, including new requirements for traditional Islamic dress, that may have increased penetrance of MS risk genes in a female-biased and RRMS-specific manner.[21,23,159,321,322]

We conclude that case study data from Canada, Scotland, and Iran, together with new mechanistic data from animal modeling, strongly support the vitamin D and estrogen hypothesis. Specifically, we propose that global declines in population vitamin D status may have been undermining a female-specific and T cell–mediated immune self-tolerance mechanism with the result that female MS incidence has been rising globally.

REVERSING THE RISING TREND IN FEMALE MS INCIDENCE

Defining Vitamin D Adequacy

Defining optimal vitamin D_3 intake and 25-(OH)D levels from a nutritional perspective has proven to be difficult.[332] Moreover, a statistical error resulted in a Recommended Daily Allowance of 600 international units (IU) per day that is too low by a factor of >10.[333] Total vitamin D (from all sources) needed to achieve 25-(OH)D (50 nmol/L) in 97.5% of the population is about 7000 IU/day.[334] There is contentious debate about 25-(OH)D levels needed for optimal biological function.[335]

Defining optimal 25-(OH)D biochemically is straightforward.[336] The human vitamin D-25-hydroxylase shows a biphasic response to a broad range of vitamin D_3 inputs (from all sources). When vitamin D_3 is low, the enzyme produces 25-(OH)D rapidly with first-order reaction kinetics indicating substrate is insufficient to supply the available enzyme. When vitamin D_3 is ≥15 nmol/L and 25-(OH)D is ≥80 nmol/L, the enzyme produces 25-(OH)D slowly with zero-order reaction kinetics indicating substrate is sufficient to enable the available enzyme to operate at maximal velocity. Thus, these parameters biochemically define the low end of optimal vitamin D_3 status.[336] Evolution optimized human biology to function with 80–115 nmol/L of 25-(OH)D as the physiological level (Fig. 10.4A).[231]

MS Prevention Study Designs

The National Multiple Sclerosis Society convened a task force to critically examine the evidence for a causal association between low vitamin D status and MS risk and to develop research study designs to evaluate the effect of vitamin D_3 supplementation on MS incidence. Participants are listed in the acknowledgments. The group was nearly unanimous in its assessment that evidence linking low vitamin D status with MS risk was sufficient to meet the Bradford–Hill criteria for causality. A few participants asserted that UV light strongly influences MS risk independently of vitamin D.[337] The Mendelian randomization study resolved the UV light versus vitamin D debate by demonstrating that genetically determined

low 25-(OH)D status strongly influenced MS risk independently of UV light exposure (Fig. 10.4C).[229] The group was nearly unanimous in its assessment that a compelling rationale exists for initiating vitamin D_3 and MS prevention studies.

The periods of vitamin D_3-modifiable MS risk acquisition were discussed. Participants agreed that the MS disease process begins long before MS becomes clinically evident.[338–341] New data from the EAE model show vitamin D_3 supplementation had no impact on established demyelinating disease.[246] The window of opportunity to modify MS risk may close after the disease process has begun but before MS becomes clinically evident. There was consensus that month-of-birth studies represent natural experiments in MS risk alteration in utero or early neonatal life, and these data support vitamin D_3 intervention beginning in pregnancy for maximum benefit.[342] Data reported in 2016 strengthened this conclusion. The Finnish Maternity Cohort data show maternal hypovitaminosis D (25-(OH)D < 12 ng/mL) correlated with a twofold increased risk of MS in the offspring compared with women who did not have hypovitaminosis D.[228] Thus, the case for maternal vitamin D_3 supplementation is now stronger. There was also consensus that migration studies represent natural experiments in MS risk alteration, and these studies support intervention throughout childhood and early adolescence for MS risk reduction.[49,343,344] The sunlight data are consistent with the migration data in support of vitamin D_3 intervention in childhood and early adolescence.[66,213,214]

The participants debated several primary MS prevention study designs. The traditional RCT study may not be feasible for this relatively rare disease with a long incubation period, but it may not be necessary. The *F:M Ratio Study* proposes that males serve as intrinsic controls for females.[8] Specifically, a vitamin D_3 intervention trial could assess the F:M incidence ratio for those diagnosed with MS. A decline in the F:M ratio would signal reduced female incidence relative to males. Such a study might be feasible in Alberta or Nova Scotia, Canada, where low vitamin D status is common, the F:M ratio is high, and historical incidence data are robust. It may not be feasible in Iran and other regions where the F:M ratio has not increased over time.

The *All Comers-All Outcomes* study would enroll any person who wished to participate via an Internet platform. Individuals would select a vitamin D_3 intake group to join and self-report their outcomes some years later. The potential to enroll high numbers of participants and generate a lot of data on multiple health conditions was the attraction for the *All Comers-All Outcomes* study. Indeed, the International Sample of People with MS study has recruited MS patients through an Internet platform to collect data on self-reported lifestyle choices and MS disease course.[345] However, many concerns were expressed including too many variables, lack of control over variables, no compliance assessment, selection and reporting bias, high attrition, low enrollment of desired populations, and the possibility that major windows of vitamin D_3-modifiable risk acquisition would be missed. For these reasons, the *All Comers-All Outcomes* study generated low enthusiasm.

The *First-degree Relatives* study envisioned enrolling high-risk adolescent and young adult first-degree relatives of an MS case.[346] Enrolling fewer participants for a shorter study period was viewed as a strength. A concern was that the *First-degree Relatives* study might miss major windows of vitamin D_3-modifiable risk acquisition. If vitamin D_3-modifiable mechanisms include epigenetic marking during gestation and purging of autoreactive thymocytes from the peripheral T-cell repertoire in childhood, then this study would miss these windows. If vitamin D_3 modifies microbial exposures that activate autoimmune T cells by molecular mimicry, the study might detect this effect. A second concern was that the *First-degree Relatives* study might enrich the study population for vitamin D_3 nonresponders, individuals with genetically determined low vitamin D status[229] or disruptions in pathways downstream of calcitriol in $CD4^+$ T cells.[171] Thus, there was a perceived high risk of a costly false-negative conclusion for this study design. For these reasons the *First-degree Relatives* study generated moderate enthusiasm.

The *Mothers and Children* study envisioned enrolling pregnant women, infants, and prepubertal children grouped by age (e.g., 0–4, 5–8, 9–12, and 13–16 years of age) who were not specifically selected for MS risk genes, relatedness to MS cases, or sex. Participants would be recruited through MS societies, obstetricians, pediatricians, day-care centers, and school systems. The study would be performed in regions where low vitamin D status is common, MS incidence is high, incidence data are robust, and a national health-care system exists with uniform MS diagnostic capability. Two vitamin D_3 intake levels would be tested; for example, a low level as recommended by the Institute of Medicine[347] and a higher level as recommended by the Endocrine Society.[335] A report in 2015 identified an egregious statistical error made in the Institute of Medicine (IOM) recommendations,[334] so prudence dictates that an expert panel determine vitamin D_3 intake levels using the best current information. The lack of selection and assessment bias, the dose–response design, the high probability of correctly identifying windows of vitamin D_3-modifiable risk acquisition with accurate effect size estimates, and the inclusion of societies, physicians, schools, and national health-care systems were viewed as strengths. Concerns expressed were the unknowns regarding number of participants and years of follow-up needed to detect an effect. These concerns would

be addressed with open-ended enrollment and assessment. Follow-up studies would be required to optimize the vitamin D_3 intervention strategy. Publications in 2015 present guidance on supplementation in childhood and recruitment and retention of urban schoolchildren into vitamin D studies.[348,349] For these many reasons the *Mothers and Children* study generated high enthusiasm.

The task force participants were unanimous in their desire to begin primary MS prevention efforts immediately. Concern was expressed that delaying action while awaiting more evidence would result in millions of young people developing an incurable and debilitating disease that may have been preventable. To accelerate progress, the participants suggested partnering with researchers and societies whose interests are in diseases like type-1-diabetes that may also be linked to low vitamin D status. Partnerships would conserve resources and eliminate redundancy of effort.

CONCLUSIONS AND RESEARCH QUESTIONS

Our conclusions regarding the nongenetic etiological roots of the rising female RRMS incidence trend and suggested intervention strategies are presented in Table 10.3. The declining vitamin D status hypothesis is the only explanation that fits the data. Low vitamin D status probably influences MS risk acquisition in gestation, childhood, and early adolescence. In gestation or very early in postnatal life, it may influence the propensity to develop MS in genetically at-risk female offspring by controlling epigenetic regulation of systemic 25-(OH)D levels (e.g., *CYP24A1* gene regulation) and/or *HLA-DRB1*15* gene expression (e.g., promoter function). This early step would represent genetic MS risk acquisition. In childhood, it may influence autoreactive thymocyte deletion or conversion to Treg cells and the release of potentially pathogenic $CD4^+$ T cells. This intermediate step would represent the beginning of biological MS risk acquisition. In the sexually mature female, it may influence the balance between Th-cell responses to microbes and Treg-cell responses that maintain self-tolerance. This late step would represent the culmination of biological MS risk acquisition. If these mechanisms are correct, then there are three windows of opportunity to modify MS risk.

We close with hope that investigators and MS societies will answer this call to action and determine whether MS is preventable. Superior scientific leadership might spare millions of young people from the neurological disease that struck Miss Elizabeth Foster in 1756. For those who would criticize the effort as premature and

TABLE 10.3 Conclusions and Research Questions

- Lying at the heart of MS molecular etiology are complex interactions between MHC class II susceptibility and resistance genes, and between risk genes and nongenetic exposures that determine deregulated interactions between APC and $CD4^+$ T cells. When, where, and how these deregulated APC and $CD4^+$ T cell interactions occur remains to be elucidated.
- The maternal parent-of-origin effect implicates events in utero or very early postnatal life in MS risk, particularly in *HLA-DRB1*15* female offspring, leading to higher-risk gene penetrance in females. The molecular details are unknown, but a strong candidate mechanism is an environmentally determined maternal influence on epigenetic marking at the *HLA-DRB1*15* locus in fetal and neonatal $CD4^+$ T cells.
- Puberty increases and pregnancy decreases female RRMS risk. E2 at about 1 nmol/L after puberty increases APC function and strengthens $CD4^+$ T-cell responses. During pregnancy, E2 at about 44 nmol/L and E3 at about 55 nmol/L acting through ERα in $CD4^+$ T cells dampen maternal pro-inflammatory $CD4^+$ T-cell responses and improve $CD4^+$ Treg-cell responses. The mechanisms are not entirely clear but likely involve estrogen influences on lineage-specifying transcription factor networks.
- Animal modeling has demonstrated a female-specific cooperative interaction between vitamin D3 and E2 in $CD4^+$ T cells. Calcitriol enhanced E2 biosynthesis, while E2 silenced *Cyp24a1* gene transcription and increased *Vdr* gene transcription forming an amplification loop. The outcome was an increase in $CD4^+Helios^+FoxP3^+$Treg cells for immunological tolerance. Whether this mechanism operates in humans and has relevance to MS is unknown.
- Female MS incidence has risen about threefold globally and >10-fold in some regions over few decades. The trend applies to RRMS but not PPMS, is most evident at high latitudes, and affects first-generation immigrants. The scope, magnitude, female bias, and rapidity of the trend imply that its etiological roots are nongenetic although epigenetic mechanisms have not been excluded. Environmental exposures together with female sex hormones are believed to be increasing female MS-risk gene penetrance.
- Candidate environmental exposures are smoking, EBV infection, and low vitamin D status. The relative contributions of these exposures may differ between populations, and there are known mechanistic interactions between them. Moreover, the precise timing of these exposures with respect to modifiable MS-risk acquisition remains to be clarified.
- The global trend toward lower vitamin D status satisfies all Bradford–Hill criteria for a dominant contributor to the global increase in female MS incidence. This trend meets criteria of strength (trend magnitude), consistency (different people, places, and circumstances), specificity (female RRMS), temporality (cause–effect time line), biological gradient (lower vitamin D status confers greater risk), plausible mechanism (estrogen–vitamin D synergy for T-cell self-tolerance), coherence (latitude effect), experiment (Mendelian randomization study), and analogy (rickets resurgence).
- An international task force convened by the National Multiple Sclerosis Society was nearly unanimous in its assessment that low vitamin D status elevates MS risk, and further that the evidence is sufficiently strong to provide a compelling rationale for initiating vitamin D_3 and MS prevention studies. The Mothers and Children MS prevention study generated high enthusiasm for its potential to correctly identify windows of modifiable MS risk acquisition and estimate effect sizes.

the scientific evidence as imperfect and incomplete, we offer this quote[27]:

> All scientific work is incomplete—whether it be observational or experimental. All scientific work is liable to be upset or modified by advancing knowledge. That does not confer upon us a freedom to ignore the knowledge we already have, or to postpone the action that it appears to demand at a given time. ***Sir Austin Bradford Hill, 1965.***

ABBREVIATIONS

Calcitriol 1,25-Dihydroxyvitamin D_3
25(OH)D 25-Hydroxyvitamin D
APC Antigen-presenting cells
CNS Central nervous system
E2 17β-Estradiol
E3 Estriol
EAE Experimental autoimmune encephalomyelitis
EBV Epstein–Barr virus
ERE Estrogen-responsive element
ER Estrogen receptor
IFN Interferon
IL Interleukin
IM Infectious mononucleosis
IU International units
MHC Major histocompatibility complex
MS Multiple sclerosis
OR Odds ratio
PPMS Primary-progressive multiple sclerosis
RCT Randomized controlled trial
RR Relative risk
RRMS Relapsing–remitting multiple sclerosis
Th T helper
Treg T regulatory
UV Ultraviolet
VDR Vitamin D receptor
VDRE Vitamin D-responsive element

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CHAPTER

11

Dietary Sodium in Multiple Sclerosis

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OUTLINE

INTRODUCTION

Multiple sclerosis (MS) is an inflammatory disease of the central nervous system (CNS) characterized by demyelination, gliosis, axonal loss, and progressive neurological dysfunction, and represents the leading cause of nontraumatic neurological disability in young adults. The pathogenesis of MS is not fully understood, but current evidence suggests that activation of myelin-reactive T cells triggers an inflammatory cascade in the CNS, recruiting other immune cells that mediate subsequent tissue destruction and pathology.[1,2] The etiology of MS involves both genetic and environmental risk factors (ERFs).[3] Monozygotic twin studies and genome-wide association studies (GWAS) have demonstrated that about 30% of MS risk can be accounted for by genetics, suggesting that about 70% is related to environmental variables and/or gene-by-environment interactions.[3,4] Moreover, epidemiological studies have documented a female-specific three- to sixfold increase in MS incidence and prevalence since about mid-1940s to 1960s.[5] This rate of change clearly implicates newly appeared ERFs that are affecting MS susceptibility preferentially in females.

A number of different types of ERFs have been associated with MS susceptibility. The most prominent of these are Epstein–Barr virus (EBV) infection, low sunlight/ultraviolet (UV) radiation exposure, vitamin D_3 (VitD) deficiency, and cigarette smoking, which have been extensively studied.[3,6] Another, novel putative MS-ERF is high dietary sodium (table salt, or sodium chloride) intake, which has long been associated with negative health outcomes, in particular with regard to cardiovascular disease.[7] Agricultural industrialization during the 20th century has dramatically increased consumption of processed foods and "fast foods" containing high amounts of fat, sodium, and various artificial food additives with poorly characterized properties.[8] Since mid-1960s, dietary sodium intake has increased dramatically in many developed countries,[9–11] temporally coinciding with increasing incidence of MS. Several recent reports have implicated high dietary sodium intake in MS risk and pathogenesis, as outlined later.

EVIDENCE FROM ANIMAL MODELS OF MS

Understanding the etiology and pathogenesis of a complex and heterogeneous disease such as MS requires the use of animal models, where environmental and genetic variables can be controlled in a precise manner, to differentiate between cause and effect. The principal autoimmune model of MS is experimental allergic/autoimmune encephalomyelitis (EAE), which can be induced

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in several animal species (most commonly laboratory mice) by active immunization with CNS homogenate or specific myelin proteins/peptides, or by adoptive transfer of CD4 T cells reactive to these antigens. As in MS, autoreactive CD4 T cells enter the CNS to initiate inflammation and pathology, leading to clinical signs. The EAE model has been instrumental in improving our understanding of MS pathogenesis, and the development of therapies.[12]

In particular, studies in EAE, together with parallel and follow-up studies in MS patients, have provided a detailed mechanistic understanding of the immunological basis of MS pathogenesis. Upon encounter with antigen-presenting cells carrying their cognate antigens (priming), CD4 Th (T helper) cells can differentiate from their naive state to distinct effector Th states/subsets, depending on the cytokine milieu during their priming, each subset characterized by the pattern of cytokines that they produce.[13] Both Th1 and Th17 cells, producing interferon (IFN)-γ and interleukin (IL)-17, respectively, are currently thought to be involved in the pathogenesis of MS.[14] Likewise, Th1 and Th17 cells are critical for EAE pathogenesis, and adoptive transfer of primed myelin-specific Th1 or Th17 cells is sufficient to elicit EAE.[14] Various molecules required for optimal Th1 and Th17 function and trafficking are also required for EAE induction, for example, transcription factors, cytokines, chemokines and their receptors, and adhesion molecules.[12] Importantly, while Th cells initiate the inflammatory cascade in CNS, other immune and CNS resident cells, such as macrophages, B cells, CD8 T cells, microglia, and astrocytes, are thought to mediate the tissue destruction and pathology, by damaging oligodendrocytes and the myelin sheath of nerve axons.[15]

Given the likely pathogenic role of Th17 cells in MS, researchers sought to identify molecular pathways that control their development. By performing transcriptional profiling, Kuchroo and colleagues identified regulatory gene networks that are critical for the pro-inflammatory capacity of Th17 cells.[16] Their analysis also revealed critical "nodes" of these pathways, which surprisingly included the serum glucocorticoid kinase 1 (SGK1), a kinase that had long been known to control sodium (Na^+) transport and homeostasis at the cellular and organismal level,[17,18] suggesting that Na^+ signaling integrated by SGK1 can promote Th17 differentiation. Indeed, it was shown that the addition of a modest amount (40 mM) of sodium chloride (NaCl) in the media to cultured naive mouse Th cells could strongly enhance their differentiation to the Th17 phenotype in a SGK1-dependent fashion.[16] Moreover, genetic deletion (knockout) of SGK1 in Th17 cells protected mice from EAE. Kuchroo and colleagues extrapolated these findings to suggest that a high intake of dietary NaCl could also enhance Th17 differentiation in vivo. Indeed, mice that were placed on a high sodium diet showed increased numbers of Th17 in the intestine and in the CNS (but not in lymphoid organs), and exhibited an exacerbated course of EAE, while neither of these phenotypes were observed in SGK1-deficient mice.[16] In a parallel study, Hafler and colleagues reported very similar results, whereby high dietary NaCl intake exacerbated EAE and increased Th17 numbers in the CNS and in the spleen.[19] Additionally, they showed that Na^+ enhanced in vitro Th17 differentiation of human cells, and identified additional molecular components required for this phenomenon, namely the transcription factor TONEBP/NFAT5, and p38 MAP kinase. Interestingly, the latter kinase had been well documented to play a role in EAE development independent of sodium.[20] Taken together, these studies in mice and in human cells suggested that high dietary salt intake may be a novel ERF for MS, and that it may act by increasing the differentiation of Th17 cells that drive or initiate the disease.

MS is a sexually dimorphic disease, where incidence is higher in females, but the severity of disease course tends to be worse in males.[5] Moreover, this disease is highly heterogeneous, at least in part due to the high level of genetic heterogeneity of human populations in the modern world.[15] Lastly, the response of blood pressure to dietary sodium is well known to be genetically controlled.[7] The initial two studies on dietary sodium in EAE used the same commonly used genetic background, C57BL/6, and did not report the sex of the animals.[16,19] To test whether the effects of dietary sodium in CNS autoimmunity were dependent on sex and genetics, Teuscher and colleagues utilized male and female mice, and two genetically distinct EAE models: chronic-progressive EAE in C57BL/6 mice, and relapsing–remitting EAE in SJL mice. The latter mimics the progression of the most common form of MS, relapsing–remitting disease. Mice were subjected to either a high or low sodium diet for several weeks prior to EAE induction, which was maintained post EAE induction. While high dietary sodium exacerbated EAE in both males and females in C57BL/6 mice, in SJL/J mice disease exacerbation was only observed in females and not males, suggesting an interaction between sex, genetic background, and dietary sodium exposure.[21] Furthermore, Teuscher and colleagues found that another C57BL/6 strain carrying a large interval on chromosome 17 derived from the 129/Sv strain showed no difference in EAE in response to dietary sodium. Taken together, these results suggested that effect of dietary sodium on neuroinflammation is regulated by an interaction between genetics and salt. This is particularly interesting, given the environmentally driven increasing incidence in MS in females, and suggests that high dietary sodium may be a candidate risk factor contributing to the increasing prevalence

of disease in women. However, the genetic control of this response predicts that the dietary sodium would represent a risk factor in certainly genetically predisposed populations, and thus may not be applicable to the whole population.

To determine the cellular mechanisms underlying exacerbation of EAE by dietary sodium, Teuscher and colleagues also examined the effects on Th1 and Th17 development. In contrast to previous studies,[16,19] exposure to high dietary sodium did not result in significant elevation of Th1 or Th17 cells, whether in gut lymph nodes, peripheral lymphoid organs, or in the inflamed CNS.[21] These results suggested that high dietary sodium can exacerbate EAE in the absence of an effect on Th17 cells. Instead, enhanced brain pathology and an increase in disruption of the blood–brain barrier (BBB) during EAE were observed with exposure to high sodium, which also correlated with a modest elevation in plasma levels of Na^+.[21] The enhanced BBB breakdown was suggested to be a negative consequence of circulating Na^+ on the sensory circumventricular organs (CVOs), specialized areas of the brain that sense circulating electrolytes, but also can serve as portals of entry for immune cells during neuroinflammation.[22,23] A potential explanation for the different subphenotypes observed between this study and the two initial studies on sodium in EAE may have to do with the fact that somewhat different EAE induction protocols were used. Importantly, the effects of sodium on clinical EAE were consistent across the different studies.

What are the other possible mechanisms whereby dietary sodium may influence pathogenesis in EAE or MS? One of the cell types that are well documented to be influenced by dietary sodium are macrophages, which also play important roles in EAE, either as antigen-presenting cells or as effector cells in the CNS. Increased dietary sodium results in interstitial retention of sodium in various tissues, in particular the skin, where it is sensed by macrophages using the same TONEBP signaling pathway as in T cells.[24,25] Remarkably, this sodium sensing by macrophages can control electrolyte homeostasis and blood pressure. Moreover, exposure to high sodium appears to influence the activation state of macrophages, activating pro-inflammatory-like, M1 macrophages, and inhibiting anti-inflammatory M2 macrophages.[26–28] A 2015 report supports the idea that the pro-inflammatory effects of dietary NaCl in EAE are in fact mediated by macrophages, rather than T cells. Klotz and colleagues confirmed the EAE exacerbating effects of a high-salt diet, and demonstrated that macrophages and microglia acquired a more pro-inflammatory phenotype upon exposure to NaCl in vitro or in vivo (via a high-salt diet), while no effects of Th cells were reported, and this phenotype did not require SGK1.[29] Lastly, NaCl appears to also impair the immune suppressive functions of T helper regulatory cells,[30] which are important in preventing disease in EAE/MS. These potentially conflicting results suggest that the effects of sodium on the immune response in EAE/MS are complex and multifaceted. More work is needed to resolve these discrepancies, and to more precisely define the cellular and molecular mechanisms underlying these phenotypes.

EVIDENCE FROM HUMAN STUDIES

So far, only one epidemiological study has explored the effect of dietary sodium intake on MS. Correale and colleagues used urinary sodium levels, a well-accepted surrogate for sodium intake, to stratify a group of 70 patients with relapsing–remitting MS into those with low, medium, and high sodium intake groups.[31] MS disease activity was measured using the Expanded Disability Status Scale (EDSS) and magnetic resonance imaging (MRI). Remarkably, compared to the low sodium intake group, the medium and high sodium intake groups had two- to fourfold higher rates of disease exacerbation, as measured by EDSS or MRI. Similar results were obtained in a replication cohort of 52 MS patients. The authors adjusted their model for effects of age, gender, disease duration, treatment, vitamin D levels, body mass index, and smoking status; thus, the dietary sodium effect appears to be independent of these variables. An interesting caveat is that there appeared to be a sexual dimorphism in the first cohort, where males exhibited significantly higher salt intakes than females,[31] a finding that has also been reported elsewhere.[32] Males with MS are known to be at higher risk for developing more aggressive and progressive disease compared with females,[33] and it is thus interesting to consider if higher sodium intakes may contribute to this sexual dimorphism.

DIETARY SODIUM: A RISK FACTOR FOR *INCIDENCE* OR *SEVERITY* OF MS?

Although several of the previously mentioned studies have made the link between increasing sodium intakes and increasing MS incidence, the evidence for sodium's effect on *incidence*, as opposed to disease *severity*, is scant. The previously mentioned epidemiological study on sodium was performed in people who already had MS, did not include a healthy control population, and reported exacerbation of already ongoing disease.[31] It would be interesting to compare sodium intakes between early onset MS patients and healthy controls. Better still would be a prospective study, measuring sodium intakes together with MS incidence, over time, but with the low incidence of MS, such a study may

be difficult. The studies in the EAE model also report exacerbated disease severity, rather than an effect on incidence.[16,19,21,29] One can argue that increased severity could translate to higher incidence, as it would perhaps push those individuals with "subclinical" MS[34] over the threshold into having clinical manifestations, but this is difficult to demonstrate definitively. It would be informative to examine the effects of dietary sodium in spontaneous EAE models, where disease incidence could be monitored over time.[35]

PERSPECTIVES AND CONCLUSIONS

Compared to other well-documented MS risk factors such as vitamin D or smoking, the role of dietary sodium in MS is still highly debatable. So far, only a single and relatively small epidemiological study has found a link between MS and dietary sodium, and this clearly needs to be confirmed in larger cohorts. Moreover, the EAE studies uniformly demonstrate a relatively modest exacerbation of disease severity, despite the fact that dietary levels of sodium in these studies were increased quite dramatically.[16,19,21,29] Lastly, the EAE response to dietary sodium appears to be genetically regulated, and thus may be absent or present in genetically diverse individuals. Therefore, when extrapolating to population level recommendations or interventions, these findings should be taken with a "grain of salt." However, given the fact that high sodium intakes already have been associated with other adverse health effects, and the fact that decreasing dietary sodium is simple and requires little to no additional cost, those individuals at high risk for MS, or those who already have MS, can easily undertake this endeavor.

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SECTION III

BEHAVIORAL MANAGEMENT OF ASSOCIATED CONDITIONS IN MULTIPLE SCLEROSIS

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CHAPTER

12

Developing and Applying the Theory of Psychological Adaptation Needs in Patients With Multiple Sclerosis*

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OUTLINE

*This chapter is dedicated to the memory of Alexander Gattas, a true gentleman loved by many.

INTRODUCTION

Multiple sclerosis (MS) is a disease affecting the brain and spinal cord which is typically defined within different descriptors, that identify the state and course of the illness.[1] It is characterized by an unpredictable course, but it often leads to substantial disability.[2] About 2.1 million people in the world are affected.[3] Within the United Kingdom this represents around 0.3% of the population. MS has a higher incidence in women than men and a peak onset between the ages of 40–50 years.[4] MS is reported to have a considerable impact on an individual's quality of life, ability to continue life roles, and the ability to work and/or undertake leisure activities.[3] MS is costly for patients, their careers, families, and society. For instance, in the United Kingdom, half yearly cost for one patient with MS has been estimated as £8397.[5] These figures are similar to other westernized countries, for instance, annual costs in the United States are $30,601 per patient.[2] For patients with MS the risk of being affected by an emotional disorder (such as depression, anxiety, or adjustment disorder) is high and can negatively impact functioning.[6] The annual prevalence of depression in patients with MS can be around 20%.[7]

Mental well-being is defined as a sense of satisfaction, optimism, and purpose in life, a sense of mastery, control, belonging, as well as the perception of social support.[8] Patients with MS experience periods of anxiety, fear, and uncertainty, which can lead to hopelessness, depression, and feelings of abandonment.[9] Such negative experiences can have a severe and negative impact on an individual's quality of life through limited opportunities to complete meaningful activities in the home or wider community, or engage in the same "role" in interactions with family, careers, and significant others.[10] In patients with MS, psychological constructs, such as hope or perceived control, are considered better predictors of adjustment than illness-related factors, for instance, remission status or the severity of symptoms.[9] Thus, it is possible that the experience of MS has a severe and lasting impact on a patient's mental well-being.

Despite the negative impact of the illness, it is possible for patients to positively influence their mental well-being. For instance, a patient's perceptions of control over their life or, those patients who are able to positively reappraise their own situation can experience better adjustment.[11] Thus, while there is reason to be concerned about the effects of MS on the mental well-being of a patient, it is important to acknowledge the ability of the patients themselves to adapt psychologically in order to cope, improve mental well-being, and function post illness-related experiences.

Evidence to support such conclusions is often generated from quantitative studies that utilize specific inventories.[12] Quantitative review-based research in neurological populations is able to consider the effectiveness of interventions or association between variables using distinct outcome measures. However, these types of review are not able to go beyond data using inductive processes or conceptualize the phenomena of interest in a different way.[13] In 2016, the development of different forms of qualitative synthesis have been able to achieve exactly this.[14] Qualitative review–based research considers rich sources of information, which can, using inductive processes generate and further knowledge, sometimes with the ability to identify new and distinct theories.[15] While such work will not claim "the truth," it does represent a "truth of truths."[16] That is, evidence that patients and researchers can relate to and test in order to consider the robustness, value, and generalizability of it.

Toward a Theory of Psychological Adjustment Needs

The theory of psychological adjustments needs (TPAN) was proposed in 2016 in a thematic synthesis of studies involving data from more than 1000 patients.[14] The theory proposes five psychological needs of patients, which impact and influence psychological adaptation. This work distinguishes these aspects from coping strategies, whereas for the purposes of this chapter we consider acknowledgment and acceptance as an aspect of psychological adaptation rather than a coping strategy. While the proposals for the TPAN were supported by research literature considering patients with MS, the review did not provide a conceptual analysis of each proposed psychological need or identify the relationship to how literature on MS supports these findings. The TPAN is represented by five core psychological needs including (1) hope in possibility; (2) acknowledgment of an MS-related event and the implications of this for the individual; (3) realizing the need for choice, independence, dignity, and purpose; (4) acting on the need for choice, independence, dignity, and purpose; and (5) psychological needs of character.

The dual purpose of this chapter is to perform a concept analysis on the psychological needs that make up the TPAN and illustrate each component of the TPAN from the perceptions and experience of patients with MS, by using a synthesis of qualitative research studies.

METHODS

The first author (A.S.) undertook the narrative review in order to identify literature useful for the dual purposes of the chapter.

Search Strategy for Identifying the Conceptual Model

An electronic database search was conducted within MEDLINE from inception of the database until November 2015. Filters on the database were used to restrict the results, aligning with the eligibility criteria. The key words used included: illness OR disease AND model OR cycle OR process OR theory AND uncertainty OR acceptance OR adaptation OR adjustment OR acknowledgment OR hope OR possibility OR control OR resilience OR defiance OR denial. Additional searches were undertaken from the websites googlescholar.com and sciencedirect.com that considered the first 20 pages of hits. Further searches were undertaken from the reference list of any included article and from searching the online profiles of key authors.

Searches for Articles on Experiences With Multiple Sclerosis

Searches for articles considering patients with MS were taken from mid-2010 reviews[13,14] and the first author's personal database. In qualitative synthesis approaches that utilize more than 15–20 studies there is a good ability to saturate the resultant themes to the level of minor codes, that is to obtain good detail about the themes that can be fully explained. Depending on the purpose of investigation and paradigmatic positioning, the benefit of using further studies within the synthesis can be questionable because saturation is achieved. Within this chapter the numbers identified were in excess of 30 articles, and therefore no further methods were considered.

Eligibility Criteria for the Conceptual Model

The following eligibility criteria were used and applied by the first author: (1) articles had to be written in English; (2) articles had to be review based; and (3) articles had to contain or focus on the following concepts: uncertainty, acceptance, adaptation, adjustment, acknowledgment, hope, possibility, control, resilience, defiance, and denial. The concepts had to be considered within a model, process, cycle, or theory and focused in relationship to the experience of illness or disease. Articles had to include one of these identified words as a MeSH term in order to be included. Also (4) articles had to be focused on humans and written in the past 8 years (2007 onward) as recent review evidence would have incorporated existing perspectives and should have furthered them. Consequently, the first author wanted to capture, represent, and further current thinking. Further, (5) full text of articles had to be available through the database and (6) the data had to be presented within a peer reviewed journal article as opposed to a book, thesis, or conference proceeding.

Eligibility for Articles Considering Experiences With Multiple Sclerosis

Articles were included if (1) they used or considered in anyway and presented at least a paragraph on one of the following concepts: uncertainty, acceptance, adaptation, adjustment, acknowledgment, hope, possibility, control, resilience, defiance, and denial; (2) they reported qualitative data that examined patient's experiences, views, or perceptions; and (3) used articles from other stakeholders [e.g., health care professionals (HCPs), careers, and family] that focused on the patient's psychological adaptation.

Synthesis Methods

The synthesis was undertaken in several phases by the first author for each identified psychosocial need, including (1) an identification and initial definition of the need from each included paper, (2) a reduction and tabular summary of each key need, and (3) a presentation of the need with examples from previous literature.

RESULTS

A total of 985 articles were located. From this the following articles were used: (a) 12 conceptual review articles to define each psychological need from the TPAN[13,17–27] and (b) 44 articles to provide a source of data detailing the experience of individuals with MS regarding each psychological need.[28–72]

CONCEPTUAL ANALYSIS OF THE THEORY OF PSYCHOLOGICAL ADAPTATION NEEDS

The results from the conceptual analysis are provided in the following by each psychological need. Each psychological need is defined, and this is followed by an illustrative example of the need identified from the experiences, views, and perceptions of patients with MS.

Psychological Need: Cognitive Needs for Psychological Adaptation

Two of these psychological needs focus on a patient's cognitive need for psychological adaptation, which

include the concepts of acknowledgment and hope in possibility.

Definition of Acknowledgment

The psychological need of acknowledgment represents an awareness, realization, or coming to terms with an event relating to adversity,[18,20,26] acknowledging one's present situation within an uncontrollable or unknown future.[13] This can occur pre- or post-diagnosis. For example, a typical MS-related event could be loss of sight pre-diagnosis, hence acknowledgment would be that there is a potentially serious health problem that will affect the individual. This includes an acknowledgment of the meaning of the individual's life. It can be multidimensional as it relates to what is important and meaningful in the person.[19] Acknowledgment therefore falls on a continuum and sits between (1) rejection or denial, and (2) a complete embracement of an event related to the MS. It lies on the continuum before acceptance as acknowledgment integrates changes and allows meaning from an event that has impacted the persons' sense of self.[26,27] While the term acknowledgment implies a recognition of what has happened in the present situation, unlike chronic sorrow, it is not resigned to a certain future, linking more closely to hope in possibility.[18] For patients with MS, the need to acknowledge an event is required to a greater extent at times of change and transition.[17]

Examples From the Multiple Sclerosis Literature

From the 44 included studies, examples related to this psychological need were seen in 9 of these studies. This included the following studies.[28,32,34,35,49,56,65,69,73] For people with MS, acknowledgment included recognition that something serious or wrong could occur both pre-diagnosis or during diagnosis. For example, pre-diagnosis one woman stated, "I was in the college trampoline display team, [and] was supposed to end the display but my body didn't belong to me – nothing that you could put your finger on."[57] At diagnosis some patients experience, a sense of relief that the diagnosis is not in fact worse. As time progressed and with illness experience, patients with MS become more aware of MS through information sources, such as the MS society, peers, or the Internet. These sources helped patients acknowledge what the illness was, what it represented in their life, and the possibility of finding a cure in the future. Often however, acknowledging what the illness was doing to their health off set this development. Some individuals understood that the possibility of improvement was lost, for example, one women stated, "I kept expecting it to get better, but it didn't and kept getting worse…whatever I could do one year the next I could do less."[69] Patients often hoped that their illness did not get any worse and reinterpret this positively with appreciation.

Definition of Hope in Possibility

Hope in possibility refers to a patient's ability to see positive aspects of the future and continue living.[13,19] This could also be expressed in individuals who are dying.[25] Hope means reconciling one's current situation and circumstances with what the future may bring[13] and requires an ability to be open to both negative or positive change or outcomes.[13,25] It also means an ability to abandon goals or substitute them if needed.[26] Seeing hope in possibility enables an individual to overcome uncertainty and act as a buffer to hopelessness.[13] Possibility implies a detachment from concrete, finalized, or certain hopes.[13] Hopes can be focused on medical or technological breakthroughs and can focus patients toward being cured completely in the future.[74] The very nature of hope in possibility allows hope that could be considered as unrealistic to continue.[18]

Examples From the Multiple Sclerosis Literature

From the 44 included studies, examples related to this need were seen in 9/44 studies. These included the following studies.[28,34,50,56,60,65,68–70] Hope in possibility was associated with the focus that something may not happen or that a patient may be able to act and interact as they wished or that there could be a future that would be worth living for. While some patients could view the future as predetermined, others could identify that their fears about the future may not happen, or that during the diagnosis process they may not have MS or have a form that was more stable or less progressive. Alternatively, individuals with MS may have seen results of research trials, which provided possibilities for their lives. Patients could also hope that the illness would not progress following diagnosis. The certainty of a cure being found by medicine was expressed as a hope in possibility, for example, one patient stated, "the only hope I have seen on the internet is from stem cell research in Bristol."[69] Hope in possibility could be severely challenged by the experiences patients have, for example, interactions with HCPs. Hope in possibility could also be challenged by an inability to adapt or the inability to overcome the experience of an uncertain future or strong emotions, such as fear. For example, one study stated, "exercise represented more than just hope for change, it gave patients a reason to get up. Patients often reacted negatively when clinicians took this hope in possibility away."[69] Another patient stated, "My doctor would say, 'Doesn't it make you feel better that in seven years 70% of people are not in wheelchairs?' No, no it doesn't at all. It is the 30% that are [that] scares the shit out of me!"[70] Strong emotions may prevent a broader acknowledgment of what the future could be for the patient and thus prevent hope in possibility.

Psychological Need: Needs of Choice, Independence, Dignity, and Purpose

A further two needs of psychological adaptation relate to the ability of the patient to be independent following an event that is related to their illness. This includes a cognitive component and a component that relates to taking action.

Definition of Realizing the Need for Choice, Independence, Dignity, and purpose

Realizing the need for choice and independence could be seen as a process of empowerment through which someone creates an internal sense of belief and power to act and take ownership of their own situation.[20] In other words, viewing their situation in order to make the most of it.[13] It represents a willingness to take part in a process that involves activating resources[27] and a decision to engage with services to make the most of their situation.[13] It could be associated with interactions and be co-created with others,[13,21] and it may be a process which is enforced by individuals not wanting to be a burden on others and the desire to live "normal" lives.[17]

Examples From the Multiple Sclerosis Literature

From the 44 included studies, examples related to this need were seen in 28/44 studies. This included the following studies.[31,32,34–36,38,39,47,48,50–57,60,62–72] Realizing the need and assuming personal responsibility to take action was often a reaction against the uncertainty of choice of the illness experience for the patient. For example, one study stated, "With the lack of ability to predict how and when MS may affect those with the disease, many thought that they had to find their own way, and 'learn to trust their own body' (PwMS 3) rather than look to medical professionals for advice."[34] Patients who could identify this as a turning point, associated with a need for self-determination. When patients were able to feel that the MS no longer controlled their lives, they could take steps to having a greater understanding of their own needs. For example, one study stated, "With such insights [from undertaking ones own research], individuals began to realize that they had choices and could select the avenues of support, which were most appropriate to their personal needs."[54] A perception of control by patients was gained through making choices and taking the initiative of which activities to engage in such as engaging in activities that were achievable, taking responsibility for the challenges that the MS presented, identifying that the MS will not control their lives, and by being more forceful in interactions with HCPs. For example, one study reported, "The medical profession knows so little…and it's been clear to me since the very beginning that it was in my court, that the ball was in my court, that I had to figure it out because they weren't gonna."[70]

Definition of Acting on the Need for Choice, Independence, Dignity, and Purpose

As a patient, becoming and being independent is defined as a willingness to take responsibility and engage with health services and utilize treatment as well as engage in efforts to improve their health status.[20] It includes searching for information, taking action, and making changes to one's life.[13] It can also mean persevering to undertaking tasks, activities, or interactions by oneself. It is likely associated with the use of different coping strategies and mechanisms that allow independence,[20] as well as meaningful achievement as a result of engaging with treatment and being proactive.[19] When a patient was unable to independently perform basic tasks, it meant retaining dignity. For example, during interactions with HCPs, being asked by an HCP if changes were acceptable and the introduction of devices to improve function were okay was positive for the patient.[18]

Examples From the Multiple Sclerosis Literature

From the 44 included studies, examples related to this need were seen in 26/44 studies. This included the following studies.[31,33,34,37,40,44,45,47,49,52–57,60–65,67–72] Patients wanted to exercise independence in order to identify that they were still contributing to other peoples' lives in particular roles, for example, as a provider and father, as someone with responsibility, in order to experience a sense of self-worth and choice and feel valued in what they do from others. For example, one study stated, "Two other interviewees were managing to live relatively independently with the help of careers, and both identified this independence as important."[40] Close friends and family and HCPs could enhance a patient's independence. Being able to respect the patient's choice for when and how much support was given was essential to this. Negotiation with others by the patient was often required in order to make this work. There were examples when interactions could make the patient feel too dependent. For example, one study stated

> Dependency and negotiation of the degree of support needed for certain tasks seemed to be a source of tension in participants' relationships with those closest to them (their caregivers). The majority commented that caregivers did not like to watch them do things.[49]

Another study stated, "When receiving help with basic needs in daily life, the women expressed feeling violated due to being uncovered and exposed to unknown people who were there to assist them in their homes."[60] Patients needed to be independent and self-reliant, while also needing to be able to accept and consider help from others.

Psychological Need: Needs of Character

Initial Definition of Concepts

The needs of character are represented by the ability to access and use internal resources to aid mental well-being.[24] Such characteristics and inner resources include determination and a strong will or inner strength to resist the effects of the event disease or illness. This strength can be particularly valuable when facing deterioration or little possibility of change in one's circumstances.[19] These characteristics impact on a response to an event[18] and are enforced by a self-belief, inner will, or aspiration to enhance recovery[17,74] and capture a range of reactions from not accepting the implications of MS to wanting to challenge or defy the MS.

Examples From Research

From the 44 included studies examples related to this need were seen in 19/44 studies. This included the following studies.[33–37,45,48,50,51,54,55,57,60,61,63–67,69–72] Patients could express a need to be strong, brave, or to fight or battle against the illness and their circumstances. For example, one study stated:

> Stubbornness was described as a helpful trait. Striving to carry out housework as they fought their impairment helped them feel better. Recovering from a relapse of MS also was described as a fight for which the can do attitude was helpful: I say to myself: "I'm damn well not going to let this beat me"—you don't get anywhere just thinking you can't do this and you can't do that, you've got to think what you can do.[50]

A further trait was a determination not to give in or be defeated by the MS. For example, one study reported, "Ella was somewhat doubtful about her ability to take part in future art classes due to the recent decline of function in her dominant hand, but again expressed determination to try."[48] The focus of the patients was toward valued goals and activities. For example, it was important for many patients to continue to work.

DISCUSSION

This chapter has been able to provide a conceptual analysis of the different elements that make up the TPAN as well as provide evidence for how each need is expressed by patients with MS. The discussion considers the broader literature and brings some further considerations to the psychological needs associated with the TPAN.

Hope in Possibility and Acknowledgment

The hope in possibility protects an individual from the effects of uncertainty, because this type of hope embodies some level of uncertainty[75] but is accepting to what might not be rather than being associated with worry and fear of possible outcomes. Possibility allows a patient to be open to change and willing to accept what happens in the future.[13] Being able to consider positive aspects of one's future is possible for individuals who are dying.[25] The ability to acknowledge an event related to illness prevents and protects against denial-related responses.[76] Some form of acceptance or related variant on a spectrum of adaptation-related responses such as acknowledgment, positive embracement, or resignation/chronic sorrow likely protects patients against succumbing to the effects of the illness.[18] The stories that patients tell often contain a spectrum of responses that relate to both adaptation and hope. Stories that are told which do not have hope in possibility and acknowledgment may be limited.[13,77,78]

Realizing the Need for Choice, Independence, Dignity, and Purpose and Acting on the Need for Choice, Independence, Dignity, and Purpose

Independence, choice, and dignity are clearly represented as fundamental aspects of living that patients with MS want to retain. This is supported by previous research in individuals with neurological illnesses, for example,[19,81] and may be more prominent as the illness progresses toward the end of life.[18] Research has identified that dependency can be linked with feelings of being powerless or feeling ineffective at tasks.[82] Also, when patients are dependent on others, negative feelings can be generated from nonfriendly interactions.[83] Results from the this review illustrate that there is a point where life may be perceived as not worth living. This point was directly associated with independence and dignity. HCPs are called to help patients self-manage and empower them with knowledge,[84] as this aids their independence. This is achieved by providing choice for the patient[85] and respecting their identity and privacy.[86] However, within the dignity-driven care paradigm, researchers[87] have suggested the need to balance the provision of respect, the support for autonomy, and preference for the patient with the need not to abdicate professional responsibility. In essence, HCPs have a duty to moderate and influence decision-making processes where the patients' decisions or choices will cause harm or are not possible.

Supporting the Perception of Perceived Control

The perception of control is regarded as a crucial dimension that can influence a patient's ability to access coping resources and strategies.[88,89] It is a factor that moderates the relationship between coping and adjustment,[90] as well as a key factor identified within the common-sense model of illness threat.[91] A perception of reduced controllability can promote a greater use of escape-based coping strategies.[92] Thus, the perception of higher levels of controllability is linked to coping with MS.

Psychological Needs of Character

The need to defy one's circumstances is a central psychological factor, which influences the proposed processes of adaptation. The ability to defy is reliant on important psychological constructs including courage,[27] motivation or agency,[93] resilience,[94] and determination.[19] The importance of will power and self-control as a central role of self-regulation has been identified previously,[95] although both will power and self-control can be vulnerable to depletion.[96]

Limitations

Several limitations are acknowledged: (1) the searches undertaken were narrative in nature and more systematic processes could have been used to acquire a broader spectrum of information and sources; (2) the theory has been developed on a limited amount of information generated primarily from literature in neurology; (3) to what extent the theory can be considered within other populations, situations, and experiences needs further consideration; and (4) the examples of each psychological construct may not have been considered fully by the synthesis and thus may have limited generalizability.

CONCLUSION

This chapter has provided a conceptual definition for the different domains that make up the proposed theory entitled the TPAN. An understanding of the TPAN can enhance HCP interactions with people with MS. By understanding the psychological implications of diagnosis and coping with a variable and deteriorating disease, it is possible to support patients to manage their condition in a way that is acceptable to them and in doing so promote their chances of obtaining a positive state of mental well-being and functioning.

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CHAPTER

13

Assessment, Consequence, and Clinical Implication of Asymmetry

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OUTLINE

INTRODUCTION

The etiology of multiple sclerosis (MS) is complex, unpredictable, and unsystematic nerve demyelination,[1] axonal deterioration,[2] and neuroaxonal loss.[3] Consequently, MS impairs the ability to transmit neurological signals throughout one's nervous system. The implications of nerve transmission disturbances include, but are not limited to, a multitude of functional symptoms such as abnormal gait, deficient balance, muscle weakness, spasticity, foot drop, and fatigue.[4–11] Furthermore, the pathophysiology of MS can generate asymmetrical effects where one side of the body has more severe functional impairments. It follows that these bilateral differences have significant clinical importance. Nevertheless, bilateral asymmetry has yet to be comprehensively quantified. Additionally, the lack of knowledge about the impact asymmetry has on activities of daily living (ADL), quality of life (QOL), and exercise tolerance creates challenges for the prescription and implementation of rehabilitation.

The following sections focus on: (1) the limited current research whose primary aims were quantification of asymmetry in individuals with MS by assessing limb-to-limb differences; (2) the consequences of asymmetry as it pertains to balance; (3) the consequences of asymmetry as it pertains to gait; (4) the secondary complications of asymmetry as it relates to injury and health care costs; and (5) clinical assessment of asymmetry.

Current Asymmetry Research

Lower-limb skeletal muscles are commonly affected in individuals with MS.[12–15] Since MS unsystematically affects sides of the body, and lower-limb muscle impairment is common, individuals with MS are predisposed

to the development of asymmetry in lower-limb skeletal muscle. Asymmetry has the potential to create strength and functional imbalances, leading to deleterious impacts on gait and balance, predisposing individuals to falling. In addition, lower body asymmetry can compromise function and increase sedentary behavior, which profoundly impacts health, ADLs, and QOL.[1] ADLs such as walking require sufficient synchronization of bilateral motor unit recruitment and discharge rates. Typically, the legs are recruited bilaterally and limb preference may switch depending on the complexity and movement conditions.[16] This suggests that, in the case of MS, if motor activation is compromised in one limb, compensatory strategies in recruitment patterns shifting to the stronger/less affected limb may be necessary.[17] The inability to bilaterally modulate and produce motor discharge rates appropriate during exercise could result in reduced exercise capacity and increased levels of muscle fatigue. This can have debilitating effects on exercise capacity, and physical function, as well as further increase the interlimb difference. In addition to these neurological (central) limitations, a variety of metabolic processes across limbs may play significant roles limiting exercise capacity.[12,18–20] For example, some researchers suggest that limitation to exercise capacity could be related to altered mitochondrial function, changes in skeletal muscle fiber type and/or enzymes related to energy metabolism. Without knowing the relative contributions of these potential bilaterally variant factors, it becomes difficult for clinical professionals to choose the most meaningful assessments to identify bilateral differences and subsequently prescribe optimal therapeutic interventions.

Despite the general acknowledgment of asymmetry in MS, a plethora of research spanning a few decades has compared muscle function and performance between MS and controls without systematically investigating asymmetry. Interestingly, the majority of previous studies of physical function were based on observations from one limb (leg or arm) or muscle group/s with some researchers only testing a single side (left or right) of the body.[12,21–24] Since MS unsystematically affects the body, data interpretation can be clouded by unsystematic increases in sample variances, and drawing comparisons across studies becomes difficult as well. Within the past decade, investigators have started to systematically test for asymmetries by stratifying sides of the body as "more" or "less" affected by MS to gain insight into the ramifications asymmetry can have on function.

Examples of researchers that have paid particular attention to asymmetries through the direct comparison of limb-to-limb differences in individuals with MS include White and Dressendorfer (2005), Chung LH et al. (2008), Larson and White (2011), Kalron A, Achiron A, Dvir Z (2011), Larson RD et al. (2013), Sandroff BM, Sosnoff JJ, Motl RW (2013), Larson RD et al. (2014), Rudroff T et al. (2014). These studies are discussed in the following paragraphs, highlighting the outcomes related to asymmetry.

White and Dressendorfer (2005)[25]

This was a case study of a 38-year-old female with relapsing–remitting MS. Single leg cycling was performed to determine interlimb differences related to exercise tolerance and metabolism. Maximal isokinetic testing of the quadriceps at 60°/s was performed to assess maximal muscle strength.

The individual exhibited very good aerobic capacity when whole-body measurements were taken, but showed considerable differences between legs during the single leg tests. The left leg (more affected by MS), exhibited much lower values for many physiological measurements compared to the right leg during the single leg cycling test. The left leg's VO_{2max} was 0.77 L/min lower compared to the right and the peak workload was 20 W lower. Maximal isokinetic voluntary contraction of the left quadriceps produced 22% less torque than the right quadriceps, which confirmed the participant's complaint of weakness in the left leg during exercise. The researchers suggested that the limited strength in the left leg may have caused the right leg to adapt to working more during aerobic activities. The right leg performed 85% of whole-body VO_{2max}, while the left leg only performed 60%. This suggests that the right leg may have compensated by increasing oxygen extraction during exercise. However, because this was a case study it offers virtually no information about the broader population. Nevertheless, it prompted further inquiry.

Chung LH, Remelius JG, Van Emmerik REA, Kent-Braun JA (2008)[14]

The aim of this study was to assess bilateral strength and limb-loading asymmetries and determine their influence on balance and symptomatic fatigue in women with MS. This study is described in greater detail in the section related to balance and asymmetry.

Larson and White (2011)[26]

Recently reduced mobility has been shown to be associated with increased risk of osteoporosis as well as the observation of low bone density increasing fracture risk in patients with MS. Commonly, bone mineral density (BMD) is assessed by measuring one proximal femoral hip; however, MS patients exhibit asymmetrical symptoms associated with the disease. Therefore, the purpose of this study was to assess total femoral neck BMD in both legs in 23 MS patients, 21 of which were females. Patients self-identified which leg was more affected by the disease prior to the study. There was no significant

difference between the right and left femoral necks; however, when researchers evaluated BMD based on the self-reported symptoms in each leg, the more affected leg had significantly lower femoral neck BMD. The difference in bone density between legs in MS patients may be associated with atypical bone remodeling due to muscle weakness and atrophy of the musculature on the weakened leg. These data provide evidence that both legs/hips should be measured in MS patients to allow for more accurate detection of BMD. More accurate detection of bone loss in MS patients provides clinicians better knowledge how to accurately prescribe rehabilitation to individuals.

Kalron A, Achiron A, Dvir Z (2011)[9]

This study focused on assessing strength and gait parameters in individuals with a first neurological event suggestive of MS. Bilateral asymmetry scores were calculated for peak isometric torque (PIT, in N m), isometric fatigue index (FI), and used to compare the groups (MS vs. controls). This study is described in greater detail in the section related to gait and asymmetry.

Larson RD, McCully KK, Larson DJ, Pryor WM, White LJ (2013)[27]

Larson and colleagues designed a study to (1) quantify limb-to-limb differences (asymmetry) in lower limb performance during lower body dynamic exercise in individuals with MS and (2) investigate whether any limb differences limited functional performance during the 6 min walk test. A total of 15 individuals participated in the study (eight MS and seven individuals without MS). The individuals with MS had an average expanded disability status scale (EDSS) score of 2.6 ± 1.6, which indicates mild to moderate disability.[28] Study participants underwent a series of single leg assessments which included maximal isometric strength of the knee extensors, limb-specific oxygen uptake, and a test of functional performance (6 min walk test). The leg that produced the greatest force was labeled "stronger" (less affected) and the leg that generated the least amount of force was labeled "weaker" (more affected).

The individuals with MS exhibited significant bilateral differences in knee extensor leg strength ($p = .004$), peak workload ($p = .01$), and VO_{2peak} ($p = .002$), whereas the individuals without MS displayed no significant limb-to-limb differences. In other words, on average the "weaker" leg in the MS group was not only weaker from a strength standpoint, but was unable to achieve the same aerobic performance as the "stronger" leg. Difference scores (stronger—weaker) were used to compare measures of asymmetry between groups. Difference scores for leg strength, peak workload, and VO_{2peak} were all significantly higher in the group of individuals with MS. Effect sizes were calculated to assess the magnitude of the differences between groups with large effects for leg strength, peak workload, and VO_{2peak} (1.1, 1.8, and 1.2, respectively).

The distance covered during the 6 min walk test by the individuals with MS was significantly less than the controls (474.3 ± 93.1 m vs. 626.9 ± 94.0 m, $p < .05$). In addition a significant correlation was observed between the distance covered during the 6 min walk test and degree of limb differences in peak power. In other words, individuals who had greater leg-to-leg differences in peak power during the cycling tests walked less distance. This study showed that individuals with MS exhibit asymmetry in knee extensor strength as well as aerobic function. More importantly, this was preliminary evidence that asymmetry had a significant impact on functional ability (6 min walk test).

Sandroff BM, Sosnoff JJ, Motl RW (2013)[29]

The aim of this study was to evaluate associations among gait parameters, walking performance, and aerobic capacity, balance, and strength asymmetries. This study is described in greater detail in the section related to gait and asymmetry.

Larson RD, McCully KK, Larson DJ, Pryor WM, White LJ (2014)[30]

The purpose of this study was to quantify potential bilateral performance disparities of the legs in individuals with MS. This study had eight individuals with MS with an average EDSS score of 2.6 ± 1.6,[28] which indicate mild to moderate disability, and eight healthy control subjects. Aerobic capacity was assessed using a graded exercise test to determine VO_{2peak}. Subsequently, participants performed single-leg cycling tests set at a predetermined intensity, which was 20% of whole body VO_{2peak} for 5 min. Maximal isometric strength of the knee extensors, was used to define the "stronger" and "weaker" leg. During submaximal single-leg cycling the MS patients performed significantly more work with their stronger leg compared with their weaker leg, while the control group experienced no difference between legs.

The stronger leg in the MS group performed significantly more work than the weaker leg (stronger leg: 6.4 ± 1.7 kJ vs. weaker leg: 4.7 ± 2.5 kJ, $p = .02$ observed in the control group (stronger leg: 9.2 ± 3.2 kJ vs. weaker leg: 9.1 ± 3.2 kJ, $p = .36$). In addition, the between leg difference (strong—weak) for total work was statistically difference between groups at $p = .02$.

The differences in work performed by each leg in the MS patients may be due to the decrement in central motor drive, affecting the body asymmetrically. Because many ADLs require coordination of both lower limbs,

such as walking, bilateral asymmetries may cause MS patients to favor one side more than the other resulting in further development of bilateral asymmetries. The weaker leg in MS patients may limit aerobic capacity and cause premature muscle failure.

Rudroff T, Kindred JH, Koo PJ, Herbert JR (2014)[31]

The objective of this study was to investigate whether glucose uptake in leg muscles was asymmetrical during a walking test in eight individuals with MS (44.9 ± 8.6 years) and eight individuals without MS (37.9 ± 8.4 years). The EDSS score for the MS group was 3.0 ± 1.6, which indicates moderate disability.[28] Maximal strength was assessed for the knee extensors and flexors and the dorsiflexors of each leg followed by a 15 min walking test, which was immediately followed by a positron emission tomography (PET) scan to measure [^{18}F]-FDG a glucose analog used to monitor cumulative muscle activity during exercise as an index of muscle metabolism.

Regions of interest (ROI) of the legs were measured to analyze the muscle tissue's glucose uptake within each leg. Differences in glucose uptake were observed between the strong and weak knee flexors and the strong and weak hip flexors in the MS group ($p < .01$). In addition the semitendinosus muscle (muscle within the knee flexors) of the stronger leg had higher glucose uptake than the weaker leg in the MS group ($p = .03$). Although the sample size was small, this study provides insight into how skeletal muscle metabolism in MS patients may affect walking ability due to leg asymmetry of glucose uptake. Overall the asymmetry in [^{18}F]-FDG and strength in the MS group suggests an increased metabolic cost during activities such as walking, which may play a pivotal role in premature fatigue during ADLs.

Consequence of Asymmetry on Balance

The ability to maintain postural stability (balance) is an integral component needed to sustain mobility and reduce an individual's risk of falling.[6,32–34] A number of factors such as reductions in strength, elevated levels of fatigue, lack of coordination, spasticity, and vestibular dysfunction have been shown to negatively impact balance.[35] Researchers have assessed balance in individuals with MS and have found that when compared with healthy controls they exhibit increased postural sway during quiet standing, limited, and slowed movement during reaching and stepping tasks, and a delayed response to postural displacement due to poor trunk control.[36] These risk factors make MS patients much more vulnerable to falls, which have a huge impact on QOL.[35] Once an individual has experienced a fall, this leads to a psychological apprehension and caution when performing normal daily tasks.[37] In addition, it has been shown that individuals with MS exhibit balance impairments even with low to moderate disability.[38]

Many of the difficulties MS patients experience with balance may be due to asymmetrical symptoms of the lower body, which could lead to the impairment in postural sway and motor gait. To date, there have been very few studies to directly examine asymmetry in MS patients and how it relates to balance. The few apparent examples are discussed in the following paragraphs.

Chung LH, Remelius JG, Van Emmerik REA, Kent-Braun JA (2008)[14]

The primary objective of this study was to quantify the magnitude of differences in bilateral strength and limb-loading asymmetries, postural control, and fatigue in individuals with MS. Secondarily this study examined the relationships between postural control, symptomatic fatigue, bilateral strength asymmetry of the knee extensors and dorsiflexors, and physical function to further understand the implications asymmetry might have on balance. Twelve women with MS (55 ± 9 years) with moderate disability (EDSS score of 4 ± 1 which indicates relatively severe disability[28]) and 12 age-matched healthy females (controls, 53 ± 9 years) participated in the study. Symptomatic fatigue was assessed using the fatigue severity scale (FSS) and the visual analog fatigue scale (VAFS). A 25-ft walk test was also used as an index of functional performance, and participants were asked to perform the walk at their "normal" pace and at a "brisk" pace. Assessments of bilateral isometric strength of the knee extensors and the dorsiflexors were performed due to their different contributions to postural stability[39] and locomotion.[40] Postural stability was also assessed with force plates, while subjects stood quietly for 20 s. Bilateral asymmetry scores were calculated and used to compare the groups (MS vs. controls). Significant differences were observed for knee extensor asymmetry scores (MS: 21.5 ± 16.2 vs. control: 9.2 ± 6.9, $p = .02$) and the loading asymmetry scores (MS: 10.5 ± 6.9% vs. control: 6.0 ± 3.0%, $p = .02$). Symptomatic fatigue and walk times were all significantly correlated to knee extension power asymmetry. In addition, center of pressure in the anteroposterior direction was significantly associated with brisk walk times and knee extension power asymmetry.

This study provides valuable information regarding the role asymmetry could play in compromising functional abilities. By improving our knowledge of asymmetric symptoms in MS patients, we can better improve our rehabilitation interventions to improve balance. For example, in extreme cases of poor balance, an assistive device, such as a cane, may be used to improve balance by providing additional support and proprioceptive information to the patient. The goal of researchers and rehabilitation specialists should be to improve physical

and cognitive function and limit the reliance on assistive devices. Currently there are many clinical interventions used by MS patients to improve balance such as resistance training, static and dynamic balance rehabilitation, sensory facilitation techniques, and dual-task training.[36] However, most of these do not include assessment and treatment of asymmetry.

Furthermore, the introduction of asymmetry testing in MS patients could have the potential to improve specific symptoms affecting each patient and allow for improved rehabilitation. Balance, along with motor gait, is a task that involves the coordination of the central nervous system (CNS), sensory, and periphery. The appearance of asymmetric symptoms in any of these three domains has the potential to cause a disruption in one's ability to properly balance, and over time could lead to a higher risk for falls and subsequent decrement in QOL.

The next section focuses on the recent studies that assess the consequences of asymmetry on gait. Recent studies that have paid particular attention to asymmetries through the comparison between MS and controls are presented.

Consequence of Asymmetry on Gait

Gait abnormalities in MS are extremely varied due to the heterogeneity of the disease. It has been observed that a reduction in lower-limb motor performance (gait abnormalities) can be identified as early as the first neurological event.[9] Common abnormalities reported include: decreased gait velocity, decreased step and stride length, increased double support time (time in which body weight is supported by both legs), decreased swing phase, and increased step variability.[4,41] It has also been noted that the energy cost of walking is increased in MS patients.[42]

In 2011, Larocca et al. conducted two online surveys of 1011 individuals with MS and 317 care partners regarding walking difficulties.[43] Forty-one percent of MS patients reported difficulty walking, and 70% of those reported that difficulty walking was the most challenging aspect of their condition. It was noted that walking difficulty had a negative impact on ADLs, social life, emotional health, and socioeconomic status. Additionally, 30–40% of care partners reported that walking difficulty placed burden on them and negatively affected their own QOL.

The exact pathophysiology of gait disturbances in MS is not fully understood, but it has been speculated that the neurological impairments associated with damage to the CNS, and in particular impaired motor control, is the primary cause. MS patients present with a wide variety of neurological impairments, and it is still not fully understood how each individual impairment contributes to the development of gait abnormalities. Researchers are beginning to investigate how functional impairments contribute to gait abnormalities. Thoumie et al., in 2005,[44] observed that hamstring strength was a significant contributor to alterations in gait as it explained 44% of the variance in gait velocity. However, there are numerous factors that could explain the variance of gait velocity. One of those factors could be bilateral differences in leg function.

Kalron A, Achiron A, Dvir Z (2011)[9]

This study characterized the association and magnitude among gait parameters, isometric strength, and fatigue in individuals with clinically isolated syndrome (CIS), suspected of having MS, up to 3 months from onset. Fifty-two individuals with CIS with an average EDSS score of 1.7 ± 1.3 indicate mild to moderate disability,[28] and 28 healthy controls participated in the study. Isometric strength and fatigue of the knee flexors, knee extensors, ankle plantar flexors, and dorsiflexors were assessed along with temporal–spatial parameters of gait. Bilateral asymmetry scores were calculated for PIT (in N m), isometric FI, and used to compare the groups (CIS vs. controls). Percent asymmetry scores were calculated. Individuals in the CIS group exhibited significantly greater asymmetry PIT scores of plantar flexors (MS: 15.85 ± 21.5 vs. control: 4.1 ± 3.38, $p = .005$) and dorsiflexors (MS: 14.93 ± 12.81 vs. control: 8.37 ± 6.81, $p = .02$).

This study is very interesting as evidence of asymmetry was observed in a group of CIS subjects. After one clinical episode, which does not necessarily guarantee diagnosis of MS, bilateral differences are evident. Therefore, early intervention to preserve optimal motor performance maybe feasible. It appears that mechanisms responsible for affecting motor performance occur very early in the disease process.

Sandroff BM, Sosnoff JJ, Motl RW (2013)[29]

This study was designed to examine associations among aerobic capacity, balance, and lower-limb strength asymmetries, walking performance, and spatiotemporal parameters of gait. Thirty-one individuals with MS and 31 healthy controls participated in the study. All subjects performed testing to assess aerobic capacity, balance, and muscle strength at the knee, walking performance, and spatiotemporal parameters of gait. Aerobic capacity and lower-limb strength asymmetries, explained significant variance in both walking performance and gait kinematics only in the individuals with MS ($R^2 = 0.23–0.58$).

SECONDARY COMPLICATIONS: INJURY AND HEALTH CARE COSTS

Over 50% of MS patients have reported falling and needing to receive medical treatment for fall-related injuries.[35,45] Of all of the reported incidences of falls 60%

are reported to have occurred during walking.[37] A large majority of falls occur while walking, which has been suggested to relate to gait abnormalities that could be from bilateral differences in lower body skeletal muscles. Individuals with MS who have been classified as "fallers" tended to be older, had a greater prevalence of assistive devices, increased disability, decreased walking endurance and coordination, greater perceived walking impairment and poorer balance than those individuals classified as "nonfallers."[46] For those individuals who are at high risk for falling research suggests that a fall prevention program should focus on developing walking endurance in order to reduce the impact of fatigue. In addition, balance has been shown to be associated with fall risk. However, MS patients have reported that altered gait patterns, as opposed to balance, caused them to fall.[47] Changes in gait pattern and limited walking ability have been described as risk factors and a correlation between limitations in walking ability and falls have been established.[48,49]

An increased risk of falling is of particular concern in patients with MS. In a 2011 study by Matsuda et al. 474 surveys were mailed to individuals with MS to investigate factors associated with falling. Of the 455 respondents, 265 (58.2%) had reported a fall in the previous 6 months. Of the individuals who reported their number of falls, 13.1% reported one fall, 44.5% reported multiple falls, with 58.5% reporting an injurious fall, and 18.9% received medical attention due to a fall. The severity of injuries ranged from sprains, fractures, and severe pain to head injury. Multivariate ordinal logistic regression showed that a combination of factors was significantly associated with falls and included use of a cane or walker, history of falls, income < $25,000, balance problems, and lower extremity weakness.[45]

In a study by Gunn et al. in 2014, the fall rate of patients with MS was tracked for a 3-month period. Of the 150 participants, 70% reported falling at least once, with three falls being the median. For all participants a total of 672 falls were recorded during the 3-month period. This equates to 18.41 falls per subject a year. An estimated 11.2% of falls were associated with reports of injuries with the majority of them being bruising, cuts/lacerations, and sprain/strains.[50]

Mount Sinai Hospital in New York conducted a 3-year study that examined the frequency of which individuals with MS visited the Emergency Department. During the 3 years, 224 individuals with MS visited the Emergency Department a total of 569 times with 33.5% on Medicaid and 12.9% were not insured. The number of visits per patient ranged from 1 to 15 with an average of 2.5 visits per patient. Of the 569 visits, 74.5% (424 visits) were related to nonneurological complaints, with pain being the most common complaint (18.3%). It is important to note that this was musculoskeletal pain and acute physical pain resulting from injury as opposed to MS-related sensory phenomena. Of those 569 visits to the Emergency Department by MS patients, about half (50.1%) resulted in a hospital admission with an average stay of 8.49 days.[51]

A study by Coleman et al. (2013) sought to estimate the impact of mobility impairment on the indirect costs of patients with MS. Of the 4288 respondents, 37% reported that they were not currently employed or in school. Those who were working reported a substantial reduction in work productivity due to complications associated with MS symptoms. Those missing work because of MS reported missing on average 9.0 ± 8.6 h of work per week or 8.2 ± 14.7 full days of work over a six-month period. Of those not working, 46.7% of patients reported retiring early. Researchers estimated that on average a patient with MS lost $1499 ± $7419 in wages per year as a result of mobility impairment. If you include lost wages due to early retirement, then that loss increases by another $29,101 ± 31,202 per person per year.[52]

In a study by Ayatollahi et al. it was found that patients with MS showed a significantly decreased BMD in the femoral neck as compared to age-matched controls.[53] This reduction in BMD is likely to increase an MS patient's risk of serious injury related to falls, as BMD scores have been shown to be a strong predictor of the occurrence of osteoporotic fractures.[54]

CLINICAL ASSESSMENT FOR ASYMMETRY

Foot drop, an abnormal decrease in dorsiflexion during the swing phase of the gait cycle, is a prominent problem for individuals with MS.[8] In other words, a decrease in the ability to dorsiflex the tibialis results in toe drag directly increasing the risk of stumbling and falling that can result in significant injuries.[38,55,56] The mechanisms for foot drop and factors that could exaggerate foot drop such as asymmetry should be assessed to minimize this symptom due to its influence on fall risk.

Research has shown that the anterior tibialis (a muscle directly related to foot drop) is affected by MS both peripherally and centrally. Muscle biopsies of the anterior tibialis muscle found that individuals with MS had fewer type I fibers and muscle fibers of all types were on average 26% smaller in individuals with MS compared to controls.[12] In addition, a 40% reduction in oxidative enzyme activity caused the muscle to rely on more inefficient energy supplies (anaerobic metabolism) were observed, which further impacts ADLs and QOL.[12] A slowing or a reduction in neural drive of the anterior tibialis has been reported in individuals with MS which translated into a 27% lower maximal muscle contraction following exercise, which

points to a reduction in overall performance in the muscle and overall metabolic demand on the muscles.[23] One area that has yet to be fully quantified is how the potential for bilateral effects on skeletal muscle influences foot drop. It is possible that once clinical assessments have been established direct interventions for metabolic asymmetries could be explored.

The reduction of skeletal muscle mass can have profound effects on individuals with MS. Yet few studies have investigated the effects of MS on skeletal muscle size. Since MS is typically diagnosed early in adulthood and patients tend to be less active than their healthy counterparts it is important to understand how the combination of the disease course (and aging) impact skeletal muscle. Of great concern, muscle weakness is typically reported to affect one side of the body more than the other,[3,14,30] and when considering the bilateral nature of gait, unilateral weakness becomes more concerning. Individuals with MS do present with weakness to a greater extent in one limb than the other. Larson and colleagues (2013) observed a near 10 kg difference in isometric knee extensor.[27] Chung and colleagues reported a significant relationship between knee extensor power asymmetry score and normal walk time ($r = 0.63$) and brisk walk time ($r = 0.61$) such that those with a greater asymmetry score in knee extensor power also had longer walk times. These data confirm the importance of addressing such asymmetry early in the disease course.[14]

Due to observations of reduced skeletal muscle strength within an individual with MS the evaluation of muscle cross-sectional area is of great importance. The limited data available do indicate that disparities may be muscle dependent. For example, skeletal muscle fibers of the tibialis anterior presented significant atrophy in three fiber types (types I, IIa, and IIax) when compared to healthy counterparts.[12] However, due to the etiology of MS, and the notion of asymmetry, a more detailed exploration of muscles is warranted.

The recognition and clinical implications of functional asymmetry in MS are only just beginning to be explored. Results of studies in areas such as strength, aerobic performance, BMD, balance, and gait all consistently indicate that asymmetry matters. The health care and clinical implications reiterate the economic justification for continued development of research programs specifically targeted as fully evaluating and treating bilateral differences.

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SECTION IV

ENVIRONMENTAL FACTORS AND EXERCISE IN PREVENTION AND TREATMENT OF MULTIPLE SCLEROSIS

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CHAPTER

14

Neuromuscular Taping and Multiple Sclerosis: Reality or Trend?

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OUTLINE

Multiple sclerosis (MS) is an autoimmune chronic inflammatory and neurodegenerative disease of the central nervous system. It is the most common disabling neurological disease in young adults. The MS Foundation estimates that more than 400,000 people and about 2.5 million people around the world have MS. About 200 new cases are diagnosed each week in the United States. Central nervous system damage results key components of disability in people with MS. A majority of patients initially show relapses of increased disease activity and worsening symptoms. These are followed by remissions in which the disease does not progress. Symptoms may improve or disappear during remission. Disease progression and clinical outcome in MS are usually monitored by the expanded disability status scale (EDSS) with scores ranging from 0 (no signs) to 10 (death).[1] MS has a vast impact on health, activity limitations, and may reduce health-related quality of life. Many symptoms associated with MS lead to continuing decline in neurologic status. Consequently, patients show limitations with negative influence on the quality of life.[2] Many studies on the quality of life in MS patients focus on medical variables such as disease progression, treatment, diagnosis, and individual variables such as gender, social and individual supports, onset of disease, comorbidity, health-related behaviors, socio-contextual factors, and other confounding factors.[3–6] Reduction of daily activity in MS patients also depends on fatigue and muscle weakness. Fatigue in MS, resulting from central causes or secondary muscle disuse and inactivity, can be very disabling because patients are required to sit, lie down, or sleep[7] and limit their social relationships, self-care activities, and jobs.[8] Fatigue treatment is currently restricted to energy-conservation strategies, cooling therapies, cognitive therapy, and pharmacological interventions including antidepressant therapy and wake-promoting agents. Fatigue may be improved by an exercise program.[9] Fatigue may also influence the ability of MS patients to exercise and limit benefit from exercise programs. The coexistence of physical and cognitive impairments, together with the unpredictable evolution of the disease makes MS rehabilitation very challenging. The main objective of rehabilitation is the relief from symptoms and improving self-performance and independence. There is evidence of the benefits of physical activity and exercise interventions in the rehabilitation of MS patients since the 1990s when Petajan et al. showed a beneficial effect of exercise therapy for people with MS.[10] Several clinical trials have examined the effect of physical therapy rehabilitation programs on ambulatory outcomes. Motor performance of MS patients improves following combined or isolated aerobic or resistance training or with aquatic training.[11] Other authors have confirmed the effectiveness of exercise therapy for this population. Exercise may increase the energy reserves available for physical activities.[12–14] and enhance neurobiological processes that could promote neuroprotection and neuroplasticity. Exercise therapy

may affect both primary mechanisms (e.g., neuroprotection or hormonal function), secondary factors related to fatigue (e.g., inactivity or comorbidity) and may reduce long-term disability.[15] Physical exercise may be an easily implemented noninvasive intervention that may have positive effects on fatigue,[12,16] health-related quality of life,[17] and muscle strength.[18] Some studies also suggest that resistance training may have direct effects on MS via modulation of blood cytokine levels.[19] Research carried out during mid-2010s suggests that the skeletal muscles serve as secretary organs that produce and release a contraction-dependent variety of cytokines mediating both direct and indirect anti-inflammatory effects.[20,21] Calle et al. suggested that higher-intensity exercise will have an important effect on resistance training.[22] Another important factor is lower body muscle strength, which is impaired among many MS patients and is a predictor of walking speed. A 2016 review shows that progressive resistance training is associated with change in muscular fitness parameters (small in magnitude) and concludes that an indication of magnitude is important for clinical research and practice by providing an evidence-based estimate of the actual benefit that exercise training confers on physiological fitness.[23] Physiotherapy has also been shown to have a positive effect on balance and mobility.[24,25]

Neuromuscular taping (NT) has become a very popular technique for treatment of various musculoskeletal and neurological disorders over the past decade. NT is known under various commercial names and has different methods of application. This technique was created by Kenzo Kase, a Japanese chiropractor in the 1970s. Kase tried to create something that was able to "reproduce the effects of the therapist's hand." The idea was to create a tissue with elasticity similar to the skin that supports muscles during movement. This tape was used during the Seoul Olympics in 1988 by athletes Japanese and then was introduced in the late 1990s from Japan to Europe and United States. The characteristics of NT were essential to the therapeutic success and to did not cause secondary damage and irritation of the skin. The tape is a cotton, latex free porous material with an acrylic adhesive coating, which is applied to create waves (Fig. 14.1). The NT is breathable, waterproof, thinner and more elastic than conventional tape, can be stretched up to 120–140% of its original length, and designed to mimic the qualities of human skin through its specific thickness and high elasticity. The taping technique is based on a tension or decompression, which facilitates natural neurophysiological processes.

NT is believed to serve several functions, including restoring correct muscle function by supporting weakened muscles, realignment of joints, and change in the recruitment activity patterns of the treated muscles. This

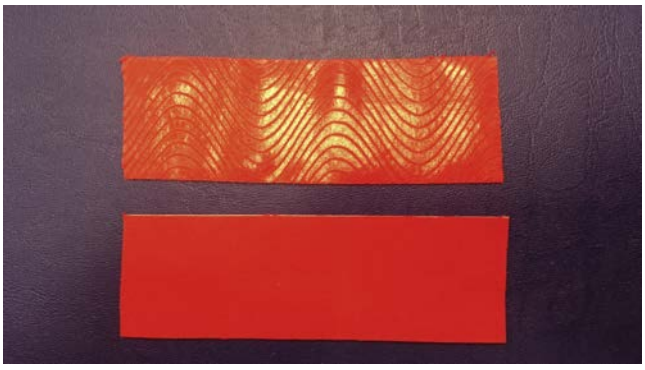

FIGURE 14.1 Neuromuscular taping: a cotton, porous with acrylic adhesive applied to create waves and latex free.

has the effect of reducing congestion by improving the flow of the blood and lymphatic fluid, and increasing afferent feedback to the central nervous system resulting in decreased pain intensity. Previous studies determined the effects of NT on cutaneous mechanoceptors and reported that its application may improve the excitability of selected muscles and joints.[26–29] Thus, when a muscle is inflamed, swollen, or stiff due to fatigue, the space between the skin and muscle is compressed with consequent activation of mechanoceptors that causes local depolarization and triggers nociceptive stimuli that communicates discomfort signals to the brain. The application of NT provides a pressure to the skin or stretches the skin, thus stimulating cutaneous mechanoceptors, causing physiological changes in the taped area.[29] We conducted a literature review for determining whether NT is effective in people with neurological disorders, especially in MS. The selection process involved screening the titles and abstracts of previously published studies. Potentially relevant articles were obtained in full text for further eligibility analysis. The following data were extracted: authors, year of publication, neurological condition of the participants, study objectives, description of the sample, description of the NT intervention used, description of the control group (i.e., placebo, no intervention, or other intervention), study outcomes, assessment times, study results, and study conclusions. Among the eligible studies, five recruited people with stroke,[30–34] four recruited people with cerebral palsy,[35–38] and four recruited people with MS.[39–42] Seven trials compared NT with no treatment, two trials compared NT with sham tape (ST), and four trials compared NT with other interventions. Other studies included interventions that ranged from other taping methods, exercises, manual techniques, and stretches. In stroke patients, some positive effects were obtained by combining ankle NT and botulinum toxin to reduce plantar flexor spasticity. Intergroup comparison demonstrated an average benefit of 5° ($p = .015$) in passive dorsiflexion in a therapeutic NT group only at 2 weeks. No other differences

were found between groups.[30] According to Kim et al. application of NT to the stroke patient before therapeutic exercise has a positive effect on improvement of asymmetric gait and walking speed compared to therapeutic exercise.[31] Ezik et al. showed an improvement of isokinetic parameters on the paretic and nonparetic side in stroke patients when NT was applied to quadriceps muscles in addition to conventional exercises for 4 weeks.[32] According to Kaya Kara et al. NT applied to children with cerebral palsy increased proprioceptive feedback and improved activity limitation and participation restriction.[35] In the other examined studies, NT was applied with the aim of decreasing spasticity by enhancing sensory inputs.[33,34] The authors suggested that this application might enhance skin receptor output, stimulating supraspinal centers, and thus improving joint position and kinesthetic senses and proper development of the motor control.[36–38]

NT in MS patients was studied in few investigations.[39–42] Cortesi et al. observed positive effects of NT on center of pressure of the ankle and balance parameters in MS patients, suggesting that NT stabilizes body posture immediately after application.[39] Mazzei et al. compared the short-term effect of NT and ST (nonelastic) for improving body-standing stability in MS patients. That study did not support the therapeutic benefits of NT on body-standing stability.[40] In our experience with MS patients NT was effective at improving motor performance and quality of life.[41] In that study, we used very restrictive selection criteria such as absence of relapses during the last 3 months, absence of rehabilitation treatment, or symptomatic drugs acting on muscular tone or fatigue for at least 2 months, and a stable disease-modifying treatment for at least 3 months. Only 19 of 20 selected patients, with mean EDSS of 3.4 ± 1.26 and affected by a relapsing–remitting form of MS, completed the study. In this study, NT, of 20-cm long and subdivided into five fan-like fringes, was applied, four times at 4-day intervals, to each patient on the weakest side of the hamstring muscles observed at gait observation analysis (Fig. 14.2). The treatment efficacy on motor performance was evaluated by the 6-min walking test (6MWT), measured at the beginning of treatment (T0), at the end of NT application (T1), and 3 weeks after the last treatment application (T2). Moreover, Short Form 36 (SF-36) health survey was administered to evaluate quality of life. The 6MWT demonstrated a statistically significant improvement between the baseline and the subsequent assessments. A significant change was already evident at the end of last application (T1), and was maintained without variation through T2.

The aforementioned study had several limitations. In particular, it was carried out on a small sample as an open-label trial and did not investigate the muscle strength and resistance of lower limbs. Our first study

FIGURE 14.2 Neuromuscular taping, subdivided into five fan-like fringes, applied on hamstrings muscles.

showed positive effects both muscle groups of quadriceps and hamstrings suggesting a likely general effect of NT. These results induced us to evaluate strength of both muscle groups. For this reason, we used the isokinetic test for evaluating lower limb muscle peak torque. The encouraging results observed allowed us to develop a randomized controlled trial with a larger sample, placebo control, and instrumental evaluation.[42] In this study, we investigated effectiveness of NT on leg muscle strength and motor performance compared with ST groups. The target muscle was quadriceps, that play an important role in controlling knee motion, stability, and impact loading,[43] and presented a greater deficit to isokinetic test evaluation.

In this subsequent study, 40 relapsing–remitting MS patients were recruited and randomly assigned to NT or ST groups. All patients followed the same inclusion criteria as the previous study. All patients underwent to the treatments five times at 5-day intervals. In the ST group, we applied an elastic and unextensible tape formed by silk (Rayon tissue) with high tensile strength (Fig. 14.3).

In NT group was applied on quadriceps muscle an NT 20-cm long and subdivided into five fan-like fringes (Fig. 14.4).

Each patient was evaluated at baseline (T0), at the end (T1), and 2 months after the end of the treatment

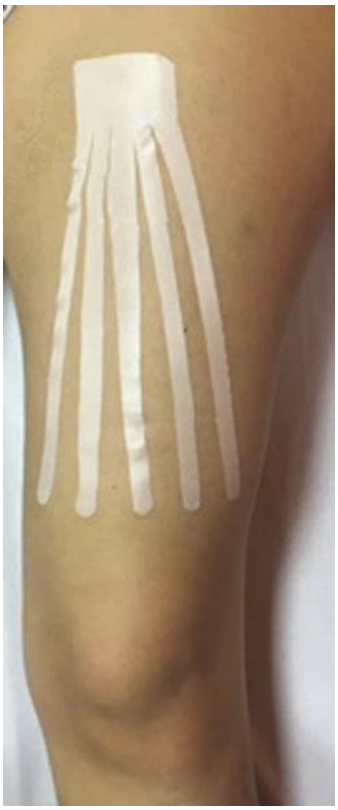

FIGURE 14.3 Sham taping: an elastic and unextensible tape made of Rayon tissue and applied like NT.

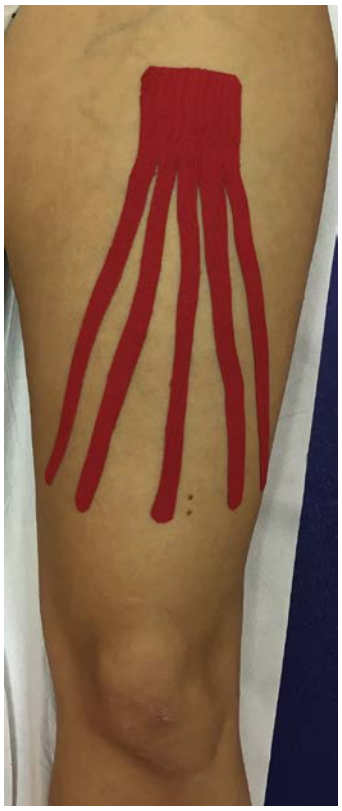

FIGURE 14.4 Neuromuscular taping, subdivided into five fan-like fringes and applied on quadriceps muscles.

(T2) with 6MWT and isokinetic test. The isokinetic dynamometer performs muscle exercises at a constant speed across the entire range of motion. The isokinetic device determined objectively the patient's condition and was used to monitor the improvements during the rehabilitation treatment. The isokinetic test was performed in knee flexion–extension in concentric contraction. Both groups completed two different exercises test. The first one evaluated quadriceps and hamstrings strength by 5 repetitions of flexion–extension with angular speed of 90°/s; the second one evaluated quadriceps and hamstrings endurance by 10 repetitions of flexion–extension with angular speed of 180°/s. Peak torque of quadriceps and hamstrings, measured in newton/meter, was evaluated and the parameters of the weaker limb were compared with the healthy one. The results showed that in NT group a significant change of peak torque of quadriceps and hamstrings was already evident at the end of the last application (T1), and was maintained through T2 time. Besides, delta peak torque T1–T0 and T2–T0 between two groups were statistically significant in quadriceps ($p = .007$; 0.000) and hamstrings ($p = .011$; 0.007). The difference between two groups at 6MWT was not statistically significant but in the NT group we noticed an increasing trend about the distance run. From data evaluation in both groups, it is possible to observe differences between the two treatments. As a matter of fact, we believe that effects induced by the NT application may provide a positive influence on muscle strength as demonstrated by improvements in isokinetic test. MS patients present with a combination of muscle weakness or muscle imbalance, decreased postural control, muscle spasticity, muscle strength, poor voluntary control, and body malalignment.

There have been a few studies that have suggested the effectiveness of the NT on musculoskeletal disorders, muscle strength and motor performance in neurological disease. In particular in MS, poor information exists on the effectiveness of NT in conjunction with other therapeutic treatments to improve functional performance and muscle strength. The limits of this study include the following: (1) it investigates only the immediate effects of NT—therefore, the effects cannot be generalized to middle- to long-term outcomes; (2) no comparisons with other interventions or control group are made. As in other studies that involve rehabilitation, standardization and randomization are particularly difficult and may be affected by specific biases such as inclusion bias and clinical confounders. Thus far, few published studies seem to confirm the positive benefits of NT when used as an adjunct to neurorehabilitation, but most of them were not randomized controlled trials and involved small samples of participants. The difficulties in investigating this topic are probably related to the intrinsic variability of these diseases. From a clinical point of view, NT can be tailored to the specific needs of each

single patient; on the other hand, this adaptability implies a lack of repeatability that could introduce a potential bias from a scientific point of view. Furthermore, most of the analyzed studies payed more attention to parameters such as static balance, regardless of functional outcomes, quality of life, depression, and anxiety. At the moment NT might be considered a "trend" because the existing studies are few and show partial and ambiguous results evaluating different variables and analyzing only short-term effects of NT. This approach does not allow a test of the real potentialities of NT at medium- and long-term on the possible functional outcomes. The present findings should encourage researchers who, as of 2016, are providing MS population rehabilitation programs. Future studies should investigate whether the increase in muscle strength, motor performance, and quality of life are correlated to application of NT.

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CHAPTER

15

Constraint-Induced Movement Therapy: When Efficacious Motor Therapy Meets Progressive Disease

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OUTLINE

MULTIPLE SCLEROSIS: A PROGRESSIVE DISEASE THAT IS RESPONSIVE TO CONSTRAINT-INDUCED MOVEMENT THERAPY

Multiple sclerosis (MS) was first described by Charcot and Vulpian in 1866.[1] MS is a combined inflammatory and degenerative disease that affects neurons, the cells of the brain and spinal cord that transmit information, enable sensation, create perception, and allow purposeful control of the body. Covering these neurons is a fatty acid layer called the myelin sheath that helps neurons rapidly transmit information to other neurons. MS slowly destroys these myelin sheaths and axons, disrupting processes in both the brain and the spinal cord. MS seems to affect about 2.5 million people globally and 400, 000 people in the United States,[2] and its prevalence is globally rising.[3–9] The etiology of the disease has yet to be discovered. Most of the symptoms of the disease present between ages 20 and 40 years, though they can begin under age 10 years or in middle age. There is no single symptom that directly points to an MS diagnosis; rather, a series of signs commonly lead to its diagnosis. MS is typically a gradually progressive disease. The disease

most commonly advances over many years in alternating stages of attack and stability of disease, while in other forms the disease continually progresses without attacks.

Possible Causes

Although the cause of MS is still not certain, research suggests a combination of both environmental and genetic susceptibility. A large meta-analysis has confirmed the long-held notion that prevalence, incidence, and mortality of the disease vary by geographical latitude gradients.[10] This latitude variance is possibly accounted for by the contribution of vitamin D insufficiency and its respective immunomodulatory role in the peripheral immune system.[11] Ultraviolet-B solar radiation produces vitamin D in the skin. This radiation decreases as sun exposure decreases near the poles. MS incidence conversely increases toward the poles.[12] Vitamin D's correlation with disease prevalence and its immunoregulatory role[13,14] seem to make it an ideal candidate for MS treatments. Although there has been no evidence that increasing patients' serum vitamin D levels benefits outcomes,[15] higher serum vitamin D concentrations have nonetheless been associated with a more favorable disease course.[16]

A genetic basis for the disease is suggested by the 25–30% concordance rate in monozygotic twins as compared with 5% in dizygotic twins. The genes associated with the major histocompatibility complex involved in antigen presentation may be involved. So far, only one gene has shown a strong association for the disease—the HLA-DRB1 gene, specifically the HLA-DRB1*1501 allele. This allele increases disease risk by three times.[17]

There also seems to be a link between MS and Epstein–Barr virus (EBV) infection, a very common virus present in about 95% of the adult population. How EBV links to MS is not exactly known, but it seems that the infection may facilitate the disease mechanisms by altering the immune system.[18] EBV may share genetic homology with a myelin-targeting antigen to trigger antibody reproduction. Strong support for an infectious etiology comes from the spike in MS cases in the Faroe Islands following British military occupation during World War II.[19,20] Convergent findings come from the increased incidence of MS among migrants to Australia from the United Kingdom and Ireland who were older at the time of emigration, compared to younger migrants.[21] This suggests that the environment of the United Kingdom and Ireland exposes individuals to a time-dependent risk factor for MS, such as a contagious pathogen.

A frustrating aspect of MS research is the unavailability of animal models that closely approximate the course and pathology of the disease.[22] Especially puzzling is why MS afflicts only humans among the primates. One suggested explanation is the uniquely human deficiency in the ability to synthesize sialic acid *N*-glycolylneuraminic acid. As a result, the only exposure to this compound in humans comes from chronic red meat consumption. It has been suggested, therefore, that red meat consumption could cause this compound to enter the nervous system and then to trigger antibody release that could attack the nervous system.[23]

Finally, it is noteworthy that MS affects about twice as many women as men,[24] with an increasing incidence partially attributed to occupational changes, smoking, obesity, birth control, and delayed childbirth.[25] In women with MS, the rate of relapse declines during pregnancy and then returns to the prepregnancy rate about 3 months post-partum.[26] These observations hint at a hormonal influence on the presentation of MS.

Clinical Presentation

The clinical presentation of MS usually includes a host of symptoms. These may include but are not limited to: somatosensory disturbance, blurring or loss of vision, and general pain. Fatigue in MS patients is considered one of the main causes of decreased quality of life[27] and is reported in at least 75% of patients at some point of their disease.[28,29] Ninety percent of patients with MS develop lower urinary tract symptoms after 10 years of disease progression.[30] Cognitive impairment has been estimated to affect 54–65% of MS patients over the course of their lifetime,[31,32] though some argue that this percentage range is inflated as a result of study participants being commonly recruited from academic medical centers.[33] Depression is also prevalent among MS patients,[34–36] more so than in the general population.[37] Similar to other diseases, depression decreases quality of life[38] and increases suicide risk[39] for these patients.

MS usually progresses in one of four clinical patterns: relapsing–remitting MS (RRMS), secondary progressive MS (SPMS), primary progressive MS (PPMS), and progressive-relapsing MS (PRMS).[40] RRMS, which accounts for about 85% of MS cases, causes transient increases (relapses) of specific symptoms. These relapses are followed by extended periods of symptomatic recovery (remissions). Relapses may last from few days to weeks while remissions may last months or years. Most RRMS patients eventually devolve into SPMS and their symptoms gradually worsen as their remission periods diminish. In PPMS, patients do not have explicit attacks or remissions but rather continually slow worsening of symptoms. Finally, PRMS patients have both the progressive worsening and the sporadic relapses.[41]

Diagnosis

Because no single feature or test sufficiently or accurately diagnoses MS, neurologists typically look for isolated symptoms that recur weeks or months apart. MS is characterized by a unique pathology that is visible on

magnetic resonance imaging (MRI) with or without the intravenous administration of contrast material (gadolinium), particularly in the white matter. In fact, MRI is perhaps the most sensitive diagnostic test for MS, because about 95% of patients diagnosed with clinically definite MS show MRI abnormalities.[42] The MRI criteria for brain abnormality require at least three of the following: (1) one gadolinium-enhancing lesion or nine T2-hyperintense lesions if there is no gadolinium-enhancing lesion, (2) at least one infratentorial lesion, (3) at least one juxtacortical lesion, and (4) at least three periventricular lesions. This four-parameter MRI model has been found to better predict clinically definite MS than traditional models, such as by Paty et al.[42] and Fazekas et al.,[43,44] that are less sensitive and specific in their lesion characteristics. It should be noted, however, that the degree of clinical disability is unrelated to the extent of abnormality seen in MRI. In fact, some patients with clinically benign presentations have had extensive MRI abnormalities and vice versa.[45] This so-called clinical–radiological paradox may in part reflect the compensatory effects of adaptive neuroplasticity[46] (described in the following sections), as well as present technical limitations in conventional neuroimaging.[47]

Clinical criteria for diagnosis require symptoms to fulfill dissociation of disease progression in both time and space.[48] Preclinical evidence is commonly used to aid in MS diagnosis. Abnormalities detected in oligoclonal bands of cerebrospinal fluid and increased immunoglobulin G are considered the primary laboratory findings that support diagnosis, while other procedures like tissue-imaging techniques and evoked responses can extend clinical examination.[49] A more detailed analysis of the diagnostic scheme can be found in the guidelines from the International Panel on the Diagnosis of MS.[50]

Treatments and Clinical Management

There is no known cure for MS. Rather, neurologists aim to reduce the inflammatory neural damage, particularly to the myelin sheath of axons and the axonal integrity, to help delay relapses as long as possible. Because no drugs prevent demyelination, treatments for acute disease relapses focus on symptomatic control. Intravenous high-dose steroids and glucocorticoid therapy (such as a 3–5-day regimen of methylprednisolone) can diminish inflammation and pain. Glucocorticoids, though not a long-term solution for patients, can regulate the cytokine network in patients and augment production of the immunosuppressor cytokine IL-10.[51] Other acute treatments include plasma exchange to decrease the concentration of circulating serum antibodies, and intravenous immunoglobulin for clinically isolated syndromes and RRMS. Additionally, several disease-modifying agents, notably interferon beta[52] and glatiramer acetate,[53] among about a dozen alternatives, can ameliorate MS relapse rates. Mark and Geng summarize many treatment options and respective drug therapies[54] and describe several clinical restorative processes in MS such as cell therapies, central nervous system (CNS) neuromodulation, and even physical exercises.[55] This chapter focuses mainly on a physical training method, constraint-induced movement (CI) therapy, which has been adapted from stroke patients and was founded from a basic neuroscience model of disability.

CONSTRAINT-INDUCED MOVEMENT THERAPY

CI therapy promotes improved function in part through harnessing mechanisms of endogenous neuroplasticity. Accordingly, we first provide some background on neuroplasticity.

Neuroplasticity

During the mid-19th century, Paul Broca attributed distinct cognitive disorders of his patients to the effects of circumscribed brain lesions that were found at autopsy. This association essentially founded the modern concept of functional mapping of the cerebral cortex. By localizing function, Broca notably had established a topographical view of the human brain.[56] "Broca's areas" helped propel the traditional view in neuroscience that spanned the first 75 years—that the mature CNS has little, if any, capacity to reorganize and self-repair in response to an injury or insult. Although some researchers disagreed,[57–60] the adult CNS was believed to exhibit no plasticity.[61–63] Even though the human brain constantly takes in, processes, and synthesizes new information, the original reasons for such a static view of the CNS are not known, although the common observations that severe CNS injuries tend to have permanently disabling effects following usual care, and abrupt focal injuries (such as from stroke or penetrating missile injuries) become converted to permanent cystic spaces (holes), likely contributed.

However, following recent investigations (some of which are described in the following section), it has become widely accepted that the adult CNS is capable of intrinsic modification. This cortical reorganization is now referred to as brain plasticity. Although neuroplasticity has far-reaching implications and various definitions that range from microscopic synaptic alternations in conductivity to more macrolevel anatomical changes in CNS structure, this chapter emphasizes the latter in adult humans.

Measuring CNS Plasticity

Patients with progressive neurological diseases, like MS, exhibit differences in brain activity when compared with those without CNS injury. One study, for instance, found that RRMS patients had greater amounts of spontaneous brain activity (resting state) in the insula and

superior temporal gyrus ipsilateral to the more-impaired upper extremity compared with controls.[64] Patients with hemiparetic MS have exhibited greater functional magnetic resonance imaging (fMRI) activation than controls in motor areas ipsilateral and contralateral to the more-impaired arm during a sequential finger-to-thumb task.[65] A third found that reduced task-specific reduction in fMRI activation of motor areas follows trained thumb movements in individuals with MS compared to controls.[66]

Functional imaging methods require patients to perform a task during MRI data acquisition. For patients with movement disorders, the patterns of movement can vary significantly between data acquisition periods, which may challenge the consistency of findings for tasks that are based on motor responses. Structural imaging acquisition parameters, in contrast, do not require patient activity and are therefore preferable for identifying neuroplasticity changes among patients.

The white matter integrity of the CNS of MS patients is reduced, as indexed by a magnetic resonance neuroimaging technique called diffusion tensor imaging (DTI). DTI, developed in 1986, has been established as a validated measure of white matter integrity.[67–69] It is based on sensing the nonrandom movement of water molecules in the brain.[70] Free water typically moves in an isotropic fashion (i.e., its molecular diffusion is not directionally restricted), thus it is equal in all directions. In contrast, the structure of CNS white matter more considerably restricts water diffusion, and thus it is said to move relatively anisotropically. One of the primary output measures of DTI is the scalar fractional anisotropy (FA) value, which ranges from 0 to 1, where large FA values represent diffusion of water largely in a common direction, while low FA values indicate more random water diffusion.[71] In very compact white matter tracts FA is close to 1 and approaches 0 in the relatively unconfined cerebrospinal fluid compartment.[71] Changes in FA values reflect altered cellular structural properties of myelin content[72] and axonal density.[73] In addition to FA, three other primary diffusion indices are used: mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD). Increases in local myelin content decrease MD and RD values, while post-insult compensatory mechanisms increase AD values.[74]

Voxel-based morphometry (VBM) is another structural imaging technique, which measures density (and potentially, the volume) of gray matter of the whole brain using T1-weighted structural MRI. Conceived in 1995,[75] VBM compares the local concentration of gray matter between two groups of subjects by analyzing the values of voxels, the three-dimensional pixels that compose the digital MRI image. After spatially normalizing high-resolution MRI images of multiple subjects who belong to either of two groups into one stereotactic space, gray matter, white matter, and cerebrospinal fluid are segmented and separated. The images are then smoothed, and voxel-wise parametric statistical tests compare the images of the groups for anatomical differences in gray matter density.[76] Rather than strictly referring to conventional anatomical areas, VBM uses images and their scalar measurement properties to test for local differences wherever they may occur. As a result, VBM is not restricted by anatomical structures and can test for differences anywhere in the brain.[77] VBM is now an established tool in morphometry.[78] Many VBM studies have found statistically significant structural brain differences between different patient populations (e.g., when comparing two different diseased groups, comparing diseased patients to healthy controls, or evaluating changes in the same group at two different time points).[79-81]

Origins of CI Therapy

In the 1970s, several research laboratories discovered that the adult mammalian CNS also has at least some capability to reorganize functionally after insult.[82–86] We now know that in the CNS there is to some degree a spontaneous recovery of function post-injury (although due to the lack of advanced experimental techniques, this spontaneous recovery received little attention during this period).

The relationship between brain plasticity and spontaneous functional recovery and its potential therapeutic manipulation to improve functional recovery is, therefore, important to help patients who have suffered neurological damage. A fundamental body of research that addresses this question is that of somatosensory deafferentation in monkeys. Surgical abolition of somatic sensation via severing all sensory nerve roots that serve a single forelimb in monkeys inhibits the animals from using that extremity in the free-life situation. The surgery prevents all afferent input from the limb from reaching the brain. Consequently, cortical reorganization takes place over the entire arm area of the cortex following such somatosensory deafferentation of an upper extremity in a monkey.[87] Investigations using magnetoencephalography (still another functional imaging technique) have supported the occurrence of post-illness cortical reorganization in humans.[88] Use-dependent cortical representation studies in monkeys also have found that increased use of a body part results in functional expansion of that cortical area.[89–92]A similar training-associated cortical reorganization has been found to occur in humans, as well.[93–97] Contrarily, there also seem to be reductions in the cortical representation of the more-affected extremity following stroke, perhaps as a result of its reduced use.[98,99] During the 1960s Taub and colleagues discovered that two techniques prompted the experimentally treated monkeys to use the affected, deafferented limb: prolonged restraint of the unaffected forelimb and shaping of training of the affected forelimb.[100] The following

techniques and relevant protocols have been translated from the primate methods to humans, establishing some of the basic procedures and the key theoretical bases of CI therapy.

Prolonged Restraint

After all dorsal spinal nerve roots of a single limb are surgically abolished, resulting in its loss of somatic sensation, the primate does not use the limb in the free situation,[101–105] even though the ventral motor roots are structurally intact and thus, it would appear, retain the capability of moving the limb. The monkey can adequately accomplish all routine activities of daily living (feeding, grooming, and climbing) with the other three limbs and so gives up using the deafferented limb, perhaps because the absence of sensation in the limb makes using the limb unreliable in the untrained situation. Therefore, the monkey has no incentive to put the deafferented forelimb to use because of behavioral compensation by the other limbs, which is called learned nonuse and is reviewed later. However, experimentally restricting movement of the unaffected forelimb, such as with a straitjacket, results in the primate's regaining use of the deafferented extremity.[102,106,107] Although the use is clumsy and unlike its presurgery state, it is still effective and used for multiple purposes. This change, from an inactive limb to a functionally useful one, occurs in hours, even if the limb has not been used for years.[108,109]After a week of restraint, the switch lasts for the remainder of the animal's life.

In humans, a padded or protective safety mitt is used to provide the restraint. This mitt allows the less-affected arm to be freely moved proximally while prohibiting the use of the hand and fingers in the patient's activities of daily living. In this way safety is maintained by allowing individuals to brace and balance themselves. Participants, by signing a behavioral contract, agree to wear the mitt on the less-impaired hand for a target of 90% of waking hours during the treatment period.

Shaping

Many investigators have employed behavioral techniques in animals to improve a motor deficit due to neurological damage.[110–113] Another method of facilitating using a single deafferented limb is training.[101,102,106,108,109,114–118] Termed more specifically shaping, this tremendously improves motor ability of the deafferented limb in the life situation.[107,108,114,117] This operant training method requires behavioral or motor tasks to be approached in small, progressively more demanding steps. As a result, the relative improvement at each incremental step is small, though the total absolute improvement is large.[119–124] In the primate experiments, there was an almost complete reversal of disability.[117,125,126] For example, in one experiment involving pointing at visual targets, Taub and colleagues were able to achieve the desired function (pointing at a target without view of the limbs) by gradual shaping. The animal was first allowed to see its limb throughout the movement, then only pointing finger, and finally only the target without view of the limbs.[117]

Shaping is very flexible and personalized. Patients are shaped by urging them to strive for their personal best on a task and are praised for their success. It provides a channel from the training sessions to activities of daily living and may generalize to improving spontaneous limb use on activities other than those specifically trained.

Transfer Package

During mid-2000s, in addition to shaping and physical restraint of the less-affected arm—which were the original essential components of CI therapy developed with the deafferented monkeys—the "transfer package" (TP)[127–129] was included to induce transferring gains in motor function from the laboratory therapy to the home setting. The experimental monkeys did not, of course, have (nor need) a transfer package, because restraint of the unaffected forelimb immediately placed the animals into a "do or die" situation—they had to use the deafferented forelimb, or else they would perish in their home environment. TP procedures, which are explained later, also require intact language skills, which are not available to monkeys. Neurologically impaired humans, by contrast, typically do not face such survival challenges during CI therapy owing to the protective effects of medical oversight and societal support. Because their survival is not at risk, patients who undergo CI therapy might therefore not maximize in their recovery of more-impaired limb use without such additional procedures. It is worth noting, however, that one case report described a "natural" CI therapy result, when a woman fell soon after she had had a stroke. The stroke had markedly paralyzed her left side while the fall had fractured her right arm, which had to be casted for orthopedic recovery and in addition could not be used because of marked pain.[130] Thus, the patient automatically was forced to use her paralyzed left arm for self-care or else be functionally armless. Despite not undergoing formal CI therapy in her rehabilitation hospital, she gradually recovered full use of her premorbidly nondominant left arm, which she relied on even a year later owing to sustained pain and difficulty using her right arm. The clinicians who cared for her had never seen such dramatic recovery of arm use in relation to the marked amount of initial paralysis.

The TP is a set of behavioral procedures that are routinely used in various other behavioral interventions

(in particular, curbing substance abuse), but which are generally left out of physical rehabilitation. It includes seven key components: (1) daily registration of use of the more-affected arm in the home environment by means of a structured interview; (2) home practice of a number of activities of daily living; (3) daily problem-solving discussions with a therapist to overcome perceived barriers to using the more-impaired extremity in the home; (4) a signed behavioral contract with agreed-on activities for the more-affected arm; (5) restraint of the arm in the padded mitt for 90% of waking hours (and removed before coming in contact with water); (6) a patient-kept daily diary that details compliance with the therapeutic program and summarizes the patient's activities in the life situation; and (7) weekly telephone review of adaptation to the home environment between the patient and therapist for the first month after the end of therapy. Our laboratory's research has shown that the TP is critical for maximizing therapeutic gains and neuroplastic effects of the therapy.[81] By heavily involving patients in their treatment procedures, the TP most likely gives patients a sense of responsibility and ownership in their treatment, and motivates them to adhere to therapist-set guidelines that help bolster their improvement.

Massed Practice

Massed practice, or extended repetitive movement training, is an important feature of CI therapy.[131–133] The optimal amount of massed practice is undetermined.[134–136] At present CI therapy involves repetitive, behaviorally relevant practice of increasingly difficult tasks (shaping) for several hours a day over 10 to 15 weekdays.[134–137] A therapist continuously monitors the participant to ensure intense practice. Massed practice is considered to be crucial to achieving the effective therapeutic factor in CI therapy,[131,132,138] and a precursor for the success of the TP. Regardless of the amount of massed practice, it seems that the sensory experience of patients determines whether enhanced cortical plasticity occurs.[89] Thus, massed practice may be an important factor in the long-term improvements in motor function of patients following CI therapy.[124,139,140]

Possible Mechanisms Behind the Efficacy of CI Therapy

The Learned Nonuse Phenomenon

Although not completely understood, it is generally accepted that a significant neurological insult, depending on the location of injury, may lead to reduced movement or perception. Monkeys experience chronically reduced use of the experimentally deafferented upper extremity without the intervention of an efficacious training procedure.[108,109] Immediately following the surgery, the monkeys unsuccessfully attempt to use their deafferented extremity, but such efforts are usually futile and result in incoordination, falling, and general motor failures. This general motor failure serves as a behavioral punishment against making further efforts to use the limb,[141–143]and the fact that the animals can survive on three limbs in the laboratory setting only further strengthens this nonuse by acting as a positive reinforcer. This punishment/reinforcement loop results in the animal never learning that its affected limb is potentially useful, even after many months into the post-deafferentation recovery period. This behaviorally conditioned suppression of movement is termed learned nonuse.

Intervention via physical restriction of the non-deafferented limb gives the monkey a choice: it can either use the deafferented limb or lose the ability to carry out basic tasks like feeding, ambulating, and many more activities of daily living. The usual outcome is that this constraining behavior prompts the monkey's use of its deafferented limb and eventual overcoming of the learned nonuse phenomenon. After the restriction device is left on for several days or more, the deafferented limb gains enough functional competence to compete with the non-deafferented limb upon removal of the restrictive device. If the device is removed before this critical period, then the monkey resumes relying on the non-deafferented forelimb as the primary, if not only, limb for upper extremity use. Since its inception, several studies have been carried out to test the learned nonuse hypothesis in monkeys, both pre-[125,126]and post-natally.[108,109] Animals that had a forelimb unilaterally deafferented prenatally used the deafferented extremity on the first day of extrauterine life for support during sitting. The animal's ability to use the deafferented limb improved as the animal matured.

Use-Dependent Plasticity

More recent findings in the literature have suggested that reorganization of the brain, or changes in the area and topography of cortex devoted to a specific motor or sensory function, may also be responsible for the benefits elicited by CI therapy. It has been found that increased use of a limb results in an expansion of the cortical representation of that limb in both monkeys[89–92] and in humans.[93,94,144] Additionally, it has been shown that cortical representation of an extremity that has been affected by a neurological insult improves after training the extremity intensely.[145] Structural changes in tissue have been observed due to increased inputs. For instance, London cab drivers have been found to show an increased posterior hippocampal volume which is associated with their level of cab driving compentency.[146] (The routine adoption of GPS devices, however, may theoretically thwart such hippocampal gains.) Other, more physically coordinated tasks, such as juggling,

have been found to increase the density of gray matter in the anterior occipital regions or the cortex.[147]

Physiological neuroplastic changes, such as the in the rate of metabolism,[148] blood flow,[149] and excitability[98] in regional brain activity have also been found following CI therapy. Using transcranial magnetic stimulation, one group found that the cortical area of representation of the abductor pollicis brevis (a muscle in the hand) was almost doubled in the infarcted hemisphere following CI therapy.[98] Neuroplasticity due to CI therapy has also been identified using fMRI techniques. In one study functional activation differences following CI therapy were found in bilateral cerebellum, somatosensory cortex, and premotor cortex of the lesioned hemisphere which were related to measure of motor ability.[150] Another study found that the relationship between fMRI activation during CI therapy and functional gains due to therapy could suggest using fMRI to predict treatment gains from therapy.[151] This use-dependent plasticity of the brain may serve as a mechanism for the long-term benefits patients receive from the therapy.

CI Therapy Applications

CI therapy has been found to produce large, clinically significant improvements in motor function.[128,133,152] It was first shown to be efficacious in treating chronic stroke hemiparesis[153] and has since been applied to several other diseases including cerebral palsy,[154–157] traumatic brain injury,[158] phantom limb pain,[159] focal hand dystonia in musicians,[160] and MS.[161] There is therefore much evidence to support the efficacy of CI therapy for the treatment of motor deficit to extremities after CNS damage. CI therapy produces motor improvements in the laboratory and real-life situations[153,162,163] and has been replicated in several laboratories.[134,139,164,165] Perhaps most important, CI therapy has undergone multiple placebo-controlled single-site trials[128,153,163] and a multisite randomized national clinical trial, the Extremity Constraint Induced Therapy Evaluation (EXCITE).[152,166] In the EXCITE trial, 220 patients were randomly assigned to either CI therapy or to typical care. The EXCITE trial found significantly larger improvements in more impaired arm use of the patients in the CI therapy group than those of the control group. This functional improvement was sustained over a 2-year follow-up period.[167]

CI THERAPY IN MS

Recent work from our laboratory has investigated applying CI therapy to MS. To date, the only randomized controlled trial of CI therapy in adults with MS and chronic upper extremity hemiparesis has been at the University of Alabama at Birmingham by our laboratory. Enrolled between February 2010 and July 2013, these 20 patients were referred by local MS specialists or referred themselves from seeing the trial notice at ClinicalTrials.gov (NCT01081275) or our laboratory website. Half of the patients were randomly assigned to receive CI therapy while the other half received a set of holistic, mostly physical treatments collectively called complementary and alternative medicine (CAM). Both of the groups received the same amount of therapist contact throughout their treatment.

Complementary and Alternative Medicine

CAM is sought by one-half to three-fourths of MS patients.[168] The top six most used CAM therapies in MS include reflexology, massage, yoga, relaxation, meditation, aromatherapy, and acupuncture.[169] A national survey of 3140 MS patients in the United States found that an average of about five CAM therapies were sought by each MS patient.[170] Besides dissatisfaction with conventional MS medicines,[171] patients often turn to CAM therapies because they desire more holistic care[172] and believe that traditional medicine's lack of a cure leaves CAM as their only hope.[173] However, CAM therapies have very mixed and inconsistent effectiveness.[168,174,175] In fact, some herbal CAM treatments have been noted as potentially harmful, because their immune-stimulating properties may facilitate disease progression.[176] The techniques used in our comparison of CI therapy and CAM included a 10-day course of treatments not anticipated to be harmful: aquatic therapy,[177] massage,[178] gentle yoga,[179] and relaxation techniques (mindfulness training[180]).

CI Therapy Protocol

The CI therapy procedure given included the four major components of the treatment that are mentioned in more detail in a preceding section: (1) intensive training of the more-impaired arm in our laboratory for 3 h/day for 10 consecutive workdays; (2) training by the behavioral technique of shaping; (3) use of the transfer package (an extra 0.5 h per day) to transfer improvements made in the laboratory to real-world situations at home; and (4) a padded restraining mitt worn for a target of 90% of waking hours. The full CI therapy protocol can be found elsewhere.[127]

Clinical Outcome Measures and Results

In our laboratory, CI therapy treatment efficacy is measured using the Wolf Motor Function Test (WMFT),[153,181–183] and the Motor Activity Log (MAL).[153,184–186] The WMFT is a reliable and valid laboratory motor capacity test that measures speed of movement by the more-impaired arm on 15 standard movements that are made on the

therapist's request.[152,181,187] The MAL is a scripted, structured interview that measures spontaneous use of the more-affected arm in the life situation.[153] The patient rates each item in the interview on a scale of 0–5, with 0 being no use of the more-affected arm and 5 being normal use. The MAL is also reliable and valid[188,189] and correlates well with accelerometry readings that objectively measure arm movement in the life setting.[190]

The improvement in real-world use of the more-impaired arm (i.e., on the MAL) was about 5 times larger in the CI therapy group than the CAM group. In contrast, both groups improved similarly on their in-laboratory motor capacity (i.e., on the WMFT), which suggests that factors that promote improved motor capacity are not closely tied to those that promote recovery of real-world paretic limb use.[191]

MRI Imaging Analysis

VBM analysis found that the group receiving CI therapy showed profuse increases in gray matter in bilateral sensorimotor cortices and the hippocampi. Statistical, whole-brain analysis of the MRIs showed that only the CI therapy group showed significant changes in cortical structures.[192]

Given the finding that CI therapy for hemiparetic MS induces increases in gray matter volume bilaterally in the cortex, our laboratory sought to determine whether these changes corresponded with parallel changes in white matter. Additionally, DTI has been able to detect white matter abnormalities in individuals with MS not present in healthy controls,[193,194] and identify tract-specific changes due to disease progression[195] or physical therapy.[196] Significant clusters of increased FA were seen in the posterior corpus callosum contralateral to the more-affected arm and in the superior occipital gyrus ipsilateral to the trained upper extremity. The ipsilateral superior temporal gyrus had a significant cluster of increased AD, while MD and RD decreases were seen in the contralateral corticospinal tract. When the statistical threshold was lowered, additional clusters of FA increases were observed in the corticospinal tract contralateral to the trained upper extremity and the superior temporal gyrus white matter ipsilateral to the trained upper extremity. There were also AD increases in the corpus callosum. As in the previous study, we again observed significant pre- to post-treatment changes in the brain structure of the CI therapy group but not in that of the CAM group.[197]

Impact

In our study both the CI therapy group and the CAM group improved after the intervention, though the real-world motor improvement of the CAM group was much less than that of the CI therapy group. So far, CI therapy is the only physical training program that has shown marked improvement in cortical as well as subcortical structure of MS patients. It is important to note that neither VBM nor DTI can describe specific physical or microbiological changes that may be occurring in the CNS. Nevertheless, animal models of CI therapy in adult female Lewis rats have exhibited an increase in synapse formation.[198,199] Because of its progressive nature, MS forces a lifelong burden onto patients and their caregivers. These results suggest that CI therapy may be a significant, life-altering treatment affording patients neuroplastic benefits and real-world motor skill gains that serve to reduce some of the burdens of this progressively debilitating disease. Future work should determine whether these preliminary findings may be replicated in larger and more diverse populations of persons who experience the debilitating effects of MS.

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CHAPTER

16

Physical Activity Behavior in Multiple Sclerosis: Definition, Rates, Outcomes, and Safety

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OUTLINE

Multiple sclerosis (MS) is typically described as an immune-mediated disease of the central nervous system (CNS) that is triggered by an environmental stimulus in genetically susceptible persons. The pathogenesis of MS involves intermittent periods of inflammation (i.e., relapses) that result in demyelination and transection of axons (i.e., lesions) in the brain, brain stem, spinal cord, and optic nerves. The degree and location of damage within the CNS result in a heterogeneous expression of outcomes, including progression of disability, impairments in function (e.g., walking and cognition), worsening of symptoms (e.g., fatigue and depression), and compromised quality of life (QOL) and participation. The progression of MS, for example, is typically described based on the accumulation of neurological disability in various functional systems (e.g., pyramidal or cerebellar) over time based on expanded disability status scale (EDSS) scores.[1] There is strong evidence that MS results in walking impairments whereby over 85% of patients experience significant problems with ambulation,[2,3] and 40–50% of patients demonstrate cognitive impairments (e.g., slowed cognitive processing speed) based on neuropsychological assessment.[4] The symptoms of fatigue[5] and depression[6] are further common among the majority of persons with MS. Such manifestations of MS clearly undermine QOL and restrict participation in daily activities of living.

Importantly, MS influences lifestyle behaviors, particularly physical activity, which can be considered a behavior with both preventive and restorative properties in MS. Indeed, the rate of physical activity is exceptionally low in MS,[7] and its curtailment has been positively associated with disability[8] and other outcomes (e.g., volumes of gray matter structures in CNS)[9] in cross-sectional and longitudinal research. Physical activity further has been the focus of behavioral interventions wherein it has improved outcomes such as walking,[10] depression,[11] and fatigue[12] in randomized controlled trials (RCTs). To that end, physical activity has been recognized as one of the most important lifestyle behaviors for preventing and managing the consequences of living with MS.[13]

This chapter reviews research on physical active rates, outcomes, and safety in persons living with MS. We first define physical activity behavior, and then present data on the rates of physical activity in MS. The chapter next reviews cross-sectional and longitudinal research wherein physical activity is considered a putative "protective" lifestyle behavior, followed by RCTs wherein physical activity is considered a putative "restorative" lifestyle behavior. Finally, the chapter concludes with a discussion of the safety profile of physical activity in MS. The objective of this chapter is to provide a selective summary review of evidence regarding physical activity as a lifestyle behavior in MS.

DEFINITION OF PHYSICAL ACTIVITY

There is a history of confusion regarding the accurate definition and description of physical activity. For example, physical activity is often confused with the related concept physical fitness, whereas at other times physical activity has been confused with disability and impairment.[14] This necessitates a clear definition for guiding the current chapter. Physical activity has been defined by Bouchard and Shephard[15] as "any bodily movement produced by contraction of skeletal muscles that results in a substantial increase in energy expenditure over resting values." This means that physical activity must include the movement of relatively large muscle groups that expend a considerable amount of energy over and above resting levels (e.g., walking). Physical activity, further, is a behavior, and there are many categories of physical activity behaviors including leisure-time physical activity, exercise, sport, occupational work and chores, and transportation. Of note, leisure-time physical activity is a type of physical activity undertaken during an individual's discretionary time that results in substantial energy expenditure (i.e., a physically active lifestyle). Physical activity may be undertaken for a variety of reasons (e.g., fun and enjoyment or transportation), but one of the main objectives involves improving morbidity or health outcomes (e.g., depression).

Exercise is a subset of leisure-time physical activity with an explicit objective of improving one's level of physical fitness. Exercise is a behavior performed on a repeated basis over an extended time period and can be described based on its type (i.e., what kind), intensity (i.e., how hard), frequency (i.e., how often), and duration (i.e., how long); volume (i.e., amount) and progression (i.e., advancement) are two other descriptors of exercise.[16] The expected outcome of exercise is an improvement in one's physical fitness defined as a set of attributes or characteristics that describes one's physical work capacity (i.e., the ability to do or perform physical work). There are five categories of physical fitness including cardiorespiratory (i.e., aerobic capacity), morphological (i.e., body composition), muscular (i.e., strength), metabolic (i.e., substrate metabolism), and motor (i.e., agility). Importantly, physical fitness outcomes are often included in RCTs as a manipulation check on the exercise intervention itself (i.e., confirmation that participants did engage in an appropriate volume of physical activity).[15]

Collectively, physical activity and exercise are behaviors, whereas physical fitness is a characteristic that reflects adaptations associated with regular participation in such behaviors. Exercise is a specific type of leisure-time physical activity. This chapter focuses on physical activity, including exercise, and its association with major manifestations of MS, because this lifestyle behavior can be targeted by focal interventions for preventing, restoring, and managing consequences of MS.

RATES OF PHYSICAL ACTIVITY IN MS

There has been longstanding concern about the safety of physical activity in MS, and this, combined with the negative consequences of MS (e.g., walking impairment and fatigue), would seemingly portend that patients are not engaging in physical activity in a manner comparable with the general population. If so, then persons with MS have increased risk of worsening disease consequences and are not achieving the rehabilitation benefits associated with physical activity. This section provides a historical and current overview of research on physical activity rates in MS.

One previous narrative review documented that persons with MS engage in low amounts of physical activity and that this amount of physical activity is less than that of "nondiseased" or "healthy" participants.[17] The most common types or modes of physical activity among those with MS were walking and swimming, whereas the least common types of physical activity were jogging and aerobics. Persons with MS typically engaged in less than 1 h of endurance exercise or resistance training per week and between 1 and 2.9 h of walking per week, and the intensity of the physical activity commonly resulted in sweating a little and being breathless. These observations suggest that people with MS typically engage in low impact forms of aerobic activity, for relatively short periods of time per week, and at a relatively moderate intensity.

Following that review, a meta-analysis quantified the degree of difference in physical activity among persons with MS compared with nondiseased and diseased populations.[14] The researchers searched MEDLINE, PSYCHINFO, and CURRENT CONTENTS PLUS using

the key words "physical activity," "exercise," and "physical fitness" in conjunction with "multiple sclerosis." We further conducted a manual search of bibliographies of the retrieved papers, and contacted study authors about additional studies. This search process yielded 53 effects from 13 studies including 2360 MS participants and yielded a weighted mean effect size of $d = -0.60$. This effect size indicated that persons with MS were significantly and moderately less active than the overall comparison group of nondiseased and diseased populations. This effect size was moderated by several variables, and effects were largest when (1) comparing those with MS and individuals without MS or any other apparent disease ($d = -0.96$); (2) using objective measures of physical activity ($d = -1.27$); and (3) studying individuals with progressive courses of MS ($d = -0.87$). These findings are alarming given the well-documented prevalence of physical *inactivity* among the general population of adults, and provide evidence that persons with MS are less physically active than populations without MS or other diseases (i.e., general population).

During mid-2010s researchers investigated levels of moderate-to-vigorous physical activity (MVPA) in a large sample of persons with MS and controls using accelerometry as a measure of physical activity, and further compared the rates of meeting public health guidelines for MVPA (i.e., 30 min/day) between persons with MS and controls.[7] The study involved a secondary analysis of a combined data set of persons with MS and healthy controls from 13 previous investigations of physical activity. Participants with MS ($N = 800$) were recruited primarily within Illinois through multiple sources, including print and e-mail flyers and an online advertisement on the National Multiple Sclerosis Society website. Healthy controls ($N = 137$) were recruited via public e-mail postings delivered within a university community. After controlling for covariates (i.e., age, sex, education, race, and income), there was a moderate ($d = -0.68$) and statistically significant ($p < .001$) difference of 13.1 min of MVPA per day between MS and controls. There further was a statistically significant difference in the rates of meeting public health guidelines for MVPA ($p < .001$) between MS (20%) and controls (47%). This study indicates that a relatively small proportion of persons with MS are achieving adequate amounts of MVPA as part of daily life, and this is important as the benefits of physical activity may be dependent on meeting these guidelines.

Another study in 2015 examined the rates of insufficient, moderate, and sufficient physical activity in persons with MS compared with healthy controls.[18] That study involved another secondary analysis of data from participants with MS ($N = 1521$) and healthy controls ($N = 162$) who completed the Godin Leisure-Time Exercise Questionnaire (GLTEQ) as part of a questionnaire packet administered in 14 previous investigations. There were statistically significant differences in overall GLTEQ scores ($p < .001$, $d = -0.83$) and rates of physical activity ($p < .001$) between MS and control groups. The rates of insufficient (i.e., level of physical activity with minimal health benefits), moderate (i.e., level of physical activity with some health benefits), and sufficient physical activity (i.e., level of physical activity with substantial health benefits) in the MS group were 58.0, 15.2, and 26.8%, respectively. Those with MS were 2.5 times more likely to report insufficient physical activity and 2.3 times less likely to report sufficient physical activity than controls. These data further supported that the majority of persons with MS are insufficiently physically active.

Collectively, the very low rates of physical activity would portend considerable risk for worsening of many outcomes in persons living with MS. This is further exacerbated by the decline in general physical activity levels over time in MS.[19] The low rate of physical activity would support the importance of designing RCTs that target this lifestyle behavior for preventing, restoring, and managing consequences of MS.

PHYSICAL ACTIVITY AS A PROTECTIVE LIFESTYLE BEHAVIOR

There has been an abundance of research examining physical activity as a correlate of MS outcomes using cross-sectional and prospective research designs. These designs cannot provide evidence for causality, but provide an exciting vision into the potential of physical activity for reducing the likelihood of MS-disease outcomes over time. Accordingly, this section contains a selective review of research from both types of research designs for building a picture of the potential of physical activity as a protective lifestyle behavior in MS.

Cross-sectional research. Researchers have examined the association between physical activity and many outcomes in persons with MS, including volumetrics of brain regions of interest based on magnetic resonance imaging (MRI),[9] integrity of the anterior visual pathway based on optical coherence tomography (OCT),[20] disability status,[21] walking and cognitive functions,[22,23] symptoms of fatigue and depression,[24,25] QOL,[26] and participation outcomes.[27] For example, one study of 39 persons with MS examined the association between free-living, MVPA levels (i.e., min/day) and the volumes of whole-brain gray (GM) and white matter (WM) and subcortical structures of the hippocampus, thalamus, and basal ganglia based on MRI.[9] The analysis indicated that MVPA was significantly associated with GM ($pr = 0.370$, $p < .05$), WM ($pr = 0.433$, $p < .01$), hippocampus ($pr = 0.499$, $p < .01$), thalamus ($pr = 0.380$, $p < .05$), caudate ($pr = 0.539$, $p < .01$), putamen ($pr = 0.369$, $p < .05$), and pallidum ($pr = 0.498$, $p < .01$) volumes, when controlling for sex, age, clinical

course of MS, and EDSS score. These results provide the first evidence that MVPA is associated with volumes of whole-brain GM and WM and subcortical GM structures that are involved in motor and cognitive functions in MS. On a related note, another study examined the associations among objectively measured physical activity (i.e., steps/day) with the OCT metrics of retinal nerve fiber layer (RNFL) thickness and total macular volume (TMV) in persons with MS.[20] Steps/day was significantly associated with both RNFL thickness and TMV. This indicates that physical activity is associated with integrity of the anterior visual pathway, assessed by OCT, in persons with MS, and such an observation further reflects the potential of physical activity for neuroprotection in MS.

Researchers have further examined physical activity and cognitive outcomes in MS. Such research provides a reflection of physical activity and CNS functions in MS. The first study we are aware of in this area examined the associations among physical activity (steps/day), cognitive processing speed (CPS), and learning and memory in 33 persons with MS who underwent neuropsychological assessments and wore a physical activity monitor for 7 days.[23] Physical activity was significantly correlated with cognitive processing speed ($pr = 0.35$), but not learning and memory ($pr = 0.20$), after controlling for sex, age, and education. Another study examined the association between objectively measured physical activity and CPS in a sample of 212 persons with MS.[28] Participants underwent two valid neuropsychological tests of CPS, completed the Timed 25-Foot Walk (T25FW), and wore an ActiGraph model GT3X accelerometer during the waking hours of a 7-day period for objectively measuring physical activity (i.e., steps/day). Physical activity was significantly associated with CPS ($r = 0.39$, $p < .01$), even when controlling for age, sex, and education ($pr = 0.26$, $p < .01$). This association was attenuated, but still significant after further controlling for T25FW performance ($pr = 0.13$, $p = .03$). This line of research suggests that physical activity behavior is positively and independently associated with CPS, and perhaps other cognitive domains, in persons with MS, and may play an important role in reducing the risk of cognitive dysfunction as it does in other outcomes in MS.

There has been some interest in physical activity and comorbid conditions in MS, particularly cardiovascular comorbidity, based on its prevalence and influence on disease progression and other MS outcomes. To that end, one study examined the possibility of a linear, inverse association between physical activity and the number of self-reported cardiovascular comorbidities in a sample of 561 persons with MS.[29] The bivariate correlation analysis indicated that there were statistically significant, inverse associations between the number of self-reported cardiovascular comorbidities and objectively measured ($r = -0.192$, $p = .0001$) and self-reported ($r = -0.151$, $p = .0001$) physical activity. Those associations remained significant in additional analyses controlling for confounding variables (i.e., age and gender). Another study compared subclinical atherosclerosis and arterial function between individuals with and without MS matched for age, sex, and body mass index, and examined the association with physical activity in MS.[30] There was a significant difference ($p < .05$) in resting forearm blood flow (FBF), peak reactive hyperemia, central pulse wave velocity, and arterial compliance (AC) between the MS and control groups. Physical activity was associated with peak FBF and central pulse wave velocity, but not resting FBF and carotid AC. Physical activity further differed between groups and accounted for group differences in arterial function. These data suggest that physical activity might be associated with the many consequences of MS through an influence on comorbid conditions such as cardiovascular disease.

Collectively, this body of research establishes that physical activity is associated with outcomes ranging from CNS-related changes detected via MRI through symptoms, comorbidity, and QOL outcomes, among others, in persons living with MS. These data and the associated research designs cannot support inferences of causality, but provide an initial and exciting basis for considering the putative protective role of this lifestyle behavior in MS.

Prospective research. There are fewer studies of physical activity and its association with MS outcomes over time, and the existing studies have focused on disability status,[8,31] walking function,[32] symptoms of fatigue and depression,[33] and QOL.[34–36] For example, one study examined premorbid physical activity as a predictor of change in disability over a 24-month period in 269 persons with relapsing–remitting MS (RRMS).[8] The analyses indicated that there was a significant, linear increase in disability scores over time ($p = .0015$), and premorbid physical activity significantly predicted the linear change in disability scores (standardized $\beta = -0.23$, $p < .005$). By comparison, current physical activity (standardized $\beta = -0.02$, $p = .81$), gender (standardized $\beta = -0.06$, $p = .54$), age (standardized $\beta = 0.05$, $p = .56$), duration of MS (standardized $\beta = 0.11$, $p = .15$), and treatment with disease-modifying therapies (standardized $\beta = -0.03$, $p = .77$) did not predict change in disability scores. This research highlights the possible role of physical activity behavior for lessening disability progression over time in persons with RRMS.

On a related note, another study examined change in physical activity as a behavioral correlate of short-term disability progression in persons with MS over a 6-month period.[31] Panel analysis indicated associations between baseline physical activity and disability (standardized $\beta = -0.41$, $p < .001$) and 6-month change in physical activity and disability progression (standardized

$\beta = -0.09$, $p = .025$). The associations were independent of sex, age of MS onset, clinical MS course, and occurrence of a relapse. Such findings provide preliminary support for a reduction in physical activity as a behavioral correlate, but not necessarily cause, of short-term disability progression in persons with MS.

Another study examined the hypothesis that change in lifestyle physical activity would be inversely associated with change in walking impairment over a 6-month period in persons with RRMS.[32] The panel model fit the data and, as expected, identified the direct effects between baseline physical activity and walking impairment (standardized $\beta = -0.31$) and follow-up physical activity and walking impairment (standardized $\beta = -0.16$). The second path coefficient, of note, indicated that a 1 standard deviation unit change in physical activity was associated with a 0.16 standard deviation unit residual change in walking impairment. The finding supports the possible importance of increasing free-living physical activity as a behavioral approach for forestalling walking impairments in adults with RRMS.

Researchers in 2014 conducted a prospective panel study and examined the relationship between changes in physical activity and health-related quality of life (HRQOL), based on the SF-36, across a 6-month period in 292 persons with MS.[36] The panel model represented an acceptable fit for the data. The standardized path coefficients were statistically significant between follow-up physical activity and follow-up physical function ($\beta = 0.12$, $p < .005$), role-emotional ($\beta = 0.16$, $p < .01$), vitality ($\beta = 0.13$, $p < .001$), and social function ($\beta = 0.12$, $p < .05$). Those who reported a change (increase or decrease) in levels of physical activity over 6 months reported a change (improving or worsening, respectively) in HRQOL on four of eight domains on the SF-36, independent of disability status, MS clinical course and duration, age, and sex. The observed pattern of relationships supports the possibility that changing physical activity through an intervention might yield desirable changes in HRQOL.

One final prospective study examined symptoms of depression, fatigue, pain, self-efficacy, and social support as possible intermediaries in the pathway between changes in physical activity and QOL across a 6-month period in persons with MS.[34] The initial analysis indicated that change in physical activity was associated with a statistically significant and small residual change in QOL ($\beta = 0.07$). The subsequent analysis indicated that change in physical activity was associated with residual changes in fatigue ($\beta = -0.17$), pain ($\beta = -0.13$), social support ($\beta = 0.07$), and self-efficacy ($\beta = 0.11$). The residual changes in fatigue ($\beta = -0.13$), pain ($\beta = -0.09$), social support ($\beta = 0.18$), and self-efficacy ($\beta = 0.10$), in turn, were associated with a residual change in QOL. The observed pattern of relationships supports the possibility that physical activity is indirectly associated with improved QOL through pathways that include fatigue, pain, social support, and self-efficacy in individuals with MS.

Collectively, this body of research provides evidence regarding physical activity and its association with changes in outcomes over time, and provides a prospective, longitudinal basis for physical activity possibly reducing the likelihood of deleterious outcomes over time. This is of note for disability status, walking function, symptoms of fatigue and depression, and QOL; additional research is necessary for confirming the cross-sectional associations between physical activity and outcomes associated with CNS structure and function (i.e., cognition) in longitudinal data. This is necessary for even stronger conclusions about physical activity and putative protective effects in MS.

PHYSICAL ACTIVITY AS A RESTORATIVE LIFESTYLE BEHAVIOR

The literature on physical activity as a restorative behavior is largely based on RCTs of exercise, as a type of physical activity, and its influence on outcomes in persons with MS. This section focuses on meta-analyses, where possible, as these provide the most complete picture in a quantitative manner regarding the outcomes of physical activity in MS. The section further includes literature reviews when applicable.

Physical fitness. There has been extensive interest in the effects of exercise on cardiorespiratory capacity (i.e., aerobic capacity often measured a peak oxygen consumption) and muscular strength (e.g., often measured as an estimate of 1 repetition maximum) in persons with MS. One systematic review in 2013 provided summary conclusions regarding exercise and its influence on measures of cardiorespiratory capacity and muscular strength in persons with MS.[37] The review included 54 studies that were identified through a systematic search of 2498 articles. Of note, 9 of the 54 studies included in that review reported an improvement in aerobic capacity, and 5 of those 9 studies provided level 1 evidence (i.e., high-quality RCTs) of aerobic exercise improving aerobic capacity in MS. The review further reported that 5 studies describing 4 RCTs provided level 1 evidence for supervised, progressive resistance training improving muscle strength in MS. Collectively, there is evidence for beneficial effects of aerobic and resistance exercise on cardiorespiratory and muscular components of health-related fitness, respectively, in persons with MS.

One meta-analysis provided a quantitative synthesis of RCTs examining the effect of exercise training on muscular and cardiorespiratory fitness in persons with MS. The researchers searched PubMed, Google Scholar, and Web of Science for all relevant articles published up

to October 2014,[38] and included only RCTs that examined the effect of exercise training on muscular and/or cardiorespiratory fitness parameters. The initial search yielded 1501 articles, and 62 were reviewed in detail with 20 RCTs meeting the inclusion criteria and providing enough data for effect sizes (ESs) (Cohen's *d*). The mean ES was 0.27 standardized units for muscular fitness outcomes and 0.47 standardized units for cardiorespiratory fitness outcomes. The mean ES was not heterogeneous for muscular or cardiorespiratory fitness outcomes (i.e., no effect moderators). The cumulative evidence indicated that exercise training was associated with changes in measures of muscular (small in magnitude) and cardiorespiratory (moderate in magnitude) fitness among persons with MS.

Walking mobility. The loss of walking mobility is a hallmark feature of disease progression in MS,[3] and represents one of the most burdensome features of the disease.[2] To that end, one meta-analysis examined the overall effect of exercise on walking mobility among individuals with MS.[10] The researchers searched electronic databases (e.g., MEDLINE and PSYCHINFO) for published exercise training studies over the period of 1960 through November 2007. Studies were selected that measured walking mobility before and after an intervention that included exercise training in persons with MS; this meta-analysis included pre–post experimental designs and RCTs. Forty-two published articles were reviewed, and 22 provided enough data to compute ESs. Sixty-six ESs were retrieved from the 22 publications with 600 MS participants and the weighted mean effect size was 0.19 standardized units. The effect of exercise was strongest when the research was conducted in a supervised exercise facility, with a mean effect size of 0.32 standardized units. Overall, the cumulative evidence indicated that exercise was associated with a small improvement in walking mobility outcomes among those with MS, and this improvement can be optimized with exercise performed in a facility under supervision.

Fatigue. Fatigue is a common and burdensome symptom of MS and has major implications for worsening of neurological disability and other symptoms such as depression, pain, anxiety, and cognitive impairment.[5] Fatigue further can contribute toward cessation of employment over time in MS.[5] There is evidence that exercise can increase energy and reduce fatigue levels in the general population.[39] There is some concern, however, that exercise might actually worsen fatigue in MS by taxing one's energy levels and reserves. To that end, researchers have undertaken meta-analyses[12,40] examining the effect of exercise on symptomatic fatigue in persons with MS.

One meta-analysis[12] searched electronic databases (e.g., Web of Science, PubMed, PsycInfo, and Google Scholar) for articles published between 1960 and October 2012 using key words such as "fatigue," OR "tiredness," OR "energy" AND "exercise," OR "physical activity," WITH "multiple sclerosis." The researchers further performed manual searches of references from retrieved articles and other literature reviews. The search resulted in 311 articles, and 17 articles met the inclusion criteria and provided enough data to compute ESs. The weighted mean ES from the 17 RCTs containing 568 persons with MS was 0.45 standardized units. There was minimal evidence for heterogeneity of the average effect size. Overall, this meta-analysis indicated that exercise resulted in a moderate reduction in fatigue compared with control conditions. Such results would suggest that exercise is effective for improving, rather that worsening, fatigue in MS.

Another meta-analysis compared multiple types of fatigue management interventions, including exercise, education, and medication, in MS.[40] The researchers searched PubMed, Embase, and CINAHL through August 2013 using terms such as MS, fatigue, and energy conservation. The search identified 230 citations, and yielded 18 rehabilitation and 7 pharmacological trials for inclusion in the meta-analysis. The meta-analysis indicated that rehabilitation interventions (i.e., exercise and education) were more effective than pharmacological interventions. Indeed, exercise (10 studies of 233 people with MS) yielded a mean ES of 0.57 standardized units and education yielded a mean ES of 0.54 standardized units (8 studies with 662 MS cases), whereas pharmacological interventions had a mean effect size of 0.07 standardized units (7 studies of 604 people). This meta-analysis conforms the previous observation that exercise is effective for reducing fatigue in MS.

Depressive symptoms. Depression and depressive symptoms are quite common and burdensome among those living with MS and have major implications for cognitive impairment, QOL, and compliance with disease-modifying agents in MS. The American Academy of Neurology further reported insufficient evidence regarding the efficacy of antidepressant medications or therapies for managing depression in MS.[41] By comparison, there is a substantial body of research supporting the antidepressant effects of exercise in the general population of adults,[42] but reviews of similar evidence in MS have been equivocal.[43]

To that end, researchers performed a meta-analysis quantifying the overall effect of exercise on depressive symptoms in MS.[11] The meta-analysis included an initial search using PubMed and a follow-up search using EBSCO Host, Web of Science, and Scopus for RCTs of exercise and MS over the period of 1960 through November 2013. The researchers further undertook manual searches of references from retrieved articles and other literature reviews. That search process yielded 25 articles on depression and exercise in MS, and there

were 13 RCTs that met inclusion criteria and yielded data for ES generation. The weighted mean ES was 0.36 standardized units, and there was minimal evidence for heterogeneity. One follow-up meta-analysis of adults with neurological disorders including MS indicated that the effect of exercise training on depressive symptoms is larger when interventions met physical activity guidelines for minutes per week of MVPA.[44] Overall, the meta-analyses indicated that exercise resulted in a small improvement in depressive symptoms compared with control conditions, and this could be improved by meeting physical activity guidelines. Such results would suggest that an appropriate exercise regimen can be beneficial for managing symptoms of depression in MS.

Quality of life. QOL is often compromised in persons with MS. Indeed, persons with MS have lower QOL than nondiseased populations and those suffering from other serious diseases including inflammatory bowel disease, ischemic stroke, and rheumatoid arthritis.[45,46] Such a profound effect of MS on QOL might be associated with the uncertain and unpredictable nature of a currently incurable disease with onset during the most productive years of one's life.[45,46] The effect of MS on QOL might further be associated with the worsening of symptoms and functional outcomes in MS.[45,46] This underscores the importance of identifying methods of managing this downstream and often inevitable consequence of MS.

One study quantified the overall effect of exercise on QOL among those with MS.[47] The researchers searched MEDLINE, PSYCHINFO, and CURRENT CONTENTS PLUS for the period of 1960 through November 2006 using the key words exercise, physical activity, and physical fitness in conjunction with QOL and MS. The researchers further conducted a manual search of cited references and contacted study authors about other publications. Twenty-five journal articles were located and reviewed, and 13 of the 25 provided enough data to compute ESs. One hundred and nine ESs were retrieved from the 13 studies with 484 MS participants and yielded a weighted mean effect size of 0.23 standardized units. The mean effect size was heterogeneous, and the effect was largest with MS-specific QOL measures and aerobic exercise training. Collectively, the cumulative evidence supported that exercise, particular of an aerobic or cardiorespiratory nature, was associated with a small improvement in QOL.

Summary. Overall, there is evidence that physical activity, particularly exercise training, can improve outcomes ranging from physical fitness through QOL. This indicates the restorative potential of exercise, and perhaps physical activity more broadly, in MS. We do note one RCT of a lifestyle physical activity intervention reported improvements in symptoms such as fatigue, anxiety, and depression, as well as QOL,[48] walking and cognition,[49] and body composition,[50] as well as reducing sedentary behavior[51] in MS. This is important as it suggests that exercise training and physical activity have restorative potential in MS.

SAFETY OF PHYSICAL ACTIVITY IN MS

There has been a history of concern regarding the safety of physical activity in persons with MS. Historically, those with MS were frequently advised against participation in physical activity based on the possibility of disease exacerbation and worsening or progression.[52] This is somewhat surprising considering the even longer history of recommendations for physical activity participation (e.g., walking for health benefits) in persons with MS.[53] The controversy over safety still persists as few researchers have specifically focused on documenting the safety of physical activity in MS; the focus commonly has been on health outcomes. Indeed, little has been done to summarize and describe the risks (e.g., relapses and other adverse events; AEs) associated with physical activity. This is critical for informing decisions and recommendations regarding the safety of this behavior in MS. To that end, one article provided a systematic review of relapse rates and other AEs reported in RCTs of exercise in MS.[54] The researchers searched electronic databases for RCTs of exercise training in MS. The researchers calculated the rates of relapse and other AEs in exercise and no-treatment control conditions, and estimated the relative risk of relapses and AEs for exercise versus control conditions expressed as the ratio of the two rates. There were 26 studies reviewed for the reporting of relapses and other AEs. Those studies collectively included 1295 participants with MS. The rates of relapse across the studies were 4.6% and 6.3% for exercise and control conditions, respectively. The rates of other AEs across the studies were 2.0% and 1.2% for exercise and control conditions, respectively. Overall, there were only 13 AEs reported among all of the exercise conditions. The common AEs reported with exercise were back and joint pain (i.e., musculoskeletal injuries) and acute illness, and the musculoskeletal injuries often occurred with resistance training. The ratio of rates yielded a relative risk of relapses for exercise training compared with control of 0.73, whereas the relative risk of AE for exercise training compared with control was 1.67. This indicates that exercise was associated with a nearly 25% decrease in the risk of relapse, and the risk of AEs with exercise was similar with that reported in healthy populations. This evidence should reduce concerns regarding the safety of physical activity in MS. This evidence further supports recent statements that physical activity is a safe intervention without reported side effects and serious adverse events and does not increase relapse rate.[55]

CONCLUSION

This chapter documents (1) low rates of physical activity participation in MS; (2) cross-sectional and longitudinal associations between physical activity and outcomes of neuroprotection through QOL in MS; and (3) RCTs of exercise training effects on fitness through QOL in MS. Such a review supports the idea of physical activity as a putative "protective" lifestyle behavior as well as a putative "restorative" lifestyle behavior. Importantly, physical activity is safe for people with MS, and persons living with MS should be encouraged toward lifelong participation in physical activity behavior if there are no other medical contraindications. Such a behavior incorporated into one's lifestyle portends considerable benefits for persons living with an unpredictable, incurable progressive neurological disease of an autoimmune origin.

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CHAPTER

17

Looking Beyond Neurological Impairment in Patients With Multiple Sclerosis During Exercise Intervention: Evidence for Muscular, Cardiac, Pulmonary, and Metabolic Dysfunction Related to Exercise Intolerance and Prognosis

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OUTLINE

INTRODUCTION

Multiple sclerosis (MS) is a neurodegenerative disease of the central nervous system that predominantly affects young to middle-aged adults. This disease, which results in heterogeneous and complex symptoms, including spasticity, weakness, visual disturbances, walking and coordination impairments, tremor, ataxia, sensory problems, and bladder disturbances, is characterized by myelin, oligodendrocyte, and axonal loss in the brain, brain stem and spinal cord, and white matter lesions.[1,2] In addition, depression, fatigue, and cognitive dysfunction are also common in patients with MS (pwMS), even early in the disease course.[3–5] Due to the debilitating character of these symptoms, physical activity is often significantly reduced,[6,7] which may further exaggerate muscle weakness, fatigue, reduced functional capacity, and associated health risks.[8–10] Such a vicious cycle in pwMS leads to further impairment in functional capacity and/or worse prognosis.[11,12]

Multidisciplinary rehabilitation intervention (including physiotherapy, occupational therapy, psychological and coping programs, cognitive rehabilitation, speech therapy, and therapy to improve fatigue) and exercise therapy is a cornerstone in the treatment of MS: pwMS significantly benefit from such intervention.[13–25] The primary goal of clinicians in such rehabilitation programs is to minimize limitations in activity and participation by improving or compensating for the neurological deficits mentioned earlier, in order to reach the highest possible

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level of independence and maintain/improve the quality of life.[13,14]

Since mid-2000s, however, evidence has been accumulating that pwMS also experience significant muscular, cardiac, pulmonary, and metabolic dysfunction. Next to the neurological deficits mentioned earlier, such anomalies may independently contribute to elevated morbidity and mortality, and increased risk for hospitalization.[11] In addition, these anomalies may lead to exercise intolerance in pwMS. In other populations with such dysfunctions (e.g., patients with heart and lung, or metabolic disease), exercise intervention is frequently a cornerstone intervention. Interestingly, pwMS with these dysfunctions are often excluded from exercise intervention studies, which may possibly explain why current exercise recommendations do not (or very limited) take this into account.[26–28] In fact, many clinicians and rehabilitation experts dealing with pwMS are often unaware of the potential presence and clinical repercussions of such anomalies.

Therefore, in this chapter (1) muscular, cardiac, pulmonary, and metabolic function in pwMS is described in greater detail and (2) the influence of exercise training on these functions are covered. It will be shown that MS is associated with a significantly increased incidence and prevalence of muscular, cardiac, pulmonary, and metabolic dysfunction, which may independently lead to exercise intolerance and worse prognosis. In addition, it will be shown that the impact of exercise training on these dysfunctions in pwMS remains to be studied in greater detail, and/or that these anomalies are not easily remediated by exercise training. The latter finding suggests that optimization of exercise prescription is urgently warranted in pwMS.

MUSCLE DYSFUNCTION IN MS

Muscle wasting and a decrease in muscle strength is often reported in pwMS, and is well known by most clinicians or rehabilitation experts. Such muscle weakness persists even after adjusted for fat-free mass in some studies, whereas other studies report no differences between pwMS and referent subjects after such adjustment.[29–45] It thus remains a topic of debate whether muscle weakness in pwMS originates from decrements in muscle quality or quantity, or both. In extent, strength deficits may also be of muscular (altered skeletal muscle fiber characteristics) as well as neural (central activation) origin.[29,32–35] Next to whole-body muscle wasting, pwMS experience significant differences in muscle strength between legs.[46,47] This asymmetry varies from 2% to 30%[46] and results in a greater muscle volume on the less affected side, suggesting a compensatory mechanism to maintain balance and posture.[44–50] pwMS are able to perform significantly more work with the stronger leg than the weaker leg during submaximal single-leg fixed-load cycling.[51] Endurance knee extensor strength and isometric knee flexor strength are reported to be main predictors for walking capacity in pwMS,[52] although this view was challenged in 2014 by the finding of a greater impact of muscle oxidative capacity on walking capacity.[53] To further understand the origin of muscle weakness in pwMS, a close evaluation of muscle morphology and biochemistry is mandatory. In this regard, it is reported that a smaller vastus lateralis type I and II skeletal muscle fiber cross-sectional area (CSA) and a selective type II(a) atrophy are present in pwMS.[31,35,54] Muscle fiber CSA is highly correlated with quadriceps muscle strength in pwMS. It thus follows that reduced muscle fiber CSA relates to muscle weakness in pwMS and that changes in skeletal muscle characteristics in pwMS may affect physical functioning.[37] Next to these morphological muscle anomalies, many biochemical abnormalities are often present in pwMS. For example, lower succinate dehydrogenase activity, delayed phosphocreatine resynthesis after isometric exercise (indicating impaired skeletal muscle oxidative capacity), increased basal muscle adenosine monophosphate-activated protein kinase alpha (AMPKα) and mammalian target for rapamycin (mTOR) phosphorylation (which was independently related to MS), blunted intramuscular metabolic responses during isometric or endurance exercise, complex-I deficiency in skeletal muscle mitochondria, and slowed exercise-onset oxygen uptake (VO_2) kinetics (meaning that skeletal muscle oxidative capacity is lowered) are present in pwMS.[31,35,55–61] In addition, resting muscle oxygen consumption in gastrocnemius muscle is higher in pwMS, compared with healthy controls, and is even higher in patients with lower walking ability, compared to pwMS with better performance, suggesting that peripheral muscular adaptations occurred to maintain mobility.[62] These data convincingly indicate disturbed skeletal muscle cell biochemistry and adaptation to acute exercise in pwMS. Although physical inactivity and sedentarism[63] could contribute to such muscle weakness and dysfunction, it is an attractive hypothesis to assume that biochemical and morphological skeletal muscle abnormalities are also related to anomalous molecular signaling pathways. It was shown in 2015 that even when physical activity is acutely restored (by an acute endurance exercise bout), muscle 5′ AMPKα and mTOR signaling (which is important for muscular mitochondrial and myofibrillar biogenesis, respectively) remained significantly disturbed.[59] The latter findings may indicate that significant anomalies in muscle biochemistry are present in pwMS, and that such dysfunction may not be related to lowered physical activity only, but intrinsically to the pathophysiology of MS.

Fortunately, significant improvements in muscle characteristics and muscle function are elicited by participation in exercise interventions in pwMS. In particular, improvements in muscle strength, endurance, and mass, and neuromuscular function or neural drive are experienced after (progressive) resistance[64–75] or combined (resistance and endurance) exercise training,[76,77] together with reductions in motor fatigue.[78] Importantly, improvements in muscle function are even greater after high-intensity exercise training, as opposed to low-to-moderate intense exercise training.[76] These findings suggest that these changes in muscle function are exercise intensity related, and challenge the widely upheld belief that pwMS should not engage into high-intensity exercise training.[76,77,79] At the cellular level, resistance training[54] and high-intensity (interval) training[76] are known to increase muscle fiber CSA and lean tissue mass.

The findings and insights mentioned in the preceding paragraphs should stimulate clinicians and rehabilitation experts to optimize clinical practice. To develop individually optimized rehabilitation programs, pwMS should be systematically screened for muscle function, and training modalities should be adjusted accordingly. For example, since abnormalities in muscle fiber size as well as in muscle oxidative capacity are present in pwMS, it is suggested to offer endurance as well as resistance training to counteract both anomalies. Moreover, it may be speculated to offer nutritional support in adjunct to resistance exercise training: it should be examined whether the supplementation of amino acids during resistance training would lead to greater clinical benefits (in terms of muscle mass and function) in pwMS. To improve skeletal muscle oxidative capacity, it is an appealing hypothesis to offer high-intensity exercise training sessions in pwMS. Indeed, when high-intensity interval training programs are followed by pwMS, markers for muscle oxidative capacity will increase with significantly greater magnitude, as opposed to the commonly applied low-to-moderate intense endurance training programs.[76] Furthermore, the ingestion of beta alanine may also assist in the capability to exercise at greater intensities, and hereby contribute to greater improvements in muscle oxidative capacity.[80]

In conclusion, muscle weakness and wasting, disturbed skeletal muscle biochemistry, and composition are present in pwMS. Some of these anomalies are (partly) remediated by exercise intervention, although more data are needed to fully understand whether the applied exercise interventions fully remediate all known muscular abnormalities. Opportunities to further increase the effectiveness of exercise intervention on muscle mass and function are present, by adaptations in exercise prescription and/or administration of food supplements.

PULMONARY DYSFUNCTION IN MS

Notwithstanding the greater likelihood for the development of severe lung complications in pwMS,[81] lung function anomalies are often overlooked or not closely evaluated in clinical practice. However, reduced pulmonary/respiratory inspiratory and expiratory muscle strength and/or diffusion capacity, collectively leading to an impaired pulmonary function, are often present in pwMS, even at the early onset of the disorder.[82–87] pwMS with a higher level of disability are even more respiratory compromised by further lowering in pulmonary function and respiratory muscle strength.[84] Such impairment in pulmonary function is known to increase the likelihood for ineffective cough, retention of secretions and inability to maintain clear airways, development of atelectasis, and pneumonia.[88] Pulmonary dysfunction at rest may independently lead to exercise intolerance in pwMS: in pwMS significant relations are present between resting pulmonary function and exercise tolerance.[89,90] It is thus important to systematically examine the pulmonary system and adapt medical treatment accordingly in pwMS. This will very likely lead to improved medical treatment.

The examination of the pulmonary function during exercise is challenging in pwMS, but worth the effort. Elevated carbon dioxide (VE/VCO_2) equivalents during submaximal exercise (meaning that the efficiency for pulmonary CO_2 elimination is impaired), elevated oxygen uptake (VE/VO_2) equivalents during submaximal exercise (meaning that the efficiency for pulmonary O_2 uptake is impaired), and elevated dead space ventilation (Vd/Vt ratios) during peak exercise (meaning that relatively lowered alveolar ventilation occurs) are often observed in pwMS.[90–93] However, in these studies elicited exercise intensities or subject characteristics were significantly different between pwMS and healthy controls, or few pulmonary parameters were assessed. In the examination of pulmonary function during exercise, however, proper matching of these factors is of key importance and a whole range of pulmonary parameters have to be assessed to be able to unravel the pathophysiology leading to pulmonary dysfunction during exercise. When matching endurance exercise intensity (at 63% of predicted maximal heart rate (HR) or 3.1 mmol/l blood lactate level) and subject characteristics, elevated dead space/tidal volume (Vd/Vt) ratios, equivalents for oxygen uptake (VE/VO_2), carbon dioxide output (VE/VCO_2), and end-tidal oxygen pressures ($PETO_2$), and lowered end-tidal pressures for carbon dioxide ($PETCO_2$) are noticed in pwMS.[94] Elevated exercise $PETO_2$ and lowered exercise $PETCO_2$ in pwMS suggests elevated partial arterial O_2 pressures and lowered partial arterial CO_2 pressures, respectively. A reduced pulmonary gas exchange efficiency in pwMS during exercise

(elevated VE/VO_2 and VE/VCO_2) can point toward a ventilation–perfusion mismatch.[94] An abnormal diffusion capacity can be thought to contribute to such ventilation–perfusion mismatch. In line with this reasoning, a significantly lower diffusion capacity has been observed in pwMS.[82,83] A compromised gas exchange during exercise consequently leads to elevations in VE/VCO_2 and VE/VO_2, and altered $PETO_2$ and $PETCO_2$.[94] Correlations between elicited exercise intensity (exercise blood lactate content), VE/VO_2 ($r = 0.42$), and $PETO_2$ ($r = 0.37$) ($p < .05$) are present in pwMS.[94] It thus seems that an impaired O_2 uptake efficiency in pwMS is related to early anaerobic metabolism during exercise. However, cardiovascular dysfunction (explained later in this chapter) may also impair ventilation–perfusion match in pwMS. Finally, a ventilation–perfusion mismatch during exercise in pwMS is also hypothesized to be due to diaphragmatic dysfunction or disturbed respiratory coordination.[95–97] Leaving the exact etiology of ventilation–perfusion mismatch during exercise in pwMS aside, such mismatch could trigger the pulmonary drive, but also cause desaturation (i.e., significant reduction in SaO_2%, due to hypoxemia).[90] This may relate to increased ratings of perceived exertion during exercise in pwMS.[94] It is thus important to realize that pulmonary dysfunction during exercise not only relates exercise intolerance, but also to increased sensations of fatigue during exercise in pwMS.

Some studies showed that expiratory muscle strength training leads to enhancements in respiratory muscle strength, as evidenced by increased maximal expiratory pressures,[85–87,97] whereas one study reported improvements in forced vital capacity after 4 weeks of endurance exercise intervention.[98] Unfortunately, a ventilation–perfusion mismatch or pulmonary dysfunction during endurance exercise in pwMS is not remediated by a 6-month training intervention (combination of strength and endurance training exercises),[94] despite significant improvements in exercise tolerance. Due to the low number of studies examining the impact of exercise intervention on pulmonary function in pwMS, greater effort to examine this clinically relevant topic is warranted.

Based on the evidence mentioned in the preceding paragraphs, it is thus important to systematically screen pulmonary function in pwMS, especially during exercise, and adapt medical treatment accordingly. In extent, exercise prescription may require significant adaptations to counteract this pulmonary dysfunction in pwMS. For example, inspiratory and expiratory muscle training (inspiratory muscle training against low-to-moderate inspiratory resistance, or expiratory muscle training against low-to-high expiratory resistance, or breathing exercises combined with certain upper body movements) significantly improves pulmonary function at rest in pwMS.[87,99,100] However, whether such specific exercise affects the pulmonary function during exercise as well in pwMS has not been studied. In fact, as long as the etiology of a ventilation–perfusion mismatch during exercise in pwMS remains elusive, it is difficult to propose conclusively effective treatments. It thus follows that the etiology for ventilation–perfusion mismatch during exercise in pwMS should be examined in greater detail so novel therapies can be implemented.

In conclusion, impaired pulmonary function and significant pulmonary dysfunction during exercise are present in pwMS. This dysfunction relates to exercise intolerance and worse sensations of exercise. The pulmonary dysfunction is not easily remediated by exercise training intervention, even though specific respiratory muscle training may be a more promising intervention to counteract pulmonary dysfunction in pwMS.

CARDIAC DYSFUNCTION IN MS

pwMS are prone to a greater risk for the development of ischemic heart disease and heart failure,[11] leading to premature cardiovascular death and hence a lowered life expectancy.[101] It is speculated that the increased incidence of these cardiac diseases in pwMS is related to physical inactivity, inflammatory processes, higher prevalence of smoking, and obesity.[11] Regardless of the exact pathophysiology leading to cardiac anomalies in pwMS, it seems fair to stimulate clinicians to screen for cardiac anomalies in clinical practice in order to improve care and treatment of MS. Typically for MS, two major cardiac abnormalities can be discovered: impaired left ventricular function and a disturbed cardiac autonomic control.

The cardiac function has been studied in pwMS by echocardiography, magnetic resonance imaging, or radionuclide angiography at rest. Most studies report significant left and right ventricular dysfunction in pwMS.[102–105] This cardiac dysfunction is characterized by a reduction in cardiomyocyte high-energy phosphate content,[103] left and right ventricular ejection fraction,[102,104,105] cardiac stroke volume,[105] impaired left ventricular relaxation,[102] and abnormalities in ventricular dimensions (i.e., wall hypertrophy).[102] It is believed that these abnormalities in cardiac function are due to cardiac autonomic dysfunction,[102,105] although the contribution of the intake of anticholinergic, α-blocking, or tricyclic antidepressant drugs also seems to elicit disturbances in cardiac function.[102] Anomalies in cardiac autonomic function in pwMS can be observed by measuring HR and blood pressure responses to Valsalva maneuver, deep breathing and active changes in posture, and/or changes in blood pressure during sustained handgrip.[106–109] It is currently thought that cardiac autonomic dysfunction results from demyelinating plaques that damage the vasomotor centers in the brain stem or

interfere with autonomous nervous system descending fibers in the spinal cord.[106–109]

These abnormalities in cardiac function and cardiac autonomic control significantly affect cardiac function during exercise in pwMS. A higher resting and (steady-state) exercise HR, and significantly lower oxygen pulse (VO_2/HR) during exercise is commonly observed in pwMS, the latter indicating a lowered stroke volume and/or peripheral oxygen extraction capacity.[92,93,110,111] In other populations, such impaired left ventricular function during exercise could elicit arterial pulmonary hypertension that would further elevate VE/VCO_2 and alter $PETCO_2$ during exercise and thus mimic pulmonary dysfunction (as explained previously).[112] As a result, first signs of cardiac dysfunction during endurance exercise can be detected by abnormalities in pulmonary parameters in pwMS. This, however, requires great insight into ergospirometry testing and interpretation. Moreover, a lowered cardiac stroke lowers cardiac output (in case of similar or decreased HR); such lowered cardiovascular reserve will definitely lead to severe exercise intolerance. The early (within the first 20 s) HR increase at initiation of endurance exercise is significantly slowed in pwMS, and this impairment in HR increase speed correlates significantly ($r = 0.64$) with walking capacity in pwMS.[111] A slower 20-s HR increase in pwMS is in support for a specific disturbance of the autonomic cardiac control.[111] Other studies also reported an attenuated HR increase during the onset of endurance exercise in pwMS[109,113] or suggested an abnormal dissociation between HR and pressor response to static work (isometric handgrip exercise).[114] In general, at onset of exercise, the rapid HR increase is determined by the withdrawal of tonic vagal activity.[115] A smaller early exercise-onset HR increase is thus believed to be related to slowed withdrawal of the vagal tone. This is linked to a disturbed central command or metabo/tetanoreflex mechanisms that precede such withdrawal. In addition, primary pulmonary arterial hypertension could be present in pwMS, especially when receiving interferon beta therapy.[116] This will also relate to abnormalities in cardiac or pulmonary function and thus limit exercise performance capacity in pwMS.

Whether exercise training intervention is capable of remediating the observed abnormalities in cardiac function in pwMS remains mostly unknown. It remains to be studied whether cardiac function, assessed by echocardiography or other medical imaging techniques, can be improved by exercise training in pwMS. The impact of 6 months of combined endurance/strength training was examined in pwMS, and it was observed that the slowed HR increase at the onset of endurance exercise (indicative for dysfunction in cardiac autonomic control) remained present, even though exercise tolerance improved significantly (as evidenced by reductions in blood lactate content at similar absolute workloads).[117] These data thus indicate that cardiac autonomic dysfunction during exercise in pwMS is not easily remediated by exercise training in pwMS. It is hypothesized that brain lesions that lead to cardiac autonomic dysfunction are permanent in pwMS, and thus persist after participation into a long-term exercise intervention.

A significantly disturbed cardiac function and cardiac autonomic control during endurance exercise is present in pwMS. Considering the significant impact of such dysfunctions on exercise tolerance in pwMS, it is evident that clinicians should systematically examine the cardiovascular system as well. Unfortunately, due to the lack of insights on the impact of exercise training on cardiac function in pwMS, we are currently significantly limited to provide optimal cardiac care to pwMS by exercise intervention. On the other hand, in patients with heart failure or diabetic cardiomyopathy, it has been observed that high-intensity interval training is sometimes better able to improve cardiac diastolic or systolic function.[118,119] Such findings are highly relevant to pwMS and should deserve further examination.

In conclusion, significant cardiac dysfunction and abnormalities in cardiac autonomic control during endurance exercise are present in pwMS. This dysfunction relates to exercise intolerance in pwMS, and is not easily remediated by exercise training intervention.

METABOLIC DYSFUNCTION IN MS

As of 2016, the metabolic profile in pwMS (blood lipid profile, glucose tolerance, fat mobilization, and oxidation) is heavily debated in the literature.[10,11,120,121] However, evidence is accumulating that pwMS more often suffer from glucose intolerance and a disturbed glycemic control, as compared to healthy persons.[120] In addition, glucose tracer ([18F]-fluorodeoxyglucose) uptake in knee and hip flexors is elevated in pwMS, while [18F]-FDG uptake is lowered in the weaker knee flexors of pwMS, indicating a greater metabolic cost of certain muscles during physical activity.[47] It is thus clinically relevant to examine metabolic function during exercise in pwMS.

Unfortunately, the metabolic function (blood lipids, glucose, and endocrine hormones) during exercise is poorly understood in pwMS, therefore, deserves greater attention in the near future. Heesen et al.[122] studied the impact of 30 min of cycling at 60% of peak oxygen uptake (VO_{2peak}) in pwMS vs. healthy controls on blood cytokine and endocrine hormone concentrations. Changes in blood (nor)epinephrine, adrenocorticotropic hormone, cortisol, β-endorphin, *interferon gamma*, tumor necrosis factor alpha, and interleukin 10 content were normal in pwMS during such exercise bout, although a trend for a hyporeactive cytokine response emerged in pwMS.[122]

It was thus concluded that metabolic dysfunction, as evidenced by these blood parameters, was not present in pwMS. However, another study highlighted a significant abnormality in the lipolytic response to endurance exercise in pwMS: due to autonomic dysfunction pwMS are less capable of triggering fat mobilization during an exercise bout.[40] In accordance, muscle fat oxidation (as indicated by respiratory gas exchange ratio, RER, in this study) is reduced accordingly in this particular condition.[40]

Notwithstanding the limited knowledge on metabolic function during exercise in pwMS, combined (resistance and endurance) exercise is known to improve glucose tolerance in pwMS, and this in an exercise intensity–dependent manner.[77,123] Once again, evidence during mid-2010s thus suggests that pwMS should exercise at greater intensities to maximize the clinical benefits of exercise intervention on glycemic control in pwMS. In addition, leisure time physical activity (which is mainly of the endurance type) is reported to be associated with lower waist circumference, blood triglyceride levels and glucose concentrations, contributing to important health-related benefits.[124] On the other hand, resistance training is only able to decrease blood triglyceride levels, while blood glucose, total cholesterol, and high-density lipoprotein cholesterol do not change.[75] It thus follows that optimal exercise prescription is mandatory to affect metabolic function in pwMS: endurance-type exercise training is preferred in this regard. In a subsequent study of Heesen et al. (see earlier section for cross-sectional observations) the impact of an 8-week endurance training program on changes in blood cytokine and hormone concentrations during acute endurance exercise was studied in pwMS.[125] No significant changes in these blood parameters during acute endurance exercise were found when following such exercise intervention.[125]

During endurance exercise, the lipolytic response, and hence muscle fat oxidation, is significantly suppressed in pwMS. This will very likely affect the RER during exercise. Because the RER can be used to determine whether a maximal exercise test is executed in cardiopulmonary exercise tests, caution is warranted in the use of this methodology in pwMS. To improve fat oxidation capacity endurance exercise training should preferentially be prescribed to pwMS (because resistance training is much less effective). In other populations (such as obesity and type 2 diabetes patients) such intervention leads to improvements in fat oxidation capacity.[126] This may then also contribute to improvements in insulin sensitivity and, hence, glycemic control. Given the elevated likelihood for development of impaired glucose tolerance in pwMS, it may be argued to add resistance training on top of endurance training, to exercise as frequently as possible or to prolong the exercise program. Such adaptations in exercise prescription are instrumental to greater improvements in glycemic control, at least in type 2 diabetes patients.[127]

In conclusion, some metabolic dysfunction is present in pwMS, both at rest as during exercise. Whether this metabolic dysfunction can be remediated by exercise intervention, remains to be addressed in pwMS, but it seems relevant to optimize exercise prescription when aiming for this target.

ARE THE OBSERVED MUSCULAR, PULMONARY, CARDIAC, AND METABOLIC ABNORMALITIES (DURING EXERCISE) SIMPLY DUE TO PHYSICAL INACTIVITY IN MS?

It is commonly assumed that the anomalies in pwMS mentioned in the preceding sections are simply due to physical inactivity and sedentarism (see Fig. 17.1). Indeed, many studies have provided compelling evidence that MS often leads to an inactive lifestyle due to difficulties in engaging into physical activities.[128,129] This physical inactivity accelerates the physical deconditioning process, which in turn makes it even more difficult to engage in physical activities. As a result, a vicious cycle of physical limitation—physical inactivity—greater physical limitation is very likely to occur in MS. It may then be deduced that muscular, pulmonary, cardiac, and metabolic function decrements are due to this physical inactivity. However, future studies should test the hypothesis mentioned earlier in greater detail and with better methodology. First, it is well known that MS is associated with systemic inflammation, oxidative stress, and vitamin D depletion, to mention few systemic abnormalities in MS.[130,131] In healthy individuals and in laboratory animals, such systemic changes are known to compromise certain muscular, pulmonary, cardiac, and/or metabolic functions.[132–135] It should therefore be examined whether the normalization of these systemic anomalies would lead to improvements in muscular, pulmonary, cardiac, and metabolic function in pwMS, even without the implementation of exercise intervention or restoration of physical activity. Second, even when clinically effective exercise interventions are implemented (as evidenced by significant improvements in endurance exercise capacity or muscle strength), not all organ functions, as discussed earlier, improve (such as cardiac autonomic and pulmonary function, and some metabolic parameters), while other organ systems are highly responsive to exercise training (such as skeletal muscles). These findings should thus confirm that not all dysfunctions, explained in this chapter, are simply due to physical inactivity only in pwMS.

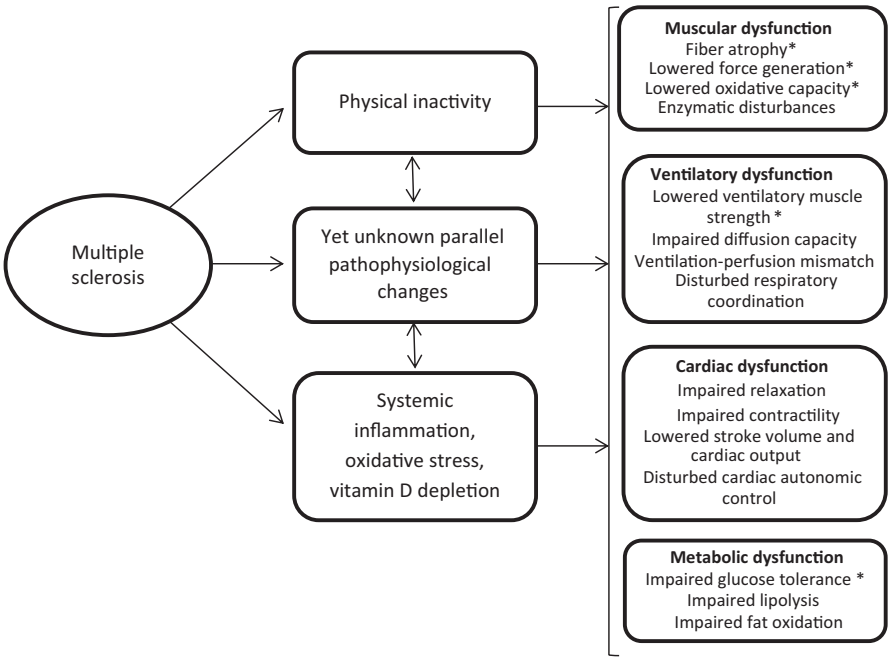

FIGURE 17.1 Muscular, pulmonary, cardiac, and metabolic function in pwMS. Outcomes highlighted by means of *may be remediated by exercise training intervention in pwMS.

CONCLUSION

MS is associated with significant muscular, cardiac, pulmonary, and metabolic dysfunction. These anomalies often relate to exercise intolerance or can lead to increased risk for the development of cardiometabolic disease, increased hospitalization rate, or reduced life expectancy. Consequently, in clinical practice pwMS should be screened systematically for muscular, cardiac, pulmonary, and metabolic function, at rest and during exercise. To further optimize exercise therapy, exercise prescription should be adapted accordingly, and it should be considered to examine the impact of nutritional support during exercise intervention.

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CHAPTER

18

Exercise in the Treatment of Multiple Sclerosis: Pragmatic Exercise Intervention in People With Mild to Moderate Multiple Sclerosis—The ExIMS Project

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OUTLINE

EXERCISE IN THE MANAGEMENT OF MULTIPLE SCLEROSIS

Multiple sclerosis (MS) has a wide range of effects on a person's health.[1] Compared to healthy individuals, people with MS have a reduced aerobic capacity, decreased muscle strength, reduced muscle endurance, impaired balance, impaired motor control, and higher levels of fatigue.[2a] People with MS can find themselves in a cyclical situation where physiological deconditioning as a result of their MS, leads to physical inactivity, which results in further physiological deconditioning.[1] The clinical course of MS is diverse. There are four standardized definitions for the clinical course of MS: clinically isolated syndrome (CIS), relapsing–remitting (RR), primary progressive (PP), and secondary progressive (SP).[2b] MS symptoms can vary from one individual to other and from one day to the next, with some individuals experiencing a rapid decline in function, while others experience a much slower deterioration.[3] The majority of research as of 2016 has focused on people with relapsing–remitting MS, with mild-to-moderate levels of disability.[2a]

Research into the use of exercise as a therapy for the treatment of MS is relatively new, with people with MS previously advised to avoid exercise to conserve energy and prevent increases in body temperature that could worsen symptoms (Uhthoff's syndrome).[4] Early research focused on rehabilitation-based physiotherapy treatments[5] and water-based exercise[6]; these studies generally had small sample sizes and lacked the robust design of a randomized-control trial (RCT). An early review[7] reported that exercise "seems to improve cardiorespiratory fitness and skeletal muscle function" in people with MS. The first RCT to explore the possible benefits of exercise for people with MS was conducted by Petajan and colleagues[8] and reported increased aerobic capacity, strength and mobility, improved bowel and bladder function, and decreased fatigue and depression, with no increase in the number of exacerbations. Research in the area has since gained momentum, with the Cochrane review on "Exercise therapy for multiple sclerosis"[9] recognizing nine RCTs of high technical quality, all concluding that exercise is efficacious for improved outcomes in MS. Further reviews[10–14] supported these findings and concluded that supervised exercise (using aerobic and/or strength exercises) training is beneficial for people with mild-to-moderate MS. Moreover, a mid-2010 review on exercise safety for people with MS suggested that exercise causes no increase in relapse rate or the number of exercise-related adverse events reported in people with MS,[15] indicating that it is both a safe and effective treatment strategy for this patient group.

Latimer-Cheung and colleagues[13] provided exercise prescription recommendations for people with MS. The guidelines are set for people between 18 and 65 years of age and with mild-to-moderate MS disability. The main recommendations are for individuals to complete 30 min of moderate intensity aerobic activity two times a week and strength training for major muscle groups two times per week.

Current evidence builds upon earlier research inferring that exercise does more than improve physiological function, it enhances the management of symptoms in MS, and could slow down the disease process, with some evidence to indicate a possible disease-modifying effect.[16]

Collectively, research to date suggests the beneficial effect of exercise for people with MS. That said, people with MS are often reported to be less physically active than the general population,[17] with symptoms being linked to physical activity levels and partially accounted for by low exercise self-efficacy.[18] This low physical activity can lead to secondary complications such as obesity, cardiovascular disease, and osteoporosis.[2a,4,10]

DEFINITIONS OF TERMS

For the purpose of this chapter exercise has been defined as a potential disruption to homeostasis by muscle activity that is either exclusively, or in combination, concentric, eccentric, or isometric.[19] Exercise is often a planned and structured activity designed to improve fitness and health.[20] On the other hand, physical activity is referred to as exercise that includes activities of daily living such as household jobs, walking the dog, and manual labor.[20]

The main clinical outcome measure used in MS is the expanded disability status scale (EDSS).[7] The EDSS is used to measure physical impairment of MS and uses the Kurtzke Functional Systems rating scale. This scale provides an ordinal score of impairment in eight areas. Based on the score in these eight categories, individuals with MS receive an overall disability rating.

EXERCISE INTERVENTIONS IN MULTIPLE SCLEROSIS TRIAL

Supervised facility based exercise programmes can offer people with MS the support and guidance needed to be more active, improving health outcomes in the short term.[21] It is therefore important that research now focused on developing a pragmatic and cost-effective exercise programmes that can have a long lasting impact on the lives of people with MS. The Exercise Interventions in Multiple Sclerosis (ExIMS) trial used a tailored program guided by the individual, with input and advice from specialist exercise scientists and physiotherapists. The majority of exercise and physical activity research studies in MS prior to ExIMS have been of poor research design, including small sample sizes, short follow-up periods, and poorly described methods. With regard to intervention design, most have included either exercising at home or in a supervised setting, with only few using a combined (supervised and home-based) approach.[22] Moreover, no previous research had looked at the impact of a combined tapered approach to exercise for people with MS, although this has been used in other clinical groups, such as prostate and breast cancer patients.[23–26] In addition, research trials in mid-2010s have included a cognitive behavioral approach in combination with an exercise intervention in people with MS.[27,28] Thus making the design of ExIMS unique, with design elements included to promote long-term exercise behavior change.

Here we report the design and implementation of a pragmatic exercise trial for people with MS. The first stage investigated the feasibility of such an approach to determine whether the approach was acceptable and safe for people with MS.[29] Following this the main trial is reporting

covering the study protocol,[30] trial recruitment,[31] and key findings, including[32] cost-effectiveness data.[33]

FEASIBILITY TRIAL

The initial trial[29] explored the feasibility of a pragmatic exercise intervention (supervised and home based) that was tailored to the individual and designed to promote confidence and motivation for long-term exercise behavior change. A total of 28 participants were recruited from MS clinics at Sheffield Teaching Hospitals NHS Foundation Trust (UK). The design was a parallel RCT. Participants attended two supervised exercise sessions and one home session per week for 10 weeks with participants followed up at 3 months. Supervised sessions were delivered one to one led by an exercise researcher. The exercise program was progressive and tailored toward individual capabilities and preferences. Each session contained a warm-up, followed by an aerobic component, tailored functional body-conditioning exercise based on individual need and a cool down.[29] The results suggested that this type of trial design is feasible and effective for people with mild-to-moderate levels of disability from MS, with excellent retention (10 weeks, 93%; 3 months, 86%) and high compliance (>75% of all sessions) alongside a good progression in training load (duration and intensity). Moreover, the initial data suggest that important improvements in exercise behavior and quality of life (QoL) benefits might be experienced by people with MS and retained for up to 3 months of follow-up. However, the impact of this type of intervention over long term should be viewed with caution as this may not reflect adherence to exercise beyond 3 months. Despite the reported benefits of exercise,[13,14] people with MS participate in less physical activity[34] than the general population and appear to find long-term adherence to exercise interventions difficult.[35] Therefore, the results from the feasibility trial suggest that a pragmatically designed and theoretically underpinned exercise intervention may have the potential to increase the likelihood of long-term exercise behavior change.

The progression of exercise load was patient led and involved increasing intensity and/or duration while maintaining an RPE (Rating of Perceived Exertion) scale score between 11 and 13 (fairly light to somewhat hard). The progression reported in this study is similar to other participant led progression rates[36] and was well tolerated (no adverse events). Exercise type was also participant led, with all participants including treadmill, rowing, and cycling ergometry in their supervised program, despite rowing previously being only recommended for well-functioning patients.[11] In addition, the most popular home exercise was walking, in accordance with previous research.[36]

Cautious consideration of the outcome data reported indicated that people with MS can experience important clinical, physical, and QoL benefits that may still be present after 3 months of follow-up, as previously suggested by McCullagh and colleague.[37] A large-scale trial, with a longer follow-up was warranted before conclusions regarding exercise benefits and maintenance could be determined.

MAIN TRIAL

Study Protocol

The trial design aimed to generate new knowledge by investigating the effects of a pragmatic exercise trial containing cognitive behavioral strategies in a large population of people with MS for up to 6 months of follow-up, reporting impact on physical activity behavior, key health outcomes including health-related quality of life (HRQoL), and cost-effectiveness. In addition, the design included participants with slightly higher levels of disability from MS (up to EDSS 6.5—constant bilateral support required to walk 20 m without resting) to determine impact on different disability levels, as suggested by Latimer-Cheung and colleagues.[13]

The study design was similar to that used in the feasibility study. However, the program was extended from 10 to 12 weeks and changed to include a tapering of contact time and an increase in home exercise sessions during the later 6 weeks to build the skills and confidence for long-term self-management.

The study was a two-arm parallel RCT. People with MS were randomized to receive either the ExIMS intervention or usual care. The intervention involved 12 weeks supervised exercise program. An exercise physiologist supervised the delivery of the intervention along with input from a neurophysiotherapist during the early stages.

Exercise Dimensions

The optimal volume of exercise for achieving health outcomes is difficult to establish and may vary from each individual. The program was designed to be pragmatic and accessible to the individual. Following is a summary of the exercise sessions for the participants including the mode, intensity, duration, and frequency.

Mode of Exercise

The program allowed the participant to have some choice in exercise preferences but aerobic exercise was the core modality of the sessions. Aerobic exercise choices included cycle ergometer, treadmill walking, elliptical trainer, rowing ergometer, and arm cranking.

Participants also performed exercises for strength, control, and balance (e.g., wall press-ups, arm curls, leg abduction, squats, knee extensions, and calf raises). These exercises were prescribed based on the individual needs of each participant after being assessed by the trial physiotherapist. Body weight, lightweights, and TheraBands were used to provide resistance. Exercise consisted of one to three sets of 5 to 20 repetitions, depending on level of disability and strength.

Intensity

Participants were instructed to complete low-to-moderate level aerobic exercise at 50–69% of age-predicted heart rate maximum or 12–14 on the Borg ratings of perceived exertion scale.

Frequency and Duration

During weeks 1–6, participants attended two supervised sessions at an exercise facility and engaged in one self-directed exercise session in their leisure time. Supervised exercise sessions were either one to one or in small groups and lasted up to 1 h. During weeks 6–12 participants attended one supervised exercise session and engaged in two self-directed exercise session.

The supervised exercise sessions incorporated cognitive behavioral techniques (e.g., goal setting and finding social support) to promote long-term participation in exercise.

Trial Recruitment

The trial took place between March 2009 and August 2012, of 349 potential participants who were assessed for eligibility; 120 (34%) were randomized to the ExIMS project. The ExIMS trial employed a comprehensive recruitment strategy to ensure the required sample size was achieved. Participants were recruited via MS hospital clinics, advertisements in the local MS society branches, and consultant invitation letters. All participants were assessed by a consultant neurologist. The inclusion criteria for the study were a clinical diagnosis of MS, as defined by McDonald Criteria,[38] with an EDSS of 1.0–6.5, aged 18–65 years, and clinically stable for at least 4 weeks. Exclusion criteria included comorbidities that prevented participants from safely taking part and already participating in structured exercise 3 times a week.

The recruitment rate of 3.5 participants per month achieved in this trial is comparable to other nonpharmacological intervention trials in MS. Previous interventions using people with MS have reported either marginally lower[39] or higher rates per month,[40] with exercise intervention trials in other clinical populations again reporting similar rates of between 2.9 and 4.0 participants per month.[41–43] This indicates that this is a realistic target to use.

Response rates were highest from targeted consultant invite letters (42.8%) and lowest from attendance at MS outpatient clinics (6.4%); despite the large number of people with MS attending clinics, many did not meet the eligibility criteria. These methods, however, should not be discounted, as the trial recruited 60% of its participants from this route, with only 29.2% coming from consultant letters and 10.8% from other trial awareness strategies. Randomization yield (number recruited/number interested) for recruitment from the MS outpatient clinics and consultant letters was similar (33.2% and 31.0%, respectively), suggesting both methods are useful in attaining targets.

The most common reason given for ineligibility to participate in the trial was already being too active (69.2%), which is consistent with results reported for similar exercise trials in cancer survivors, where 55% were too active.[41] This is surprising given the low levels of physical activity reported in the UK, with people with MS reported to be even more inactive than the general population.[45] However, those who wish to take part in an exercise study are likely to have an interest in and be more motivated to exercise. This suggests that we may not be reaching those who have limited interest in exercise and may need to look at more appealing interventions and other ways to incentivize this group to volunteer to take part in trials of this type. Options could include offering taster sessions prior to consent.

Many eligible participants (66.3%) chose not to give a reason for declining to participate. However, of those who did, travel to the site was the most commonly cited reason. Ensuring adequate travel arrangements or arranging community venues for exercise sessions could alleviate these concerns and should be considered in the design of future exercise trials in MS.

To adequately budget for recruitment to future trials it is important to not only understand where participants were recruited from, but how long it took to recruit each participant, with some community-based interventions reporting to take up to 10 h per participant to recruit.[45] This trial reported that consultant mail-out was the most efficient method of recruitment at 0.6 h per participant, with MS outpatient clinics requiring seven times this amount at 4.2 h per participant. This provides an indicator of the time allocation required for recruitment in future trials. The results from this study provide a unique insight into trial recruitment for exercise interventions in MS and may be used to inform the design of future trials of this type.

Future trials would benefit from using a comprehensive recruitment strategy that includes methods to recruit individuals less keen on exercise, to ensure that a representative sample of patients is recruited in an efficient and timely fashion. If the project is of a similar design and requires more than three patients a month

to be recruited, a multicenter trial is recommended. This would have the added benefits of testing generalizability across a variety of different settings.

Main Trial Results

The ExIMS project aimed to demonstrate whether a robustly designed intervention, containing cognitive behavioral strategies to promote long-term participation, would show an increase in physical activity levels and improved health outcomes at up to 9 months of follow-up, when compared with usual care. Secondary to this the main trial also investigates the exercise dose–response relationships and whether level of disability from MS has an impact on health outcomes. Cost-effectiveness analysis was also carried out on the trial to determine whether it would be a cost-effective intervention if implemented in the usual care pathway for people with MS.

The ExIMS project fills an essential gap in knowledge, as despite a large volume of literature in the area of exercise and MS, many questions have remained unanswered.[9,46] The majority of current trials have been of poor quality and have not involved a pragmatic approach with cognitive behavioral strategies to promote long-term adherence to exercise.[46] Moreover, none have begun to answer the questions regarding the dose–response relationship and the impact of disability status on outcomes.

The ExIMS trial reported significant improvements in exercise behavior (Godin Leisure-Time Exercise Questionnaire, GLTEQ, and accelerometer step counts), fatigue, and HRQoL at 3 months of follow-up, with significant improvements in emotional well-being, social function, and overall QoL being sustained at up to 9 months of follow-up. In addition, the cost-effectiveness analysis of ExIMS suggested that the intervention is likely to be cost-effective and provide cost benefits to the National Health Services (NHS), with even greater benefits likely to be reported if exercise was focused toward individuals more severely affected by MS.

In line with previous systematic reviews on exercise interventions for people with MS[13] ExIMS reported a significant increase in physical activity (GLTEQ and step count) at 3 months of follow-up. However, only the self-report data (GLTEQ) showed a notable sustained increase at 9 months. There are two possible reasons for this: first self-report bias could have impacted on the data reported in the questionnaire and secondly, accelerometry for people with MS can be difficult to interpret[47] and does not account for activities such as swimming, cycling, and rowing ergometry, which were reported to be popular in the feasibility trial for this study.[29] However, physiological data collected for diastolic blood pressure and waist circumference did indicate that there may still have been some increase in physical activity levels at 9 months of follow-up, with both showing significant improvement; thus, suggesting that the program may have an important long-term impact on cardiovascular health for people with MS. This is essential as people with MS are reported to have 2.4 times greater risk of death due to cardiovascular disease than the general population.[48]

In addition to the significantly increased physical activity reported for the intervention group, when compared with usual care control at 3 months of follow-up, multidimensional fatigue and most dimensions of HRQoL were also significantly improved. This is comparable with data reported in previous systematic reviews on exercise interventions for people with MS.[17,49] When physical activity was no longer reported to be significantly enhanced at 9 months, follow-up improvements were no longer noted in fatigue and some of the HRQoL domains, despite improvements in emotional well-being, social function, and overall QoL remaining significant when compared with usual care control.

Previous research suggests that improvements in fatigue and QoL can be maintained up to 3 months of follow-up even when improvements in exercise capacity have returned to normal.[38] No measures were taken in this study at 3 months of post-intervention, and it is possible that by 6 months of post-intervention these changes had diminished; thus, suggesting that continued engagement in exercise is required to maintain improvements in fatigue. This is supported by current literature that suggests that although the cause of MS fatigue is unknown it may be linked to immune dysfunction, with pilot work suggesting that aerobic exercise activates genes responsible for the immune response not observed in healthy controls. However, this disappears when exposure to exercise is removed.[50]

The study also reported that individuals experiencing the highest levels of fatigue at baseline, experienced the greatest improvements from the exercise intervention. This is comparable with the hypothesis drawn in the systematic review by Andreasen and colleagues,[49] who suggested that exercise interventions that demonstrated an impact on fatigue were those that had clinically fatigued patients at baseline. The ExIMS project also indicated that people with MS achieving high volumes of exercise during the intervention reported less pronounced improvements in fatigue, implying that an optimum level of training may exist. This finding warrants further research as data reported in a systematic review by Andreasen and colleagues,[49] suggest that at present "it is not possible to draw solid conclusions on optimal exercise duration, frequency, and intensity." It is likely that maintenance of exercise and hence fatigue and HRQoL domains during the follow-up may have been enhanced if the protocol had included additional

contact with participants in the 6-month period following the intervention. However, this additional resource would increase the cost of the intervention and may impact on the cost-effectiveness results.

The ExIMS trial did not show any significant changes in functional ability (6-minute walk test, 6MWT) or neurological impairment (multiple sclerosis functional composite—MSFC and EDSS) when compared with usual care control. However, the study was not powered to demonstrate a change in these outcomes. In addition, studies lasting less than a year have only been reported to show subtle differences in EDSS.[51]

IMPLICATIONS FOR PRACTICE

Although there has been great progression in the area, there is still a lack of quality evidence regarding exercise training and physical activity for people with MS.[9,46] Therefore, many questions, such as what is the long-term impacts of exercise; is there an optimum dose, and does this differ for different disability levels, remain unanswered.[2a,14] Current evidence is sufficient to suggest that mild-to-moderate intensity exercise is safe and effective at increasing fitness and may improve symptoms of fatigue and QoL in patients with mild-to-moderate disability from MS.[13,14] The ExIMS trial was the first robustly designed exercise trial for people with mild-to-moderate MS. The results from this project can be inferred for ambulatory individuals with mild-to-moderate disability from MS, with the effectiveness of the intervention for those with more severe disability from MS remaining unclear.

The ExIMS trial demonstrated that a pragmatic approach is effective at enhancing self-directed exercise behavior and retaining some important health outcomes at up to 6 months of follow-up and is likely to be cost-effective if implemented by the NHS.

In addition, it suggests that there is an optimum level of exercise for improvements in fatigue and that exercise is likely to be more beneficial for people experiencing higher levels of fatigue. Moreover, it indicates that some long-term benefits in HRQoL are retained at up to 6 months of follow-up. However, it is suggested that for long-term improvements in fatigue participants need to maintain the elevated levels of fitness achieved following the 3 months of intervention. Therefore, it is recommended that cognitive behavioral strategies form an essential component in the design of future exercise interventions, with further contact during follow-up required to maintain participant's confidence and motivation to exercise following the intervention. It should also be noted that the ExIMS trial is the only study to our knowledge to use an individually tailored program guided by the individual, with input and advice from both specialist exercise scientists and physiotherapists. This approach is recommended in the future for the design of exercise programs that provide people with MS with the ability to become more physically active and participate in more regular exercise.

The ExIMS project provides valuable evidence to guide the design of future exercise interventions and provides robust and detailed data to enable more comprehensive guidelines for exercise and physical activity to be drawn up. This new evidence suggests that exercise would be a valuable addition to the current treatment pathway for MS. However, further research needs to be done to embed this exercise into clinical practice.

DIRECTIONS OF FUTURE RESEARCH

To date review articles in the area of exercise and MS have consistently stated that there is a need for more high-quality RCTs, with sample sizes based on statistical power calculations[2a,14] and interventions tailored to individual's symptoms and lifestyle.[46] In addition, there is also a need for studies to take into account different disability levels and longer-term impact.[2a] For exercise interventions to have the greatest impact there is a need for future studies to use a mixed-methods approach, examining the motivational responses that determine exercise behavior and enabling the barriers to exercise participation in this population group to be fully explored,[52] with studies also designed to include cognitive behavioral strategies to promote long-term exercise behavior change.[23,52,53]

The ExIMS project begins to answer these questions. However, there are still many questions that need to be answered as most studies have involved people with mild-to-moderate disability from MS, exercising at a moderate intensity.[46] Therefore, there is a requirement for further high-quality RCTs designed to explore the following research topics:

- Exercise for people with more severe disability from MS (EDSS greater than 6.5).
- The feasibility of higher-intensity exercise for people with mild disability from MS.
- Early educational intervention to prevent rapid decline in exercise participation on diagnosis.
- The optimum type and dose of exercise for fatigue management for people with clinical levels of fatigue from MS.

Exercise for People With More Severe Disability From MS

Despite the rapid increase of research on exercise for people with mild-to-moderate MS since 2000s, research

on exercise for those with more severe disability has been sparse. The ExIMS project looked at the acceptability of a pragmatic tailored approach to exercise for people with MS (EDSS 1.0–6.5) and whether the achievable dose is different for those with more severe disability. Results suggest that although some participants at the upper limits of our inclusion criteria (EDSS 6.0–6.5) were able to achieve excellent compliance levels, with one achieving 100%, most however found attending the supervised sessions difficult, with high drop-out levels experienced in this population group, thus supporting research suggesting the need for a tailored approach to physical activity interventions, directed by disability status.[54] The challenge now is to explore the type of physical activity interventions that would be acceptable and achievable for people with more severe MS and what benefits could potentially be gained from participation in interventions aimed at decreasing sedentary behavior and increasing physical activity in this population group. Such research has the potential to have a significant impact on the lives of people with MS and their families.

Feasibility of High-Intensity Exercise for People With Mild Disability From MS

Current guidelines recommend that people with MS exercise at a moderate intensity,[9,13] as most current exercise research is conducted at this intensity.[55] Thus, meaning that even if individuals have very mild or benign MS they are still advised to avoid high-intensity exercise, as there is no current research available to suggest whether it is safe or not. This may lead to the type of scenario where an individual who is currently very active may be recommended to significantly alter their current exercise habits on diagnosis, when they may not have to. It is therefore recommended that future research investigates the feasibility of higher intensities of exercise for people with mild disability from MS to determine if it is safe and beneficial for this population group.

Early Educational Intervention to Prevent Rapid Decline in Exercise Participation on Diagnosis of MS

People with MS participate in less physical activity than the general population,[56,57] by nearly 1 standard deviation, with almost 60% of individuals with MS participating insufficiently in physical activity to provide minimal health benefits.[58] Unpublished qualitative data collected during the ExIMS trial suggests that at diagnosis people with MS currently receive little if any advice and support on what type of exercise is beneficial and that this continues long term, with health professionals and gym instructors unable to provide adequate advice. In addition, if people with MS wish to access additional information on exercise and physical activity, their preferred source is the Internet.[59] This is a resource also utilized by health care professionals wishing to promote physical activity.[60]

Qualitative research suggests that fear of making the condition worse[61] and fatigue[62] may contribute to the observed decline in physical activity and structured exercise following a diagnosis of MS.

Therefore, cost-effective strategies that provide support to individuals to help them maintain and or take up new forms of exercise and physical activity both at diagnosis and as disability levels and symptoms change are crucial. This would enable people with MS to maintain a healthy relationship with exercise that enables them to better self-manage their condition and gain maximum benefits from being more physically active.

The Optimum Type and Dose of Exercise for Fatigue Management for People With Clinical Levels of Fatigue From MS

Data synthesis from systematic reviews[13,49] and meta-analysis[15,63] suggests that exercise may provide a useful approach to managing fatigue for people with MS. However, quality research is sparse and does not enable inference across different types of MS and disability levels,[63] or what type and dose provide optimum results.[13,63] Results from ExIMS suggest that individuals experiencing the highest levels of fatigue have the potential to experience the greatest improvements, as supported by Andreasen and colleagues.[49] In addition, our data also suggests that there may be an optimum dose of exercise, with individuals achieving the highest dose of exercise during the ExIMS trial not achieving the greatest improvements in fatigue.

Therefore, it is recommended that future research explores the optimum type and dose of exercise required to gain benefits in people with MS presenting with clinical levels of fatigue.

CONCLUSION

The ExIMS project was the first robustly designed RCT to investigate the effects of a pragmatically implemented progressive exercise program in people with MS. The intervention used a unique approach that was individually tailored, employed cognitive behavioral techniques to promote long-term adherence, and was designed to contain tapered supervision, being predominantly home based in the later stages. The main outcomes suggest that this approach was not only feasible, but results from ExIMS indicate that this type of

intervention can provide significant increases in self-directed exercise behavior, fatigue, and HRQoL, with significant improvements for some domains of QoL being sustained at up to 9 months of follow-up. In addition, this intervention is highly likely to be cost-effective if implemented by the NHS.

Prior to this research, systematic reviews and meta-analysis into the benefits of exercise for people with MS have consistently highlighted a need for more robustly designed research trials, containing long-term follow-up and participants with higher levels of disability from MS. The ExIMS project has taken a notable step toward filling in the gaps in the literature, by providing data from a robustly designed exercise trial, which has recruited people with a range of neurological impairment (EDSS 1.0–6.5) and has included a longer-term follow-up (6 months).

The results provide a strong evidence base to suggest that a pragmatic approach to exercise can have important long-term health benefits that improve self-management and should encourage health professionals to motivate individuals with MS to exercise. It is hoped that exercise will now be considered as part of the treatment pathway for people with MS. However, if outcomes are to be optimized and increased levels of activity maintained, there is a need for strategies to provide continued contact between participants and the delivery team following the intervention.

Furthermore, there are still many questions that remain unanswered, as the majority of exercise research has involved people with mild-to-moderate levels of disability from MS, exercising at a moderate intensity. There is a need for more high-quality RCTs exploring the benefits of exercise for people with more severe disability from MS, and the feasibility of higher-intensity exercise for people with mild disability from MS. In addition, further details are required on the optimum dose of exercise for improvements of important health outcomes such as fatigue.

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CHAPTER

19

Yoga and Pilates as Methods of Symptom Management in Multiple Sclerosis

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OUTLINE

BACKGROUND

The earliest accounts of multiple sclerosis (MS) date back to Vikings. According to Norse legend, in the late 12th century a bishop cured a woman with acute paralysis and muscle weakness, a condition that was potentially MS. In the late 1830s, Robert Carswell reported the first case of MS, which was confirmed by autopsy. Carswell described lesions in the white matter of patients who suffered transient paralysis. From 1840 to 1870, Jean-Martin Charcot, a French physician, studied patients with periodic paralysis and upon the patient's death would examine their brains with a microscope. From these studies, he distinguished MS from Parkinson's disease.[1]

MS is a chronic neurological disorder in which the body's immune system mounts an attack against the central nervous system (CNS). Within the CNS, oligodendrocytes produce the fatty substance myelin, which wraps neuronal axons and allows for effective neuronal transmission. Demyelination is responsible for impaired neuronal conduction and, eventually leads to brain lesions. Demyelination also results in the symptoms observed in patients with the disease. The onset of the disease typically occurs in young adults aged 20–40, and is far more prevalent in females than in males.

There are four courses categorizing this degenerative disease, which were initially described in 1996 by the National Multiple Sclerosis Society in the United States. An international panel later adopted these four courses in 2013. Of the four, relapse-remitting (RRMS) is the most common diagnosis. For patients with RRMS, onset of new symptoms occurs during periods of relapse, which are followed by remission periods of varying lengths. Over time, this typically progresses into a secondary progressive (SPMS) course. The two less common forms of MS are primary progressive (PPMS) and progressive relapsing (PRMS). Individuals diagnosed with PPMS experience increasing neurological dysfunction without periods of remission from disease onset, and those with PRMS experience a steady decrease in neurological function accompanied by occasional exacerbations and worsening of symptoms. Common symptoms are varied based on which brain region is affected and may include fatigue, spasticity, impairment or loss of mobility, bladder and bowel dysfunction, chronic pain, depression, and cognitive impairment. Pain is the chief complaint for patients with MS.

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As of 2016, there is no known cure for MS. Therefore, the chronic nature of the disease makes managing disease progression and symptoms imperative for maintaining quality of life (QoL). Medications approved by the United States Food and Drug Administration (FDA) reduce the severity of relapses and the accumulation of brain lesions. These medications are designed for patients with relapsing forms of MS, and no medications have been approved for treatment of PPMS. Relapses are commonly treated with corticosteroids, which have no long-term benefits, and there is a wide range of medications commonly used to manage ongoing symptoms. However, individuals may have adverse reactions to pharmacological treatments. Some medications may produce negative side effects such as nausea, fever, headache, fatigue, depression, and psychological imbalance.

It is estimated that approximately half of patients in the United States with MS use some form of complementary or alternative treatment, and prevalence of these therapies may be even higher in other countries such as Great Britain.[2] Complementary and alternative medicine (CAM) includes a wide range of nonpharmacological interventions that are defined by the National Center for Complementary and Alternative Medicine (NCCAM) as "complementary" when used in addition to conventional medicine and "alternative" when used in place of it. Due to the complex progression of MS, the National Society for Multiple Sclerosis promotes a comprehensive approach for disease management, including exercise and complementary therapies. In this chapter, we review the benefits of Pilates or yoga for patients with MS. First we define yoga and Pilates. Then we review literature examining potential benefits for patients with MS on managing pain, mental health, fatigue, mobility, spasticity, balance, strength, bladder control, and sexual function.

THE PILATES METHOD AND YOGA

Joseph H. Pilates developed the Pilates Method in the 1900s. Joe Pilates was born in Germany on December 19, 1883. Joe's mother was a naturopath who believed in healing the body through substances and therapeutic methods that encouraged self-healing. Growing up, Joe was plagued with asthma, rickets, and rheumatic fever. From a young age he studied exercise and athletics as a means to improve his own health. He became familiar with and embraced the Greek philosophy that the body and the mind were interconnected. He firmly believed that bad posture, inability to breathe, and sedentary lifestyle were the cause of modern societies' poor health. In 1925 he moved to New York City and opened his Body Contrology studio. He defined Contrology as the "integration of mind, body and spirit." Since his death in 1967 Joe's exercise method has been renamed Pilates.

Pilates is a mind–body workout based on six principles: centering, concentration, control, precision, breath, and flow. Each exercise and movement sequence embodies these six principles. Pilates is a low-impact exercise and can be easily modified through use of springs and various apparatus. It can be performed standing, supine, prone, or seated. Pilates integrates the trunk, pelvic and shoulder girdle, and lower and upper extremities while emphasizing proper breathing and alignment.

The practice of yoga can be traced back to 1000–500 BC. The word "yoga" translated into English means "union" and is thought to be the union of the mind, body, and spirit. Though intended to be predominately a spiritual practice, yoga has become a popular exercise in modern day.

Yoga is a series of poses, postures, and movements intended to improve strength, flexibility, and balance of the mind, body, and soul. Yoga integrates breathing and meditation into each pose and posture. Much like Pilates, yoga is a low impact exercise that can be easily modified through the use of props such as yoga blocks, straps, and mats.[3]

When practiced properly, yoga and Pilates are systems of movement that can increase core strength, balance, improve breathing patterns and mental awareness, and may alleviate pain. Many of the movements in both Pilates and yoga are easily modifiable for people at varying levels of strength and stability. Based on typical outcomes from practicing yoga or Pilates, it is logical to predict that yoga or Pilates could aid in reducing some of the symptoms of MS. In the following, we examine the research by symptoms studied that concludes that yoga and Pilates constitute an effective complimentary therapy for MS.

PAIN AND QOL

QoL is a broad concept, encompassing both physical and mental health components. Pain is one physical variable capable of affecting QoL, ability to work, and mental health. Pain is a commonly reported symptom for individuals with MS and manifestations include back pain, trigeminal neuralgia, painful spasms, extremity pain, and headache.

Yoga or Pilates may reduce pain through concentration, centering, breathing, visual imagery, and the reduction of fear avoidance. It is well established that pain is processed in the brain and chronic pain changes the makeup of the brain.[4] The yoga and Pilates principles of concentration, centering, and breathing allow the patient to focus on the movement rather than the pain itself. Both practices of yoga and Pilates incorporate visual imagery, which may help to improve pain outcomes in people with chronic pain.[5] Additionally, fear

avoidance has been found to be a factor in the development of chronic pain. Yoga and Pilates offer a safe and modifiable framework for movement that may decrease fear avoidance, thereby reducing chronic pain.[6] While no mechanism of how yoga and Pilates can reduce pain has been described, a handful of studies support the idea that yoga or Pilates reduce pain in patients with MS.

A study conducted by Doulatabad et al. in 2012 examined how pain and QoL were impacted by a 3-month intervention of pain-managing yoga techniques. In this clinical trial of 60 women with MS, the treatment group reported significantly lower pain ($p = .007$) and improved QoL ($p = .001$) following the intervention.[7] Based on this study, pain-managing yoga techniques enhanced QoL by decreasing pain in patients with MS.

A review published by Yamato et al. in 2014 examined studies that explored the Pilates method in patients with varying degrees of low back pain. While this is not a direct study of patients with MS, this review can help determine potential effectiveness of the Pilates method for patients with MS. This review examined 10 trials, which spanned over 500 participants.[8] The review concludes from the 10 trials it reports, that when compared to minimal intervention, Pilates reduces pain to a moderate degree.[8]

With only a small number of studies directly examining pain outcomes from yoga or Pilates, more controlled studies with large participant groups need to be conducted in order to best understand if yoga or Pilates can improve the pain symptom of MS. However, these few studies reviewed demonstrate that yoga or Pilates could in fact help regulate pain in patients with MS.

MENTAL HEALTH AND FATIGUE

The mental health component of MS includes depression, cognition, stress, and mental fatigue. Depression, experienced by up to 50% of patients with MS, can impact cognition, willingness for treatment, and suicidal intent.[9] Yoga or Pilates could impact depression in patients with MS through regulation of the vagus nerve or through the focus on the mind–body connection that is central to both yoga and Pilates.

There has been much research since 2000s investigating the vagus nerve and its impact on emotional health. Breathing is an integral aspect of Pilates and yoga practice. Breathing has been found to have a positive effect on the vagus nerve, which can influence emotions such as anxiety and depression.[10,11] While the links between the vagus nerve and emotional health are not fully understood, this research suggests that activities like yoga and Pilates may have an overall positive impact on mental health. As yoga seeks to connect the mind and body, it is entirely possible that yoga as a treatment could reduce depression and mental fatigue. Again, no mechanism of how yoga or Pilates may regulate mental health has been explained. However, there are multiple studies demonstrating the effectiveness of yoga or Pilates on improving the mental health in patients with MS.

Results of an 8-week yoga intervention targeting depression among women with MS revealed significantly lower levels of depression (using the Beck Depression Inventory) in the experimental group following the treatment ($n = 15$, $p < .05$), and no change for the 15 participants in the control group.[12] This study suggests that yoga reduces depression in patients with MS.

Another study that examined the impact of different forms of exercise on cognition in 24 patients with RRMS, found that participants showed significantly improved reaction time following treadmill walking, cycling, and yoga compared to resting.[13] Walking corresponded to the sole significant reduction in the effect of interfering stimuli on reaction time ($p = .04$). However, participants engaged in only 20 min of each exercise, so these results may not be applicable to a longer exercise regime.

In a study of a 6-week relaxation-based yoga intervention on a sample of chronically ill patients with cancer ($n = 10$) or MS ($n = 12$), both groups of patients reported significantly lower perceived stress ($p < .001$), with a greater effect for patients with MS,[14] supporting the idea that yoga improves mental health in patients with MS.

In additional studies, researchers examined the effect of an 8-week mindfulness-based intervention (MBI) using yoga on depression, anxiety, and fatigue in participants with RRMS or SPMS.[15] Participants who received the intervention ($n = 76$) showed improved levels of depression, anxiety, and fatigue ($p < .001$) compared to the control group ($n = 74$), and results remained significant at a 6-month follow-up. This study employed an intensive intervention with weekly group meetings and "homework" for the participants to practice individually. Again, this study demonstrates that yoga improves the mental health of patients with MS.

In a 6-month randomized control trial investigating yoga's use in MS management, Oken et al. found that yoga improved levels of fatigue compared to the control ($N = 57$, $p < .001$), although the level of improvement was not significantly different than a conventional aerobic exercise regimen.[16]

Razazian et al. conducted a study examining women. Patients were divided equally among the control group, an aquatic group and a yoga group (total $n = 54$). This study measured the impact of three Hatha yoga sessions a week (60 min each) on depression (measured using the BDI and a Likert scale) and fatigue (measured using a Fatigue Severity Score) in patients with MS. Yoga was compared to aquatic exercises. At the end of the 8-week study, both aquatic exercise and yoga significantly ($p < .001$) decreased depression and fatigue.[17]

However, not all studies are in agreement about the effects of yoga on mental health in patients with MS. A small study with participants between the ages 26–50, found a 17% increase in selective attention following the yoga intervention ($n=20$, $p=.015$), although outcomes for mood and fatigue were not significant.[18] Clearly more research is necessary to understand the impact of yoga on mental health and fatigue.

Together, these studies suggest that yoga reduces mental fatigue, stress, and depression in patients with MS. Some areas that remain to be explored include whether or not this reduction holds true for both men and women, and whether these results are observable in all types of MS, and if these benefits are potentially age dependent. Because Pilates utilizes the same mindfulness techniques as yoga, it is highly likely that Pilates would also reduce mental fatigue, stress, and depression, but such studies have yet to be reported.

MOBILITY, SPASTICITY, BALANCE, AND STRENGTH

Physical components of QoL that may be impacted by MS include spasticity, balance, and strength. Spasticity, prevalent in up to 80% of MS cases, may be manifested as muscle stiffness, muscle spasms, or cramping.[2] It may also negatively affect posture, gait, and mobility.

The mechanism for yoga or Pilates improving spasticity, balance, and strength are directly through the use of poses and postures in these exercises. In yoga and Pilates, focus on poses and fluid movement can help strengthen muscles and improve postural control thereby reducing spasticity. Various poses focus on balance and strength, thereby reducing those side effects of MS directly.

Velikonja et al. found that there was no significant improvement in spasticity following a yoga intervention for 20 patients with RRMS or progressive MS.[18] However, because the trial was uncontrolled, more evidence is needed to examine the effects of yoga on spasticity.

In a study conducted by Guner and Inanici, it was found that a bi-weekly yoga program over the course of 12 weeks yielded significant improvements in balance ($p=.027$), fatigue ($p=.012$), step length ($p=.007$), and walking speed ($p=.005$) in a group of eight participants with MS.[19] While these results demonstrate yoga can improve balance and fatigue, the sample size was small, and so these results require further replication.

Another study demonstrated that a 4-month yoga intervention yielded improvements with moderate effect sizes in balance ($n=24$, $p=.002$) and functional strength ($p<.001$) following the treatment,[20] again suggesting that yoga can improve balance and strength in patients with MS.

As of 2016, one of the larger sample size studies was performed by Hassanpour-Dehkordi in 2014. This study demonstrated that yoga was better than aerobic activity at reducing fatigue, muscle weakness, and increasing strength in patients with MS. In this study 90 patients were randomly assigned to a control group, aerobic activity group, or a yoga group for 12 weeks of study.[21] QoL questionnaires were compared before and after the study. No difference was seen between the groups at the start of the study. After 12 weeks, the mean QoL score of the yoga group had a higher QoL score than aerobic exercise group ($p=.07$). Yoga and aerobic exercise both had a higher QoL score than the control group ($p<.001$).[21]

Marandi et al. explored how Pilates impacted dynamic balance in patients with MS. Three groups, namely, control, aquatic exercise, and Pilates, were created from 57 patients. These patients were monitored for 12 weeks, exercised three times a week, 1 h per session. It was demonstrated that patients in the aquatic group and in the Pilates group significantly improved ($p=<.05$) the adjusted Timed Up and Got Test (TUGT), therefore Pilates did help with dynamic balance.[22]

A later study took this finding further and explored how Pilates impacts balance, mobility, and strength in patients with MS. This study took 26 patients divided into an experimental ($n=18$) and a control ($n=8$) group. The treatment program was 8 weeks. The experimental group practiced Pilates and the control group did breathing and active extremity exercise. Improvements in balance (TUGT), mobility (Berg Balance Scale), and upper and lower muscle strength (measured with a handheld dynamometer) were reported in the Pilates group ($p=<.05$) but not in the control group.[23]

More research is necessary to understand the long-term effects of yoga or Pilates on balance, strength, and mobility in patients with MS. While several of the studies have small participant numbers, they are in agreement with larger studies, which demonstrate that yoga or Pilates increase short-term balance, mobility, and strength in patients with MS, which improves QoL.

BLADDER CONTROL AND SEXUAL FUNCTION

Components of QoL that may be impacted by MS include bladder control and sexual function. Impaired sexual function and decreased satisfaction may occur with MS. Additionally, certain pharmacological therapeutics, such as selective serotonin reuptake inhibitors, may include sexual dysfunction as a side effect.[24]

Breathing exercises may be one of the mechanisms by which yoga and Pilates improve bladder control and sexual dysfunction in patients with MS. Breathing is one of the guiding principles in both Pilates and yoga.

Breathing problems have been linked with incontinence, lower back pain, and gastrointestinal issues. Incorporating movement with diaphragmatic breathing exercises has been found to reduce incontinence in children[27] and adults. Pilates and yoga are an alternative method to integrate the practice of conscious breathing with postural alignment and exercise.

A 2012 study tested the efficacy of yoga pelvic floor exercises and breathing techniques as a complementary method of treating neurogenic bladder dysfunction (NBD) in 11 patients with MS.[25] Ultrasound scanning revealed significantly reduced post-void residual urine volume ($p = .004$), frequency of micturition was reduced by 25% ($p = .001$), and improvements in self-reported measures of urogenital distress ($p = .006$) and incontinence impact ($p = .003$). This was not a randomized control trial, thus there was no control group.

There is evidence suggesting that yoga may be an effective method for improving sexual function and physical activity among women with MS.[26] A 3-month randomized control trial with a yoga intervention focusing on slow body movement, respiration, and concentration, reported participants who received the treatment reported improved levels of both physical activity and sexual satisfaction ($N = 60$, $p = .001$).

Yoga and Pilates have been utilized as treatments for both bladder control and sexual function. While evidence that yoga and Pilates address these symptoms in patients with MS has not been well examined, evidence in persons without MS concludes that yoga and Pilates can help increase bladder control and sexual function.

CONCLUSION

Traditionally, health care providers believed that it was appropriate to advise patients with MS to limit physical activity in order to reduce levels of fatigue, although this attitude has shifted due to experimental results suggesting that exercise is beneficial for patients with MS and may not adversely affect fatigue.[28] The literature suggests that there are not only physical benefits of exercise but also benefits for mental health. Opinions about the role of complementary and alternative medicine (CAM) therapies and MBIs in MS management have shifted.[29] Treatments such as yoga, cannabinoids, and acupuncture are becoming more widely endorsed.[24]

Yoga and Pilates are regarded as safe and relatively inexpensive, and may be more accessible for patients with spasticity and impaired mobility than other forms of exercise. Yoga and Pilates may be more practical for some MS patients due to modification of poses with props, chairs, and cushions. In spite of evidence suggesting that individuals with MS may benefit from yoga or Pilates as a complementary therapy, there are inconsistencies and limitations in experimental results on this topic.

A major limitation of studies on yoga or Pilates and MS is that it is impossible to blind participants to the intervention, and since they are aware of the treatment, some participants may have an expectation of improvement, which could influence results. Additionally, it is difficult to discern whether improvement following interventions was due to the treatment or due to confounding variables. For instance, some benefits may be conferred through social interactions during group exercise or the instructor serving as an encouraging mentor. A further limitation in previous study designs is the frequency of small sample sizes and a lack of random sampling.

It is difficult to study depression as a variable because some symptoms associated with MS, such as mental fatigue and trouble sleeping, may present themselves as manifestations of depression. Also, previous studies have not been rigorous in their methodology to study this outcome or even included clinically depressed participants in their sample. Some studies may evaluate outcome on depressed mood, but not have thorough psychological assessment of depression before and after the intervention.[9]

There is little information about how the impact of yoga or Pilates may differ between the four courses of MS. Also, interventions varied in duration between few weeks and several months, but little is known about the possible impact of yoga or Pilates as a long-term method of symptom management.

It is unclear how the frequency, duration, or intensity yoga or Pilates practice may impact symptoms experienced by patients. Future research might investigate whether a certain regimen might be more effective for improving patient outcome. Also, very few trials examining yoga, Pilates, and MS have conducted follow-up studies. Future research should include follow-up measurements to investigate if outcomes remained significant or if variables had returned to their baseline values. Furthermore, longitudinal research should be done on how yoga or Pilates may impact symptom management in the different courses of MS when yoga or Pilates is incorporated into a treatment plan from early stages in the disease.

Even with the limitations in these studies, the overwhelming evidence supports yoga or Pilates as a complementary treatment for patients with MS. The review of controlled studies discussed here demonstrates that yoga reduces pain in patients with MS, and data suggest that Pilates could have the same result. Yoga and Pilates increase balance, mobility, and strength in patients with MS. Yoga reduced fatigue in patients with MS and again, it is suggested that Pilates could do the same. Yoga reduces mental depression and stress in patients with MS. Finally, a handful of

studies have suggested that yoga and Pilates could increase bladder control and sexual function in patients with MS, although more research needs to be completed in order to draw a solid conclusion. While no treatment can address all of the symptoms of MS, evidence reviewed here suggests that Pilates or yoga can address many of the symptoms of MS and should be highly considered as a complementary treatment for MS.

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CHAPTER

20

Exercise in Prevention and Treatment of Multiple Sclerosis

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OUTLINE

PHYSICAL EXERCISE

Multiple sclerosis (MS) is a chronic, immune-mediated disease of the central nervous system that is characterized by inflammation, demyelination, axonal degeneration, and gliosis. The progressive nature of MS is characterized by an increase in neurological impairments, activity limitations, and participation restrictions.[1]

People with MS typically develop symptoms in their late 20s, experiencing visual and sensory disturbances, limb weakness, gait problems, and bladder and bowel symptoms. They may initially have partial recovery, but over time develop progressive disability.[2] The deterioration of physical condition may cause a variety of complications and have a significant impact on the individual's motor autonomy, and consequently their quality of life (QoL).[3] The Kurtzke Expanded Disability Status Scale (EDSS) is a method of quantifying disability in MS. The EDSS is divided into 20 half-points ranging from 0 (normal) to 10 (death due to MS).[4] The objective of the reeducation depends on the score in EDSS. At the beginning of the disease, the therapeutic education has a fundamental place. The principal points are the control of fatigue, physical activity, vesical sphincter disorders, and so on. For EDSS score lower than 6, the program of rehabilitation depends on the symptomatology: retraining to the effort and balance, muscular strengthening, and so on. For top EDSS scores, the base of the program of rehabilitation is compensation: technical and human assistance and prevention of complications. This chapter centers on patients with scores lower than 5 in EDSS.[5]

There is evidence that people with MS engage in less moderate to vigorous physical activity, have lower peak aerobic capacity, and have lower walking endurance performance compared with healthy controls.[6]

Many of these problems are complex and need individual assessment and management strategies. These assessments and treatments need to be carried out by health care professionals with appropriate expertise in rehabilitation and MS.[2]

The best option to improve physical skills in patients with MS is physical exercise in comparison with others types of treatments (medication, no exercise, education, and so on).[7,8]

The aim of this chapter is to describe and discuss the available recommendations about various modalities of physical therapy treatment involving exercise training in patients with MS who have a deterioration of their physical skills.

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The selected documents were classified according to grades of recommendation of the DUODECIM (Finnish Medical Society Duodecim).

Fifteen articles were found, of them two are systematic reviews (one A recommendation grade and one C recommendation grade). The remaining 13 articles comprised of three A recommendation grade, four B recommendation grade, and six C recommendation grade. The findings about grades of recommendation about various modalities physical therapy treatment are shown in Table 20.1. In addition to this, we describe main outcomes measures regarding what could be used in clinical practice in patients with MS.

Most of articles listed in the table showed a significant effect on fatigue in favor of exercise therapy.[7–9,14,15,17,20,21] Another complication is balance. Lots of articles showed improvements in balance.[10–13,17,21,22] Gait was analyzed in various research studies (speed, walking mobility, walking distances).[10–17,19,21,22] Some articles showed effect of strength[16,18,19,21]; however, an article demonstrated that the addition of eccentric training to standard exercises did not result in significantly greater lower extremity strength gains.[9]

An article showed significant improvement in aerobic capacity,[20] and another in spasticity[17] as a result of these interventions.

Accordingly, it can be concluded that the improvements observed in physical condition are associated with improved QoL.[15,17,19]

It was observed that the most common outcome measure to evaluate fatigue was the Fatigue Severity Scale (FSS)[7,9] in Table 20.1. Another test for mobility assessment was Timed Up and Go (TUG).[9–11] Similarly, the Six Spot Step Test[12] and Activities-specific Balance Confidence (ABC) Scale[10,13,22] were common outcome measures to evaluate balance. Furthermore, a common assessment of Impact of Quality of Life was the Multiple Sclerosis Quality of Life Survey (MSQoL-54).[10,15]

Based on our analyses, the best option to improve physical skills in patients with MS is physical exercise in comparison with other types treatments (medication, no exercise, education, and so on).[7,8]

The most common effects of physical exercise include:

- decreased fatigue,[7,8,14]
- improved balance,[9,10,12,13]
- recovery of mobility,[13]
- improved gait speed,[10]
- walking mobility.[11,14]

The exercise training session should be supervised by a rehabilitation specialist and/or physiotherapist with expertise in MS. Treatment should take into account individual needs and preferences.[2] Based on our analysis, recommendations about exercise training session include the following:

- moderately intense[11];
- intermittent: rest breaks[14];
- progressive, maximum 60 min per session[11];
- 3 days per week over an 8-week period[11];
- divide into aerobic exercise, resistance exercise, and balance activities.[11]

Patients with MS, in general, present fatigue, motor weakness, spasticity, poor mobility disability and balance,[23] bowel and bladder incontinence, and sexual dysfunction.[24] Considering those symptoms, physical therapy treatment, including exercise- and nonexercise-based interventions has been indicated for all those patients.

The majority of evidence available in the literature about exercise suggests that such training is effective for improving fatigue. In order to reach those benefits, exercise programs should preferably include endurance and resistance exercises performed at medium intensity, following a frequency of, at least, 3 times a week during 5–12 weeks.

Nonexercise–based interventions, such as bronchial hygiene techniques and breathing retraining also present positive effects in patients with MS. In some cases it is recommended that these interventions should be combined with exercise training.

Respiratory Muscle Training

Respiratory complications have been recognized as the major cause of morbidity and mortality in individuals with advanced MS,[25] as nearly half of patients die from pulmonary complications, such as aspiration pneumonia.[26] Respiratory muscle fatigue is observed in later stages of MS, leading to disability and ultimately mortality.[27]

There is a direct connection between respiratory muscle function and the level of a patient's disability.[25]

MS-related respiratory muscle dysfunction involves both the inspiratory and expiratory muscles; however, expiratory muscle strength deteriorates earlier in the disease course than inspiratory muscle strength.[27]

Respiratory muscles are weakened and pulmonary function is affected even in the early phase of MS. Ambulatory patients with MS who have a higher level of disability have lower pulmonary function, respiratory muscle strength, and functional capacity than less disabled ones and controls.[26]

A systematic review covered training protocols that were carried out for 10 weeks to 3 months at a frequency of 7 day/week with one or two daily sessions consisting of three sets of 10 or 15 repetitions per set at an intensity of 10–60% of the subject's maximum expiratory pressure. Patients with MS showed changes in maximum inspiratory and expiratory pressures after respiratory muscle training.[25]

TABLE 20.1 Types of Physical Therapy Treatment Interventions and Main Outcomes Measures in Patients With MS

Year and References	Intervention	Outcomes
2014 Asano [7]	Exercise	FSS
	Education	MFIS
	Medication	FIS
2015 Heine[8]	Exercise therapy	Expanded disability status scale
	No-exercise therapy	
2016 Hayes[9]	STAND group: received standard exercises	Strength assessment: $p = .74$
	RENEW group: 3x/w for 12 w	Mobility assessment: TUG $p = .81$; TMWSS $p = .96$; TMWM $p = .15$; S-A $p = .02$; S-D $p = .02$; 6MWT $p = .89$
		Fatigue assessment: FSS $p < .001$
2012 Jackson[10]	Kickboxing program: 3x/w for 5w, each class lasting 1 h	Mini-BESTest: $p = .006$; ES = 0.60
		GS: $p = .003$; ES = 0.63
		Fast GS: $p = .01$; ES = 0.52
		TUG: $p = .003$; ES = 0.63
		BBS: $p = .164$
		MSQoL-54: PH composite ($p = .110$) and MH composite ($p = .213$)
		DGI: $p = .03$
		ABC scale $p = .042$
2012 Motl[11]	3x/w for 8 w	MSWS-12 ($p = .03$, ES = 0.56)
	Each training session: aerobic, resistance, and balance activities	TFW ($p = .004$, ES = 0.90)
		TUG ($p = .01$, ES = 0.72)
		FAP score ($p = .02$, ES = 0.65)
2013 Marandi [12]	Pilates exercise group	SSS test ($p < .05$)
	Aquatic training group	
	Control group	TUG ($p < .05$)
	3x/w for 12 w, 1 h for each session	
2012 Freeman [13]	Pilates group	MSWS-12
	Standardized exercise program group	Functional reach
	Relaxation program group	NRS to determine "difficulty in carrying a drink when walking"
		ABC scale: 95% confidence intervals will be produced for the treatment effect
2015 Karpatkin[14]	Continuous group	Walking distances ($p = .005$)
	Intermittent group	VAS-F ($p = .036$)
2014 Najafidoulatabad[15]	Yoga group: 8 sessions/month for 3 months; 60–90 min at each session	MSQoL-54
	Control group	Physical activities ($p = .001$)
2016 Mandelbaum	Dance intervention: 2x/w for 4 w, each class	TUG ($p = .02$)
	1 h	DGI ($p = .09$)
		ABC scale ($p = .09$)
		MSWS-12 ($p = .05$)

Continued

TABLE 20.1 Types of Physical Therapy Treatment Interventions and Main Outcomes Measures in Patients With MS—cont'd

Year and References	Intervention	Outcomes
2012 Claerbout[16]	Control group	Muscle strength (tibialis anterior, quadriceps, hamstrings, and gluteus medius)
	WBV-full group	TUG test: $p < .05$
	WBV-light group	BBS: $p < .01$
		3MWT $p < .001$
2013 Tarakci[17]	Control group: no intervention	BBS ($p < .01$)
	Exercise group: 3x/w for 12 sw, each session	10MWT ($p < .01$)
	1 h	10 steps climbing test ($p < .01$)
		Modified Ashworth Scale ($p < .01$)
		FSS ($p < .01$)
		MSQoL-54 ($p < .01$)
2013 Dalgas[18]	Control group	EMG activity: vastus lateralis
	Exercise group: 2x/w for 12 w	($p < .001$), rectus femoris ($p < .001$), and semitendinosus ($p = .02$)
2013 Carter[19]	Control group: usual care	Progression of exercise volume, intensity, and training impulse ($p = .050$)
	Pragmatic exercise group: 3x/w for 10 w, each class 1 h (aerobic exercise, flexibility, balance, and core work)	MSQoL-54 ($p = .003$)
2014 Schmidt[20]	3x/w for 12 months, each class 30 min	VO_2 peak ($p = .03$)
		FSS ($p < .03$)
2012 Learmonth[21]	Intervention group: 2x/w for 12 w, each	TFW (no statistically significant)
	Session 1 h (mobility, balance, and resistance exercises)	Physical activity ($p = .005$)
	Control group	Balance ($p = .013$)

ABC scale, activities-specific balance confidence scale; *BBS*, Berg balance scale, *Continuous group*, participants walked for 6 min without rest breaks; *DGI*, dynamic gait index; *EMG*, electromyographical; *ES*, effect size; *FAP*, functional ambulation profile; *FIS*, fatigue impact scale; *FSS*, fatigue severity scale; *GS*, gait speed; *Intermittent group*, participants walked three 2-min increments with 2-min seated rests between each increment; *MFIS*, modified fatigue impact scale; *MH*, mental health; *MSQoL-54*, multiple sclerosis quality of life survey; *MSWS-12*, multiple sclerosis walking scale-12; *NRS*, numerical rating scale; *PH*, physical health; *RENEW group*, received standard exercise and high-intensity lower extremity resistance exercise via negative, eccentrically induced work; *S-A*, time to ascend; *S-D*, time to descend; *SSS Test*, six spot step test; *TFW*, timed 25-ft walk; *TMWM*, 10-m walk test maximal-pace; *TMWSS*, 10-m walk test at both a self-selected; *TUG*, timed up and go; *VAS-F*, visual analog scale of fatigue; *VO_2 peak*, peak oxygen consumption; *w*, week; *WBV*, whole-body vibration; *WBV-light group*, same exercises of WBV-full group on a foam mat of 10 cm thickness put on top of the standard mat; *WBV-full group*, 10 sessions of WBV exercises over a period of 3 weeks. Vertical vibration with amplitude of 1.6 mm and at a frequency of 30–40 Hz; *x*, day; *3MWT*, 3-minute walk test; *6MWT*, 6-minute walk test; *10MWT*, 10-minute walk test.

Another systematic review showed that there is evidence that RMT, using pressure threshold devices, improves a number of respiratory function parameters in patients with MS. There were significant changes in inspiratory muscle strength and endurance, perception of dyspnea, and pulmonary volumes and capacities when the training intervention consisted of at least 30 inspiratory maneuvers each day, 6 days a week, for 10 weeks, at 30% of maximal inspiratory pressure (MIP), with progressive increased load. Moreover, there were significant changes in expiratory muscle strength and cough function when the training intervention consisted of at least 25 expiratory maneuvers each day, 5 days a week, for 4 weeks, at a minimum load of 40% of maximal expiratory pressure (MEP) with progressive increased load.[28]

A research study showed that RMT program improved inspiratory and expiratory muscle strength and reduced fatigue in patients with mild to moderate MS. Training was a 5-week combined progressive resistance RMT program, 3 day/week, 30 min per session.[27]

Sniff nasal inspiratory pressure (SNIP) has become increasingly popular for the measurement of inspiratory muscle strength in both neuromuscular and skeletal disorders, due to its simplicity and utility in the laboratory and in clinical environment.[29] SNIP test may help in establishing inspiratory muscle weakness.[30]

Therapeutic Aquatic Exercise Intervention

A noteworthy modality of physical exercise treatment is aquatic training. It is effective for improving flexibility, range of motion, cardiovascular endurance, fatigue level, muscle strength, mobility function (including gait and balance), QoL, and psychological well-being.[31]

The exercise program for the aquatic training included a series of water activities in the water for a period of 12 weeks and three sessions per week and 1 h per session. The plan for each session started with a 10 min of walking in the water followed by stretching, power, and endurance activities. In the final 10 min of each session, some cool-down and balance movements were performed.[12]

Further research showed that aquatic exercise enhances aspects of MS patients' QoL. In one study, patients took part in 1 h sessions 3 days a week, for 1 month. The temperature of the water was 80–84°F (30–31°C) to reduce the stiffness of the muscles and allow the patient to move easier.[32]

Also, another research study suggested that aquatic exercise training is effective in improving fatigue and QoL in patients with MS. The intervention consisted of 8 weeks supervised aquatic exercise in a swimming pool (3 times a week, each session lasting 60 min).[33]

Fitness Kickboxing Intervention

An effective modality of physical exercise is fitness kickboxing. This intervention is a nontraditional form of exercise that has gained popularity since 2000s, and improves balance and mobility. The kickboxing program was performed 3 times weekly for 5 weeks, each class lasting 1 h. At the beginning and end of each session, participants performed 5–10 min of warm-up and cooldown activities consisting of both seated and standing large amplitude rhythmic movement of the trunk and limbs, diaphragmatic breathing, and stretching.

Overall, the best option to improve physical skills may be physical exercise that is personalized, moderately intense, intermittent, and progressive. There are many modalities of training programs; physiotherapists should take into account individual needs and preferences of patients.

Another option to improve balance and mobility of people with MS is Pilates. In one approach, the exercise training program for Pilates included a series of exercise activities for a period of 12 weeks and three sessions per week and 1 h per session. The plan for each session started with 10 min of simple stretching movements to warm up followed by the main part of the exercise plan including stretching, power, muscular nervous coordination, and balance moves. In the final 10 min of each session, some cooldown stretching movements were performed.[12] Another program for the Pilates included 12 half-hour individualized face-to-face training sessions, delivered over 12 weeks, plus an individualized 15 min daily home exercise program. The exercises were designed to challenge trunk control progressively by adding a gradually increasing limb load, and/or reducing the base of support. Stretching will be undertaken prior to or during these exercises to address any malalignments.[13]

One of the recognized aims of Pilates is improved control over the core muscles, strengthening them. Changes in these structures may cause pelvic floor dysfunction.[34] Yoga training improved sexual satisfaction.[15]

Kegel exercises improve QoL, overactive bladder, and perineal contraction.[35] However, there is evidence supporting different electrostimulation techniques, which have been widely proposed to treat urinary disorders and consistent results have been reported.[36]

The following paragraphs examine the recommendations for the use of electrotherapy treatment for lower urinary tract symptoms (LUTSs) in patients with MS.

LOWER URINARY TRACT SYMPTOMS IN PEOPLE WITH MS

LUTSs are common, occurring in 50–80% of patients with MS.[37]

Urinary urgency is the most common symptom in 65% of patients with MS and urinary tract infection was the most common complication in 15% of subjects.[37] Urodynamic evaluation showed neurogenic detrusor overactivity (NDO) in 27%, detrusor sphincter dyssynergia (DSD) in 25%, and an underactive detrusor in 6% of subjects.[37] Symptoms include dysuria, frequency, urgency, suprapubic pain, fever, chills, flank pain, and hematuria.[38]

The treatment of urinary incontinence is important for social rehabilitation of the patient and thus contributes substantially to the individual's QoL.[39] The most common treatment comprises pharmacotherapy and/or intermittent self-catheterization. However, in some patients anticholinergics have troublesome side effects and intermittent self-catheterization requires motivation and is not always possible for physical or psychological reasons.[40] In 2008, intravesical injection of botulinum toxin was shown to be beneficial for these issues.[40] Different electrostimulation techniques have also been widely proposed to treat urinary disorders and consistent results have been reported.[36]

The aim of this chapter was to examine the recommendations for the use of electrotherapy treatment for LUTSs in patients with MS.

The selected documents were classified according to grades of recommendation of the DUODECIM.

Twelve articles were found (two A recommendation grade,[40,41] one B recommendation grade,[42] and nine C recommendation grade[36,43–46]). Six articles used posterior tibial nerve stimulation (PTNS),[36,42–46] three used this treatment for NDO,[36,44,45] and one of them did not demonstrate statistical significance.[44] PNTS is an effective, safe, and well-tolerated treatment for LUTS[43] and PNTS was associated with a sensory response, either alone or in combination with a motor response.[46] Another article compared the efficacy of pelvic floor muscle training versus PNTS. Both therapies similarly improved symptoms related to urgency in MS patients with mild disability. Result are shown in Table 20.2.

Five of the articles reviewed used the same parameters (200 μs, 20 Hz, 1–5 mA, intensity under the threshold of motor contraction, 1.5 times the threshold of evoking). Electrical stimulation was applied by using charge-compensated 200-μs pulses with a pulse rate of 20 Hz. Intensity level was then chosen below the threshold determining motor contraction. The stimulation amplitude was set at the maximum tolerable level according to the subject under investigation, which was usually 1.5 times the threshold for evoking plantar flexion of the toes and/or toe fanning (range: 1–5 mA).[36,43,44]

One study used rectangular biphasic current with pulse duration of 220 μs, frequency 10 Hz, and ramp up 20 s by ramp down for 4 s. The intensity was just below the threshold determining motor contraction.

Sacral nerve modulation (SNM) was a good option in the treatment of lower urinary tract dysfunction,[47,48] but the poor results observed suggest avoiding this therapy in mixed symptoms and in cases of advanced disability.[47] Urinary retention due to detrusor underactivity was not a good indication for SNM; it should be offered to MS patients with refractory urgency urinary incontinence and urinary retention due to DSD.[48] SNM improved frequency, urgency, number of pads, residual volumes, number of catheterizations, the voided volumes, and QoL score.[47,48]

Another therapy was transcutaneous electrical stimulation of the dorsal penile/clitoral nerve (DPN) for management of NDO. One research study suggested

TABLE 20.2 Main Outcomes Measures and Results PTNS Treatment in Patients With MS

Year and References	Result	ES	*p*
2009 Kabay[36]	1st IDCV	162	<.05
	MCC	79	<.05
	OBQ		<.05
	UE, UI episode, DF episode, nocturia, and Pad test:P		<.001
2014 Gaspard[42]	UQolQ score		.197
	OBQ score		.532
	Frequency of UE	−1.55	.788
2011 Gobbi[43]	DF	−3	.04
	Nocturia	−2	.002
	Post-micturition residual	−55	.02
	VV	43	.03
	KHQolQ		<.05
	UI	−3	.6
2007 Fjorback[44]	PTNS failed to SDC and no reduction of urgency		
	PP during PTNS was similar to PP during contraction in the control fillings	11	.48
2008 Kabay[45]	1st IDCV	83.5	<.001
	MCC	91.5	<.001
2014 Zecca[46]	Sensory response		<.001
	Motor response		.08
	Sensory response in combination with a motor response		.001

DF, daytime frequency; *ES*, effect size; *KHQolQ*, king's health QoL questionnaire; *MCC*, maximum cystometric capacity; *OBQ*, overactive bladder questionnaire; *PP*, peak pressure; *PTNS*, posterior tibial nerve stimulation; *SDC*, suppress detrusor contraction; *UE*, urgency episode; *UI*, urinary incontinence; *UQolQ*, urinary quality of life questionnaire; *VV*, voided volume; *1st IDCV*, volume at the first involuntary detrusor contraction.

that involuntary detrusor contractions could effectively be inhibited as well as increased bladder capacity and reduced number of incontinence episodes.[49] Although another study suggested that DPN was not effective to suppress detrusor contraction,[50] suggesting that further research will be necessary on this topic.

The articles with higher recommendation grade demonstrated that the addition of electrical stimulation to a program of pelvic floor muscle training and electromyography biofeedback induced several improvements in lower urinary tract dysfunction. The mean numbers of incontinence episodes and pad weight were both reduced. Statistically superior improvement was also observed for voided volume and post-void residual.[40,41]

There was heterogeneity in the outcomes measure. The most common outcome measures were number of leakage episodes, frequency, residual volumes,[36,40,43–50] volume at the first involuntary detrusor contraction and maximum cystometric capacity.[36,45] Some common questionnaires were King's Health QoL questionnaire,[43,47,48] Urinary QoL questionnaire,[42] Incontinence Impact Questionnaire (IIQ), and Urogenital Distress Inventory (UDI), International Prostate Symptom Score (IPSS9), perception of bladder condition (PPBC)[40] questionnaire, and Overactive Bladder Questionnaire (OAB-q).[36,40]

To sum up, there was heterogeneity in the results of various researches. The addition of active neuromuscular electrical stimulation to a program of pelvic floor muscle training and electromyography biofeedback should be considered as a first-line option in alleviating some of the symptoms of lower urinary tract dysfunction associated with MS.

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CHAPTER

21

Physical Activity and Health Promotion for People With Multiple Sclerosis: Implementing Activities in the Community

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OUTLINE

Historically, physical activity has been a controversial topic for individuals with multiple sclerosis (MS), since it was thought to increase fatigue and reduce the ability to perform activities of daily living.[1] Unknowing the long-term effects of the Uhthoff phenomenon, which is the temporary worsening of symptoms due to elevation in body temperature, contributed to the rationale, and the subsequent recommendation, for individuals with MS to avoid physical activity.[2] Unfortunately, previous "resting" prescriptions along with personal and environmental barriers led individuals with MS to a more sedentary lifestyle compared to the general population. As with any sedentary population, limited movement can lead to higher rates of chronic conditions (e.g., obesity, heart disease, and diabetes) and result in greater muscular weakness, fatigue, and overall deconditioning (USDHHS, 2008). In response, research during early 2010 shifted focus from nonprescription of physical activity to examining and promoting the known health benefits of physical activity, particularly, the unique benefits of physical activity for persons with MS. In the process of debunking previous assumptions that physical activity increases the risk of MS progression,[3] current research is now more focused on the appropriate dose or intensity of physical activity to optimize benefits for this population.[4,5]

Evidence suggests that physical activity helps to decrease secondary conditions, but it also has other benefits unique for persons with MS, many of which surround symptom management. These benefits include managing the effects on fatigue, spasticity, mobility, depression, and pain.[6–8] For example, physical activity is now considered an effective tool for reducing depression in the early stages of MS, as well as a mechanism toward

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a better outlook on life, despite a progressive and degenerative disability.

Embedding physical activity practice and health-promoting activities into the everyday lives of individuals with MS has far-reaching and known benefits. This chapter outlines the inclusion of persons with disabilities within public health and health promotion efforts from an evidence-based perspective, while providing details on health-promoting programs and physical activities for individuals with MS. We provide evidence and practical tips on implementing community-based physical activity and health promotion programs for individuals with MS and address a number of questions, such as "How do I prescribe physical activity to an individual with MS?" "What barriers do persons with MS face when engaging in physical activity?" "What physical activity programs and activities can be implemented in the community?" and "How can I encourage or promote physical activity in the community?"

INCLUSION OF DISABILITY IN PUBLIC HEALTH PRACTICE

People with disabilities, including persons with MS, have historically been excluded from public health efforts, making this one of the largest underserved populations with evident health disparities.[9] Specifically, those with disabilities experience inequity-based disparities in health outcomes, which negatively impact their quality of life (QOL) and community participation.[10] This inequity was rooted in the traditional public health philosophy, and therefore, the mindset of public health professionals did not focus on the health and wellness of persons currently living with a disability, but rather solely on preventing disease and disability.[11] Thus, for many years a negative relationship existed between individuals with disabilities, including individuals with MS, and professionals in public health. However, since mid-2000s health promotion initiatives and this relationship have improved dramatically. In 2007, the American Public Health Association established a "Disability Section," a step toward more inclusion, as it relates to public health and individuals with disabilities. More importantly, since mid-2000s, public health initiatives have shifted from preventing disability toward addressing health disparities and preventing secondary conditions among individuals with disabilities.[12,13] Secondary conditions are conditions that a person with a preexisting disability experience at higher rates than the general population and are generally regarded as preventable, such as chronic disease, obesity, and depression.[14] For persons with MS, secondary conditions are not directly related to an individual's disability, like primary conditions of muscle weakness, fatigue, speech problems, and limited mobility, but rather are preventable when the proper supports are in place.

To reduce secondary conditions and health disparities, and to promote symptom management, health promotion efforts are needed for persons with MS. Health promotion is defined as the process of enabling people to increase control over and improve their overall health.[9] This includes the promotion of a balanced state of physical, mental, and social well-being, thus focused on the pillars of health, and not just on physical health (i.e., traditional focus). For those with MS, outcomes of successful health promotion programs and the adoption of health-promoting behaviors encompass improving personal skills as well as creating supportive inclusive environments.[9] This includes, but is not limited to, accessible facilities, inclusive programs, easy access to and from programs (via transportation and otherwise), and trained and familiar staff. The public health shift in philosophy and attention to reduce disparities have opened the doors for disability and health researchers to develop and implement health promotion programs within the community for persons with preexisting conditions, such as MS.

Health Promotion for Persons With Multiple Sclerosis

Despite increases in public health efforts, health promotion programming for people with disabilities is still lacking with a clear need for further dissemination and reach of programs.[11,15–17] By way of illustration, Dixon-Ibarra and colleagues (2014) conducted a structured audit of the physical activity literature for individuals with MS using the behavioral epidemiological framework. They found that less than 10% of research efforts ($n = 13$ studies) discussed the design, implementation, and/or evaluation of physical activity promotion programs. The need to increase health promotion programs for persons with disabilities is further addressed in multiple public health initiatives, including Healthy People 2020, the Surgeon General report—The Surgeon Call to Action to Improve the Health and Wellness of Persons with Disabilities (2005)—and Vital Signs (2014), which highlights the lack of physical activity and high rates of chronic disease among persons with disabilities.[18] Moreover, the Centers for Disease Control and Prevention (CDC) as of 2016 funds 18 state disability and health programs and five National Public Health Practice and Resource Centers to improve the health and wellness of persons with disabilities. Many of these offices on disability and health provide access to physical activity programs or resources to promote healthy lifestyles for those with disabilities (resources for these centers can be found at: http://www.cdc.gov/ncbddd/disabilityandhealth/programs.html).

Physical Activity Health Promotion

Physical activity is any bodily movement produced by the contraction of skeletal muscle that increases energy expenditure above the basal level (CDC, 2016). Exercise, on the other hand, is a subcategory of physical activity that is planned, structured, repetitive, and purposeful in the sense that the improvement or maintenance of one of more components of physical fitness is the objective (cardiorespiratory endurance [aerobic power], skeletal muscle endurance, skeletal muscle strength, skeletal muscle power, flexibility, balance, speed of movement, reaction time, body composition, and so on). In short, physical activity encompasses exercise, but physical activity has broader scope and may include increasing daily energy expenditure (e.g., parking further away, walking, performing housework, and other unstructured activity resulting in body movement). At the same time, sedentary behaviors should also be emphasized in the promotion of health, as prolonged sitting has independent risks for negative health outcomes. Studies have demonstrated prolonged sitting is associated with premature mortality,[19–21] metabolic syndrome, cardiovascular disease, diabetes, cancer,[22–25] and obesity.[22,24] Fortunately, simply moving and taking breaks in sedentary behavior (e.g., standing up) has been associated with healthier metabolic profiles, triglyceride levels, 2-h plasma glucose levels, reduced upper back and neck pain, and improved mood.[23]

Physical activity research for persons with MS has consistently shown this population to be less active, and with higher amounts of sedentary activity compared to the general population.[26] The differences in activity levels may, in part, be due to the appropriateness of recommended physical activity guidelines. For example, physical activity guidelines for the general population suggest about 150 min per week of moderate to vigorous physical activity (CDC, 2016). This type of physical activity (moderate to vigorous physical activity) typically means that an individual is sweating, with an increased heart rate. Depending on severity and symptoms, many persons with MS will not meet recommended guidelines, however, increasing daily activity from sedentary to light is achievable and will also impact health. This might look like walking to the mailbox, a short walk around the block, bringing the groceries in from the car, to name a few examples.

Participation in physical activity has not been shown to increase or decrease MS progression. Nevertheless, in respect to managing symptoms of MS, physical activity has resulted in decreased muscular fatigue, weakness, contractures and spasticity, improved bowel and bladder function, decreased swelling and edema, reduced risk for diabetes and heart disease, increased muscle strength and endurance, more effective weight management, greater independence, and decreased stress and depression.

Empirical research has identified several forms of physical activities that have demonstrated a variety of physical, emotional, and psychological benefits to individuals with MS. These physical activities include but are not limited to, yoga,[27–30] Pilates,[31–33] aquatics,[34–36] endurance training,[35,36] walking,[37] aerobic exercise,[38] physiotherapy/physical therapy,[39] strength and cardiorespiratory training[40] (Ponichtera-Mulcare, 1993), and resistance training.[41]

A growing number of individuals have turned to mind–body therapies—yoga, meditation, relaxation techniques, breath work, visual imagery, hypnotherapy, and biofeedback—to manage their symptoms of MS.[42–49] One mind–body therapy that has received considerable interest and attention is the practice of yoga. The various forms and practice of yoga involve the combination of "muscular activity and an internally directed mindful focus on the awareness of the self, the breath, and energy."[50] The following four basic principles underlie the practice and teachings of yoga: (1) [the] human body is a holistic entity comprised of various interrelated dimensions inseparable from one another and the health or illness of one dimension affects the other dimensions; (2) [individuals] and their needs are unique and therefore must be approached in a way that acknowledges this individuality and their practice must be tailored accordingly; (3) [yoga] is self-empowering; the student is his or her own healer; and (4) [the] quality and state of an individual's mind is crucial to healing.

Now recognized in the Western world as a holistic and comprehensive approach to health, yoga is currently classified as a form of complementary and alternative medicine (CAM) by the National Institutes of Health and integrates the physical, mental, and spiritual components of an individual in order to improve health and well-being.[50] Since mid-2000s, research has demonstrated a variety of therapeutic benefits associated with the practice of yoga in treating many illnesses, chronic diseases, and disabilities, such as high blood pressure,[51] fatigue and mood,[52,53] depression, and respiratory function,[45] as well as increased QOL,[54] improved flexibility,[50] and increased physical, emotional, and spiritual wellness.[55] Specific to persons with MS, and compared to other exercise interventions, research has shown yoga to be as effective in improving both patient-reported and physician-rated outcomes.[27] Yoga is considered safe, relatively inexpensive, and in light of other forms of exercise, may actually be more accessible for individuals with spasticity and impaired mobility.[28] Furthermore, improvements have been evidenced in muscle tone, strength, psychological well-being, and confidence[56]; fatigue, pain, and psychosocial status[30]; balance, step length, and walking speed[29]; and sexual function.[57]

Therefore, by participating in health-promoting behaviors (e.g., physical activity), individuals with MS have opportunities to prevent and manage symptoms associated with their disability, improve their lifestyles, and increase access to their physical and social environments, which will ultimately improve their QOL.[11]

Factors Influencing Physical Activity Promotion and Community Participation

The year 2015 marked the 25th anniversary of the Americans with Disabilities Act of 1990 (ADA), a civil rights law focused on full inclusion and participation.[58] This hallmark legislation provided clear guidelines for accessibility as it relates to individuals with disabilities. This includes, reasonable accommodations in all areas of life covering, but not limited to, public transportation, public building access (e.g., fitness facilities, yoga studios, and community spaces), parking accommodations, and restroom facility accessibility. In short, the ADA prohibits discrimination against persons with disabilities on the basis of employment, state and local government, public accommodations, commercial facilities, transportation, and telecommunications. Thus, community spaces, including spaces where health promotion activities may take place, and community fitness facilities, which are open to the public, need to be accessible and display reasonable accommodations for individuals with disabilities. Although many businesses and community spaces attempt to be "accessible," there are often aspects of accessibility that are missed or differ considerably depending on an individual's disability (e.g., curb cuts provide wheelchair accessibility, but some individuals with visual impairments depend on curbs to guide their environment). Barriers to physical activity include barriers to the built and natural environments, economic issues, emotional and psychological barriers, equipment, issues related to the interpretation of guidelines, codes, regulations, and law, professional knowledge, education and training, and multiple levels of policies and procedures.[59]

Specific barriers to physical activity participation for individuals with MS include personal factors, the environment, cost, equipment, and existing policies. Personal barriers include the type of MS (e.g., relapsing remitting, secondary progressing, primary progressive, and progressive relapsing),[60,61] age, severity of disability,[7,62] functional limitations,[63] use of a cane for ambulation,[60] severity of MS symptoms,[64] fatigue,[65,66] anxiety and depression, level of cognition,[66] family to care for, and receiving a disability pension.[62] Environmental determinants/barriers include but are not limited to, walkability to shops and stores,[67] low–cost recreational facilities, social environment (i.e., social support), weather,[68] accessible gym facilities, and specific barriers within fitness-related facilities (e.g., the front desk of a facility being too high for an individual who is seated).[59] Cost barriers may include budgetary constraints based on a fixed income, but it may also include paying for facilities/services that cannot be used as a part of the membership fee (e.g., hot tubs and saunas that are inaccessible). In addition, transportation to and from activities needs to be calculated into the overall cost (e.g., accessible transportation may add additional costs). Equipment-related barriers include limited space for wheelchair access and limited accessible/adapted equipment. Finally, policy-related barriers include misinterpretations of the ADA, building codes, and other guidelines and informational barriers including information specific to accessible facilities.

Collectively, all barriers to participation, in physical activity and other health-promoting behaviors, are ultimately enabling the known disparities that have been indicated between the general population and individuals with MS. Although a barrier may be difficult to overcome for one individual with MS, the same barrier may be less of a barrier for another individual. This makes universal design or "accessibility for all" important for this population to successfully participate in physical activity and the community abroad.

HEALTH PROMOTION AND PHYSICAL ACTIVITY PROGRAMS FOR INDIVIDUALS WITH MULTIPLE SCLEROSIS

Health promotion programs have increased in numbers for persons with MS and across disability populations.[9] These programs target specific barriers, promote enabling environments, and empower persons with MS to be physically active.[69] Using and adapting available programs for this population will engage community participation in physical activity, ultimately resulting in more inclusive environments and better health outcomes for everyone, including persons with MS.

In examining existing health promotion and physical activity programs for individuals with MS, common themes emerged in respect to program application and common adaptations. This includes a focus on light activities, shorter program durations, adapted and individualized instructions, and ample modifications (e.g., multiple options within exercise programs). Various methods of implementation also exist, including Internet-based health promotion,[63] telephone counseling,[70] randomized control trials of structured exercise regimens,[2,71,72] health education,[73,74] and group wellness interventions.[68,75]

By way of illustration, one program conducted a randomized control trial for individuals with mild MS

($N = 16$), and found that brief bouts of aerobic exercise and strength training trended toward improved QOL, and improved aerobic fitness and strength. Furthermore, this study found no worsening symptoms of MS.[76] This study focused on light and moderate bouts of exercise, thus intensities lower than the recommended physical activity guidelines for the general population (150 min of moderate to vigorous physical activity per week).

In another study, a Jazzercise group exercise program was adapted and implemented for individuals with MS. The program was advertised as a *Jazzercise lite program*, offered twice per week for 45 min, and held in the early evenings, separate from regularly scheduled classes. Core components of the *Jazzercise* program were still present, like 3–4 min choreographed routines and upbeat songs, but in its *lite* version, light and moderate physical activity were targeted rather than moderate to vigorous physical activity. Instructors used clear brief instructions and demonstrations, a recommended adaptation for instructing individuals with MS. In addition, the program used two instructors, a primary instructor and a second instructor to assist in adapting the exercise program for unique individual needs, such as low impact modifications (e.g., sitting in a chair while participating in the program).[71]

In the same manner, a progressive resistance-training program ($N = 9$), made adaptations to intensity and dose for participants with MS. In this 2-day per week progressive resistance-training program, each 60-min session consisted of three resistance-training exercises for the legs, and three resistance-training exercises for the arms on weight machines.[77] The focus on six exercises limited overcomplicating multiple exercises and provided a clear focus for each 60-min session, in which one program leader and two additional instructors facilitated the individualized participant adaptations. Results from this study indicated that participants had a positive experience with the program, felt physically stronger, psychologically sharper, more confident, and less fatigued.

Another study evaluated a 3-month exercise program for individuals with MS. Program participants were randomly assigned into either an intervention group (i.e., individuals participating in the exercise program) or a control group (i.e., individuals receiving only standard care). The physical activity program consisted of meeting with personal trainers 3 times per week for a 60-min session. Each session consisted of a 5-min warm-up, about 50 min of aerobic activity performed at an intensity of about 50% of age-related maximal heart rate, and a 5-min cooldown.[74] Again, the specific physical activity focus for individuals with MS was targeted at lower intensities. Results from this study suggested that participants in the intervention group enjoyed the activity, felt better, and perceived that they worked harder.

Other physical activity interventions have been implemented via the Internet, with the potential to extend their reach far beyond individuals with MS, who live in the immediate vicinity of the intervention site. For example, one 12-week Internet-based physical activity intervention emphasized four specific points: (1) learning the benefits of physical activity, (2) goal setting and outcome expectations, (3) overcoming barriers and learning about social support, and (4) sustaining physical activity over time.[63] Improvements were experienced in respect to physical activity behavior, during and following the intervention.

In summary, simple adaptations like clear instructions, specific exercise foci, and light activities had meaningful effects and impacted aspects of QOL in persons with MS. This strongly suggests that guidelines for physical activity meant for the general population may not be appropriate for people with MS; however, relatively simple adaptations to these guidelines have direct impacts on QOL, including symptom management, for individuals with MS. In many communities, physical activity programs are readily available through commercially available and other community-based programs. Participating in the "light" aspects of a program, or choosing a modified exercise option, are both viable options for individuals with MS. At the same time, current health promotion programs target a variety of healthy behaviors, including physical activity participation, which make inclusive health promotion programs an important and excellent resource to promote health and manage symptoms for individuals with MS.

One health promotion study implemented a remote 12-week health promotion program for individuals with MS. Components of the health promotion program included fatigue management, communication and/or social support, anxiety and/or stress management, reducing alcohol or other drug use, and exercise. Participants were randomly assigned to an experimental group (i.e., initial motivational interview, personal goal setting session, and health promotion program), or a control group (i.e., initial motivational interview, personal goal setting session, but no health promotion program). This individualized health promotion program consisted of personalized coaching over the phone every other week for the duration of the 12-week program. In short, the program was successful and QOL was improved. Although various components of health promotion were studied, exercise (a form of physical activity) was the most popular health promotion activity chosen.[70]

In another study, health promotion programs were delivered in 3-h sessions over a period of 8 weeks. Health promotion content included exercise and physical activity, lifestyle adjustment, fatigue management, stress management, nutritional awareness, and responsible

health practices. The purpose of the program was to provide individuals with the necessary supports to engage in health-promoting behaviors. The results suggested success in health promotion programs with targeted and specified content.[73]

A broad range of positive health outcomes have been linked to health promotion programs and physical activity participation, including improved QOL and decreased MS-related symptoms (e.g., less fatigue, improved fitness, and improved social benefits). A number of key elements have been outlined with respect to how best practice should be designed and implemented for individuals with disabilities, such as adapted physical activity content, addressing barriers and facilitators to physical activity and other health-promoting behaviors, individualized and adapted instruction, and inclusive environments.

Practical Applications for Promoting Physical Activity in the Community

Physical activity guidelines for cardiorespiratory endurance exercise, resistance training, and flexibility designed and based on the general population may not be appropriate for many individuals with MS. More appropriate guidelines and exercise protocols have been created.[1] The following section provides practical suggestions for increasing physical activity based on current exercise recommendations, guidelines for implementing health promotion programs for persons with disabilities (Drum et al., 2008), and national public health initiatives for persons with disabilities.

The CDC (2014) published *Vital Signs*: *Disability & Physical Activity United States 2009–12*, a step-by-step guide to increasing physical activity for individuals with disabilities.[18] This guide was designed for practitioners and includes a brief schematic focused on five key points and corresponding physical activity guidelines for individuals with disabilities:

1. Know the physical activity guidelines.
2. Ask about physical activity.
3. Discuss barriers to physical activity.
4. Recommend physical activity.
5. Refer patients to resources and programs.

These guidelines provide simplified information about each corresponding key point. For example, section one addresses physical activity guidelines by first stating that physical activity participation includes individuals of all shapes and sizes, and also recommending 2½h of physical activity per week, or activities appropriate for an individual based on abilities. Section two assesses physical activity knowledge and behavior with questions such as "What types of physical activity do you enjoy?," "How much physical activity is a person currently completing?," and "How might an individual fit more physical activity into their life?" Section three, focused on barriers to physical activity (see previous section on barriers to physical activity). Learning about activities that an individual enjoys can help when brainstorming or thinking about activities to recommend. Finally, it is important to refer individuals to resources and program such as programs within the community where they live. Promoting programs in the community with trained professionals in adapted physical activity will likely lead to a positive physical activity experience and foster long-term participation for those with MS. Trained professionals are critical to making correct recommendations and adaptations and will create an inclusive and positive environment focused on individuals' abilities and strengths.

As previously mentioned, the physical activity guidelines for the general population may not be appropriate for many individuals with MS; however, it is just as important to recognize that physical activity guidelines for individuals with disabilities may be very relevant to some individuals who have MS, but less relevant for others. Here are some essential steps to give to individuals with MS before starting a physical activity program:

1. Obtain medical clearance and suggestions for exercise from a physician.
2. Seek out appropriate exercise programs (e.g., instructors willing to adapt, adapted programs, swimming, and "light" exercise programs).
3. Pace physical activity and slowly increase intensity overtime.
4. Track symptoms before, during, and after (create an activity log).
5. Discuss changes in physical activity routine or symptoms with a physician.
6. Finally, talk with the instructor about MS before starting a new class. This will help to avoid embarrassment if rest or light adaptations are needed.

In general, warming-up and cooling-down should be regular practice in any exercise regime. In fact, participating in a gentle warm-up can help to reduce occasional increases in symptoms of spasticity for individuals with MS that can occur after physical activity participation. During the warm-up, an individual should hydrate with plenty of water, gently stretch (static stretching is preferred for individuals with MS over dynamic stretching), and move the limbs through a variety of range of motion (ROM) exercises. During the cooldown, an individual should begin decreasing the intensity of exercise (i.e., start exercising at a slower pace), static stretch (static), and continue hydrating with plenty of water. This should be followed by adequate rest and monitoring for any signs of fatigue or discomfort.

During a physical activity/exercise routine, there are a number of exercises to consider choosing from. The following exercises and suggested adaptations are basic guidelines, which should be limited to persons with an *Expanded Disability Status Score* (EDSS) of less than 7.[5] For persons with a more severe disability, small increases in activity, from sedentary to light activity, are recommended, including the practice of yoga and mindfulness activities, which provide numerous benefits for all persons with MS.[78]

Aerobic Training

Types of aerobic activities might include bicycle ergometry, arm–leg ergometry, and aquatics and treadmill walking. The same exercises in a natural environment—rowing, running, swimming, road cycling—should be done with caution. These activities are recommended at least 2–3 times per week, at an intensity that is light to moderate for about 10–40 min.

Strength Training

Types of strength-training activities might include weight machines, light free weights, TheraBands and functional training exercises (i.e., use of body weight only). The use of light free weights and TheraBands, as well as functional-training exercises can all be performed at home. The number of times one strength exercise is performed is called a "repetition" and one group of repetitions is called a "set." Each exercise can be performed 8–15 times for about one to three sets. For example, for the strength-training activity of "sit to stand," an individual could complete one set of eight repetitions of standing from a chair, rest for 2 min, complete a second set of eight repetitions, rest again for 2 min, and finish up with a third set of eight repetitions. In other words, three sets of eight sits to stand. It is important to remember to rest and recover for 2–4 min after each exercise and each individual set to prevent over training and possible injury. Moreover, fatigue is a known symptom of MS, thus performing multijoint activities could be preferential for the sake of time, for example, this means that rather than strengthening a single muscle group, performing multijoint exercises will act on more muscle groups before fatigue sets in. For example, performing the sit to stand exercise instead of two to three exercises of leg extensions and leg curls. Ideally, strength-training exercises should be completed 2–3 days per week with a recommended three to eight exercises in each session (a higher priority can be on strength training of the legs).

Combined Training

A typically weekly regime might include aspects of aerobic training and aspects of strength training. If an individual is interested in a combined training program, one recommendation is to include 2 days of aerobic training per week and 2 days of strength training per week. Keep in mind that strength training should be separated with 24–48 h for recovery.

It is also important to note that these suggestions are basic guidelines that should be used on an individual basis. Each person with MS should have an individual exercise plan according to their specific needs. To date, evidence-based recommendations regarding optimal training for persons with MS are not available. These guidelines are developed based on existing literature for this population.[5]

Practical Applications: Health Promotion in the Community

The use of established health promotion guidelines for designing and implementing programs in the disability community provides additional guidance in program development, and ultimately, prescribing aspects of health promotion to individuals in the community. Experts in disability, public health, and other related fields established guidelines for conducting and implementing health promotion programs for those with disabilities. The following guidelines were identified as key components for an effective health promotion intervention/program: (1) including an underlying theoretical framework; (2) implementing process evaluation; (3) using disability-appropriate outcome measures; (4) including all stakeholders in the development and implementation of the program; (5) considering the beliefs, practices, and values of the targeted group; (6) making the program socially, behaviorally, programmatically, and environmentally accessible to participants; and (7) creating an affordable program. These guidelines are strongly encouraged to use as the best available set of practices for implementing health promotion programs for those with disabilities.[10] The use of these guidelines helps to develop effective translational programs for those with MS.

One university based adapted physical activity program exemplifies the use of physical activity guidelines.[79] Participants, with MS, visit the adapted physical activity program 2-days per week (for 10 weeks each term), with about 20 participants in each respective session. Each participant in the program is matched-up with a university student volunteer, who reviews the participants profile, gets to know the participant, and provides individualized exercise instructions. Exercise instruction includes adapted strength, endurance, balance, and flexibility training. In addition, each program session consists of warm-up and cooldown exercises. In addition, a graduate program coordinator in adapted physical activity, oversees all aspects of project administration. Thus,

exercise programs and goals are reviewed and edited by an expert in the field of adapted physical activity. In addition, program participants have had an opportunity to opt into a health promotion program, specifically created for individuals with MS.[79] The specific health promotion program used a health behavior theory (i.e., Social Cognitive Theory; Bandura, 1989), and individuals with MS were included in all aspects of program development, design, and evaluation. The program was implemented for 8 weeks, and consisted of one 60 min meeting each week. Health promotion program content included goal identification, goal setting, addressing barriers and facilitators to health-promoting behaviors, and social support. This program also directly connected with the adapted physical activity program, whereby the health promotion program took place immediately following the adapted physical activity program. This is important to note, because it assisted in overcoming a consistent barrier to participation—transportation (e.g., participants did not need to scheduled additional transport to attend the program).

CONCLUSION

Health-promoting behaviors range in nature and include eating well, staying physically active, engaging in good social supports, and staying active with the community. Traditionally, individuals with disabilities have been left out of population-based health initiatives, even though the benefits of physical activity and health-promoting behaviors do not discriminate. In fact, the benefits are often amplified in disability populations.[80] The period 1990s and 2000s have shown improvements in respect to health promotion initiatives for individuals with disabilities, yet there is still considerable room for improvement. Physical activity is one health-promoting behavior that should be emphasized in the treatment of MS for symptom management and to improve overall QOL. It has been supported across the literature that physical activity and mind–body therapies, such as yoga, have known benefits to individuals with MS. This chapter highlights physical activity health promotion programs used for those with MS and their strategies for increasing healthy behavior and provides practical tips for promoting physical activity in this population. Despite additional barriers to physical activity participation, proper supports and modifications to activity programs may prove to be the most successful at increasing activity in this population. Moreover, beyond amplified benefits of symptom management, individuals with MS need health promotion programs and community-based programs for the same reasons that the general population does, to stay well, active, and part of the community.

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CHAPTER

22

Interdisciplinary Treatment of Patients With Multiple Sclerosis and Chronic Pain

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OUTLINE

PAIN AND MULTIPLE SCLEROSIS

Chronic pain, a common experience in patients with multiple sclerosis (MS), is a highly debilitating condition that contributes to significant social, emotional, physical, and economic losses.[1] Patients with MS rate pain as one of their most debilitating symptoms, one that has been associated with adverse disease outcomes, decreased quality of life, and increased disability.[2]

The co-occurrence of chronic pain with MS can lead to diagnostic and treatment challenges. Particularly, MS can give rise to a variety of sensory-perceptual disturbances and neuropathic pain phenomena; in addition, it further complicates the course of already-existing pain syndromes. In turn, the experience of pain can influence the course of MS.[3] Generally, pain is associated with psychological distress and decreased daily functioning in areas including recreation, family responsibilities, self-care, occupation, sexual behaviors, and social activity.[4]

It is not surprising, then, that the co-occurrence of chronic pain and mood disorders such as depression is highly common in patients with MS. Given the complexities of chronic pain and MS, it is necessary to consider an interdisciplinary treatment approach to target and manage all the different components that lead to, and maintain, chronic pain in the population with MS. Rehabilitation programs designed for patients with chronic pain offer a comprehensive approach to managing symptoms and more importantly, improving quality of life.

Prevalence

Current estimates indicate that about 29–86% of individuals with a diagnosis of MS report physical or psychological pain.[5,6] Of note, this variability in prevalence estimates may be attributed to the range of research methodologies employed to define pain classification, location, severity, duration, and impairment. Other factors may include variation in the study samples, unreliable data collection, and different sampling methods.[3] Despite the wide range in estimates of prevalence, it is clear that pain syndromes in the population with MS are quite common and can have a significant impact on a person's quality of life and course of disease.

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Pain Classification

"Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage."[7] Pain can be classified as acute or chronic. Generally, acute pain can be triggered by a specific injury or disease progress; it tends to be time limited, well localized, and continuous. Treatment of acute pain focuses on the cause, and it usually resolves once the injury subsides. On the other hand, chronic pain, by definition, has a duration of 6 months or more, persists beyond the healing process, and can be considered a separate condition altogether.[3]

The types of pain experienced by individuals with MS vary depending on the area of the brain that is affected. O'Connor identified four types of MS-related pain: (1) continuous central neuropathic pain, (2) intermittent central neuropathic pain, (3) musculoskeletal pain, and (4) mixed neuropathic and nonneuropathic pain.[8]

Central neuropathic pain is experienced in the absence of any psychiatric disorder or peripheral neuropathic pain.[9] It is consistent with a lesion of the central nervous system (CNS) and is estimated to affect 27.5–58% of patients with MS.[10] Most commonly, central neuropathic pain includes extremity pain, trigeminal neuralgia, and Lhermitte sign. *Extremity pain*, also known as "dysesthetic" extremity pain, is the most common type of pain experienced by patients with MS. It is often described as a continuous "burning" pain, typically experienced bilaterally, on legs and feet, and usually worsens at night. It is one pain subtype that can be exacerbated by physical activity.[8] *Trigeminal neuralgia* affects the trigeminal or fifth cranial nerve, which is one of the most widely distributed nerves in the head. It is a chronic pain condition that is 20 times more common in MS than in the general population. It has been found to affect younger patients with MS in particular.[8] Lastly, *Lhermitte sign* is characterized by a brief sensation of "electric shock" related to neck movement, felt in the back of the neck, lower back, or other parts of the body. It is usually triggered by neck flexion, or other forms of sudden movement. Prevalence rates ranges from 9% to 13% of patients with MS.[8]

Other forms of pain commonly encountered in the population with MS include painful tonic spasms (PTS), back pain, headache, and pain associated with optic neuritis. PTS are a specific type of episodic spasm that is associated with MS. These tend to occur several times per day, lasting less than 2 min, and can be accompanied by a somesthetic aura. Common triggers include touch, movement, hyperventilation, or strong emotions.[8] Although prevalence is thought to be high among patients with MS, there is variability in the literature in terms of defining criteria. *Back pain*, another common presentation in individuals with MS, is generally musculoskeletal in origin and can be exacerbated by prolonged periods of sitting or activity. Prevalence rates are estimated between 10% and 16% of the population with MS.[8] Additionally, the frequency of headache has been reported to be higher in the population with MS than in the general population, with a prevalence estimate of 54% of newly diagnosed patients.[8] *Migraine headaches* are a common presentation in patients with MS, and it is estimated that about 26.8% of those experiencing headaches will have migraines.[8] Finally, pain related to *optic neuritis* is a common occurrence among many patients with MS. However, many studies examining the prevalence of pain have excluded pain associated with optic neuritis; therefore, specific information about prevalence is not widely available. One reason for this could be attributed to diagnostic criteria, which have been controversial and not widely used.[11] Indaco et al. described that 8% of patients enrolled in their study experienced painful optic neuritis.[12] Stenager found that none of the 117 participants enrolled reported any form of optic neuritis pain.[6] Certainly, this is an area where further research is needed.

PSYCHOLOGICAL ASPECTS OF PAIN

As previously mentioned, the experience of chronic pain in the patient with MS can have significant implications in terms of the individual's quality of life. Moreover, the condition can be further complicated by the presence of comorbid depression, anxiety, or both.[13] In studies of chronic pain in the general population, it has been shown that nearly half of the individuals with chronic pain endorse symptoms of anxiety and depression.[13] Likewise, epidemiological studies show that depression is two times more likely in individuals with chronic illness than in those without.[14] In individuals with chronic pain conditions, it is estimated that 30–54% also have depression.[15] In turn, depressive symptoms may even contribute to the emergence of chronic pain in certain populations. For instance, Schwenk et al. found that 73% of former professional football players with high depression scores also endorsed higher pain severity. The relationship between mood and pain can be complex.[16] It is thought that emotions associated with chronic pain such as anxiety, stress, depression, anger, and fatigue may decrease the body's production of natural analgesics; consecutively, these emotions may increase the level of substances that amplify the perception of pain.[1] To complicate matters further, somatic symptoms of depression such as lethargy, anhedonia, and sleep disturbance along with motivational factors, can lead to decreased levels of activity, which can result in a deconditioning process. We will discuss this concept in further detail in the next section.[1] Aside from exacerbating the condition of pain, bouts of inactivity can lead to weight gain and related complications.

Although research is limited in the area of chronic pain, depression, and MS, there is evidence indicating that psychological distress is reported at higher rates in patients with MS who also experience chronic pain.[17] In several large-scale studies assessing the prevalence of major depression in MS, estimates indicate that it occurs in 25–51% of cases, or at three to fourfold higher levels than in the general population.[14] The etiology of depression in MS is a highly debated topic with an array of theories ranging from depression being a result of experiencing a chronic debilitating disease, to it being another consequence of neurologic damage present in the CNS due to demyelination.[14] Regardless of the etiology, it is evident that treating depression is a key aspect of successful management of chronic pain conditions in patients with MS.

Given the multidirectional nature of the development and perpetuating factors of chronic pain in MS, the treatment approach should reflect the complexities of this condition and aim for an individualized plan of care that targets symptoms from a biopsychosocial perspective.[18] In the following section, we will outline the interdisciplinary approach to managing chronic pain in patients with MS.

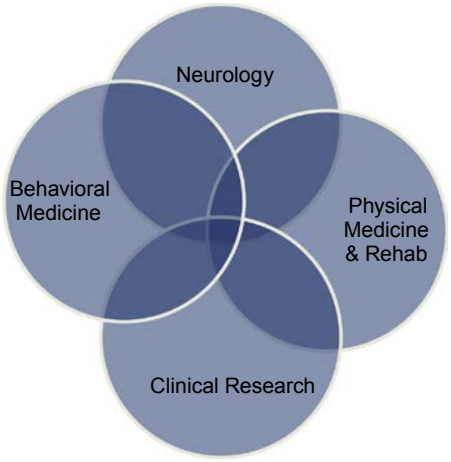

FIGURE 22.1 An interdisciplinary approach to treating pain in patients MS.

AN INTERDISCIPLINARY TREATMENT APPROACH FOR PAIN AND MULTIPLE SCLEROSIS

Assessment

Conducting a thorough assessment is essential for the development of an adequate and comprehensive treatment plan. The ultimate goal in the treatment of chronic pain is improving functionality, providing stabilization of symptoms, and maximizing relapse prevention. Furthermore, given the high psychological and pain comorbidities in the population with MS, it is essential to adequately screen, assess, diagnose, and treat co-occurring conditions such as depression and anxiety.

Treatment Approach

There is a growing body of literature showing evidence supporting a multifaceted interdisciplinary treatment approach for the management of chronic pain. Such programs can result in significant improvements in pain, depression, and daily functioning among individuals with MS.[9] Interdisciplinary pain rehabilitation programs have been shown to reduce pain and improve mood and functioning in patients with chronic pain conditions[9,19] (see Fig. 22.1). In contrast with multidisciplinary approaches to treatment, interdisciplinary teams work from an integrated as opposed to a parallel model of care. This treatment method usually involves a team that includes pain management physicians, psychologists, nurse specialists, physical therapists, vocational counselors, and pharmacists.[19] Typically, patients undergo a thorough screening and assessment process by all members of the team. Each individual case is then presented to the entire core team to formulate a tailored treatment plan. The plan takes into account the patient's individual needs and goals, which must fit his or her abilities or expectations. It is not uncommon for individuals to want to find a cure or complete eradication of their pain, so a primary goal should be to provide education and to address treatment expectations. Generally speaking, realistic goals for therapy may include the reduction, but not complete resolution, of pain; improvement in physical functioning, including mood and associated symptoms; the development of coping strategies; medication management; and improvement in functioning. Thus, the goal is focused on the rehabilitation of the patient, as opposed to the complete eradication of pain. Stemming from the biopsychosocial model, Kerns et al. describe a multidimensional approach to the assessment and management of MS-related chronic pain. This approach includes the initial and continued assessment of medical interventions, the evaluation and strengthening of coping strategies, the utilization of psychological interventions, the incorporation of physical interventions (e.g., exercise), and the support of patients' use of complementary and alternative medicine interventions.[18]

Sullivan et al. evaluated the effectiveness in a population with MS of a program developed at the Cleveland Clinic for the treatment of chronic pain.[9] The Chronic Pain Rehabilitation Program (CPRP) consists of a 3- to 4-week intensive outpatient program with many components shown to be important in managing chronic pain and improving functioning and quality of life. It includes physical therapy, occupational therapy, group

and individual psychotherapy, relaxation training and biofeedback, and medication management, including medically supervised discontinuation of opioids, benzodiazepines, and other addictive substances. Based on admission screening, substance abuse treatment is available and provided as needed. Other services to consider include vocational counseling, medical/surgical consultations, anger management and assertiveness training, biofeedback training, behavior modification training, and family therapy. In certain cases, the incorporation of pharmacotherapies for symptom management may be warranted. In addition to the integration of all these components, there is evidence to support the effectiveness of exercise and physical activity in the management of chronic pain and related conditions. Next, we will describe in further detail some of the most common components of pain rehabilitation programs.

Psychotherapy and Behavioral Approaches

Psychotherapy—which can include group and individual therapy, couples therapy, and sexual dysfunction counseling—can help patients learn about the role of healthy interpersonal relationships and how they can affect and consequently reduce the experience of pain. A specific form of psychotherapy, which has been found to be effective in the treatment of chronic pain as it relates to MS, is cognitive behavioral therapy (CBT).[20–23] This approach focuses on altering patterns of negative thoughts and dysfunctional attitudes and beliefs to encourage the emergence of healthier and more adaptive thoughts, emotions, and behaviors in the individual.[20] The main components of the treatment involve psychoeducation, skills acquisition, cognitive and behavioral rehearsal, and generalization and maintenance.[24] Third-wave CBT such as acceptance and commitment therapy (ACT) have shown some efficacy in the management of chronic pain.[25] One of the major goals of ACT is to mitigate the effects of stress, and it emphasizes mindfulness, attention to the present, and a commitment toward wellness.[25] However, one limitation within the literature is the lack of consistency in treatment across studies, small sample sizes, variability in outcome measurement approaches, and lack of replicated studies.

Additional behavioral methods include training patients in stress management and relaxation strategies. Relaxation training is an effective tool in the management of chronic pain, and can include techniques such as guided imagery, progressive muscle relaxation (PMR), biofeedback, and mindfulness approaches. *Guided imagery* can include a variety of techniques. More commonly, guided imagery contains an element of simple visualization and direct suggestion using imagery, metaphor, story-telling, and active imagination. *PMR* is a relaxation technique that focuses on the systematic tensing and releasing of groups of muscles throughout the body with the purpose of learning to monitor and control muscular tension. *Biofeedback* is a strategy that incorporates instruments that monitor various physiological functions (e.g., heart rate) as a way to regulate various physiological processes. It can include electromyography, electroencephalography, galvanometry, and temperature.[19] The Association for Applied Psychophysiology and Biofeedback provides a standard definition of biofeedback:

> Biofeedback is a process that enables an individual to learn how to change physiological activity for the purposes of improving health and performance. Precise instruments measure physiological activity such as brainwaves, heart function, breathing, muscle activity, and skin temperature. These instruments rapidly and accurately "feed back" information to the user. The presentation of this information – often in conjunction with changes in thinking, emotions, and behavior – supports desired physiological changes. Over time, these changes can endure without continued use of an instrument.[26]

One of the advantages of using biofeedback in the context of pain and MS is that it teaches individuals how to regulate their autonomic nervous system as it relates to the stress response. This reinforces to the individual the importance of the mind–body connection when learning alternative strategies to manage chronic pain.

Other mindfulness approaches may consist of a variety of different components including meditation. There are many types of meditation practices, but one commonality is the incorporation self-observation of mental activity from a nonjudgmental stance as a way to promote mental calmness. Mindfulness meditation has been shown to be effective in a variety of settings including the management of chronic pain. Mindfulness-based stress reduction was first developed by Kabat-Zinn in 1979 and has been utilized for both stress and pain reduction.[27] Lastly, breathing techniques such as diaphragmatic breathing focus on enhancing awareness of breathing rate, rhythm, and volume.[25] The goal of most breathing techniques is to maximize physiologic responses to stress by enhancing the parasympathetic response. These techniques are often used as an adjunct to other relaxation strategies.

Physical Therapy

Physical therapy teaches patients proper body mechanics and is intended to improve strength, flexibility, endurance, and functionality. Specific treatment protocols are tailored for the individual based on patient history, a physical examination, and oftentimes a review of laboratory and imaging data. It may include specific exercise sequences, manual therapy and manipulation, mechanical devices, patient education, and the use of physical agents including heat, cold, and electricity. One of the main goals of physical therapy is to restore mobility to improve functional ability.

Occupational Therapy

Occupational therapy is used to improve performance on tasks at home and at work. It is geared toward reducing stress and helping patients gain confidence in performing daily activities as they relearn how to perform daily tasks. Moreover, some occupational therapists have a specialty in cognitive rehabilitation. Similarly to physical therapy, instruction in proper body mechanics, which can reduce muscle strain, also helps patients gain a sense of self-efficacy in performing daily activities.

Physical Activity

More traditionally, the treatment of pain conditions has consisted of adopting long periods of inactivity and resting; however, there are supportive data showing that regular exercise is an effective approach to managing chronic pain.[1] There is a process referred to as "deconditioning" that occurs when individuals rest due to pain or fear of pain. In turn, this leads to more inactivity, which results in suboptimal physical condition.[1] Deconditioning can also be characterized by a lowered exercise tolerance (e.g., walking on a treadmill) and quicker fatigue with usual daily activities (e.g., making the bed). Avoidance behaviors reinforce pain-related fear, which only furthers suffering, dysfunction, and disability[28] (see Fig. 22.2). According to Vlaeyen's fear avoidance model of pain, individuals avoid movement and physical activity due to fear that they will increase or worsen pain.[29] Decreased activity in addition to increased anxiety and pain perception only lead to further deconditioning and increased pain, which reinforces the vicious cycle.[28]

Physical activity can be broadly defined as any bodily movement generated by skeletal muscles resulting in energy expenditure. Exercise is a biochemical, social, and physical activity that can be manifested in a variety of forms, with the purpose of training or developing the body to promote physical health. These include aerobic activity, anaerobic activity, flexibility, coordination, and relaxation. Aerobic exercise consists of physical activity sustained for long periods with the training designed to increase the efficiency of the oxygen transport system. Examples include distance running, cardiovascular training, walking, and playing sports such as basketball or soccer.[28] In contrast, anaerobic exercise consists of high-intensity work sustained for a short period, with the purpose of increasing muscular strength, for example, weight lifting and strengthening core muscles. Lastly, a third type of exercise helps increase flexibility, coordination, and relaxation, and may include activities such as stretching, ballet, and yoga.

Among the many benefits of exercise are the production of endorphins, which can help to effectively block pain and produce feelings of relaxation.[30] Given the strong relationship between mood and pain perception, this is an important consideration. Regular physical activity is also linked to weight loss and weight management, which has important implications for the prevention of chronic pain. Lastly, regular exercise can help strengthen core muscles, which support bones and cartilages and help keep joints flexible, relieving stiffness and acting as a natural brace for large bone structures commonly associated with pain.[31,32] Interestingly, there is research supporting that even

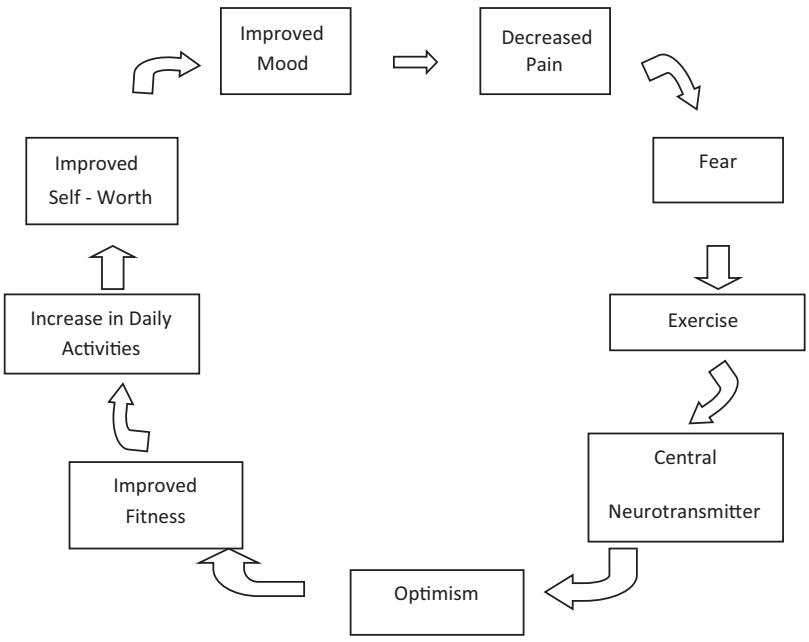

FIGURE 22.2 The upward cycle of activity of a patient with pain.

exercise carried out for a brief period of time can have an immediate positive impact on mood. In a previous study by Sullivan et al., participants in the CPRP were asked to complete measures of mood prior to a 10-min walk on a treadmill. Results showed that participants reported subjective improvements in mood following the intervention.[33]

Medication Management

An additional treatment component to be considered for some individuals includes the utilization of pharmacological agents to manage symptomatology associated with chronic pain. For example, in the management of MS-related spasticity, muscle relaxants such as baclofen or tizanidine may be considered. For myofascial pain, trigger point injections with local anesthetic and/or steroids can provide significant and immediate relief. In regard to the management of neuropathic pain, there are a variety of commonly used pharmacologic treatment options including antiepileptics, antidepressants, muscle relaxants, and topical analgesics in the form of patches and creams.[3] Lastly, there have been studies showing support for the use of ketamine, an *N*-methyl-D-aspartate receptor agonist, in the management of chronic pain conditions.[19–34] Of note, the use of pharmacological agents in pain management should not be employed as a stand-alone treatment, and its consideration in an individual's treatment plan should be seen as an adjunct to additional components. The use of more traditional pain medications such as opioids in the management of MS-related pain is controversial, given that success rates are poor and that such medications tend to lose effectiveness over time.[18] Furthermore, the use of these agents can have a negative impact on other aspects associated with pain; for instance, some of the medications can increase fatigue levels and lead to cognitive problems, which could further exacerbate or worsen mood and aggravate the pattern of inactivity.

SUMMARY AND CONCLUSIONS

Chronic pain is a common symptom described by patients with MS. In addition, the presence of mood disorders generally accompanies chronic pain, making depression also a highly common symptom of MS. Both conditions contribute to significant complicating issues in a person's life. Given the complexities of chronic pain and MS, the clinician should work to look at pain as a multifaceted disease. Assessment and treatment should encompass a multifaceted approach based on the biopsychosocial model. Effective management of chronic pain in MS should aim to address common comorbidities associated with chronic pain including mood disorders. Treatment should be within an interdisciplinary model with a focus on managing pain, not eradicating, and on improving functioning, both physically and emotionally.

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SECTION V

DRUGS OF ABUSE, ALCOHOL AND TOBACCO, AND DISEASE OF MULTIPLE SCLEROSIS PATIENTS

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CHAPTER

23

Alcohol and Tobacco in Multiple Sclerosis

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OUTLINE

INTRODUCTION

Multiple sclerosis (MS) is a chronic immune-mediated, inflammatory demyelinating disease of the central nervous system (CNS) that affects both the brain and spinal cord. The cause of MS is not fully understood, but the disease is believed to relate to an abnormal immune response in individuals who are genetically predisposed. The association of personal predisposition and environmental conditions may result in an immune-mediated inflammation of the CNS culminating in neuronal loss. The disease usually manifests as bouts of neurological disabilities and later progresses to dysfunction of many areas of the CNS. It can affect motor and sensory functions, coordination, cognition, vision, and sphincter control. It is often associated with fatigue, pain, depression, anxiety, and sleep disorders, thus leading to considerable personal, social, and economic losses.[1]

Lifestyle/environmental factors may have an important role in determining the risk of MS. These are harder to accurately study and quantify than are genetic factors. However, it is important to identify these potential determinants of risk and worse outcomes in MS, since they are potentially preventable.[2]

Depression, anxiety disorders, social withdrawal, and cognitive deficits often affect the quality of life of patients with MS[3]. Chronic diseases like MS may negatively affect friendships and family relations. Patients may find themselves out of work while still relatively young and may find it difficult to deal with relapsing and/or progressive neurological disabilities. Ultimately, patients with MS may resort to alcohol, smoking, illicit drugs, and even reckless behavior as part of their lifestyle choices.[4,5] If these personal choices are associated with worse outcomes from the disease, then preventing or minimizing such harmful decisions is an important part of the treatment. The present study reviews the potential influence of tobacco smoking and alcohol misuse in MS.

METHOD

The authors independently searched for the terms "alcohol" and/or "tobacco" and "multiple sclerosis" or "MS" in the Medline, PubMed, Lilacs, SciELO, and Google Scholar databases. There was no time limitation on the search. Summaries of articles in any language containing those words in English (in the title, keywords, or abstract) were reviewed independently. The authors selected the articles with relevant information on the potential influence of tobacco smoking and alcohol on the development of or outcomes from MS. Only articles presenting data were included in the review. The review

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was conducted systematically, in accordance with the PRISMA-P guidelines.[6]

RESULTS

Articles discussing the role of lifestyle habits in relation to MS were recent publications, typically from the 21st century. Those presenting data on patients are discussed individually in the following sections.

Alcohol

Sixteen potentially eligible studies were identified through the search strategy, and six articles were excluded after assessment of the complete text. Ten original articles published between 2004 and 2015, which assessed the increased consumption of alcohol associated with MS, were included in this review.[8–17] The first article identified dates from February 2004.[8] A summary of the data from these articles is given in Table 23.1. Most studies show alcohol misuse in patients with MS, but the rate of alcohol abuse varied from 3%[17] to 40%.[14]

Despite the numerous health and social consequences of alcohol misuse, routine screening and intervention for people with MS remain uncommon. Brief screening and advice to reduce or refrain from alcohol use can be incorporated into the regular course of medical care.

TOBACCO

From a public health perspective, the impact of smoking and passive smoking on the risk of MS may be considerable. Preventive measures to reduce tobacco smoke exposure are therefore essential Table 23.2.

Nineteen potentially eligible studies were identified through the search strategy, and five articles were excluded after assessment of the complete text. Fourteen original articles published between 2011 and 2015 were selected for the review.[17–30] The first article identified dates from July 2011. This consisted of a population-based case-control study that estimated the influence of passive smoking on the risk of MS.[18]

Most articles have suggested that regular smoking is associated with greater disease severity and faster progression of disability. The risk seems to be higher among men and is associated with the dose and duration of tobacco use. However, the risk of developing MS after the initial manifestation of clinically isolated syndrome was not associated

TABLE 23.1 Studies Assessing the Use of Alcohol in Patients With MS

Author	Year	Country	Population	Assessment	Main results
Quesnel and Feinstein[8]	2004	Canada	140 patients with MS	Associated psychiatric diagnosis	Higher lifetime prevalence of anxiety, suicidal ideation, and substance abuse and a family history of mental illness
Bombardie et al.[9]	2004	United States	708 patients with MS	Questionnaire on the use of alcohol in the previous month	The prevalence of alcohol misuse was 14% in this population of patients
Turner et al.[10]	2009	United States	2625 patients with MS	Retrospective questionnaire on life habits	The prevalence of alcohol misuse was 13.9% for those who developed MS
D'hooghe et al.[11]	2012	Belgium	1372 patients with MS	Follow-up epidemiological data on large databases	Consumption of alcoholic beverages, coffee, and fish was inversely associated with the progression of disability in relapsing-onset MS, but not in progressive-onset MS
Foster et al.[12]	2012	United States	272 patients with MS 151 control subjects	Retrospective assessment of alcohol consumption over 15 years	The duration of alcohol consumption was associated with disability and MRI measures in MS
Massa et al.[13]	2013	United States	(a) 92,275 women followed up from 1980 to 2004 (b) 95,051 women followed up from 1991 to 2005	Follow-up epidemiological data on large databases	Alcohol consumption was not related to the risk of developing MS
Beier et al.[14]	2014	United States	157 patients with MS	Cross-sectional study, individual interviews	Excessive consumption of alcohol by 40% of patients

TABLE 23.1 Studies Assessing the Use of Alcohol in Patients With MS —cont'd

Author	Year	Country	Population	Assessment	Main results
Hedström et al.[15]	2014	Sweden	(a) 745 patients with MS and 1761 control subjects (b) 5874 patients with MS and 5246 control subjects	Follow-up epidemiological data on large databases	Dose-dependent inverse association between alcohol consumption and risk of developing MS (statistically significant for both genders)
Weiland et al.[16]	2014	Australia	2469 patients with MS	On-line platform	Most (61.5%) consumed less than 15 g alcohol weekly; few (0.8%) drank large amounts
Fragoso et al.[17]	2015	Brazil	168 patients with MS 168 control subjects	Cross-sectional study, individual interviews	Control subjects had significantly higher alcohol consumption

MS, multiple sclerosis; *MRI*, magnetic resonance image.

TABLE 23.2 Studies Assessing the Use of Tobacco in Patients With MS

Author	Year	Country	Population	Assessment	Main results
Hedström et al.[18]	2011	Sweden	695 patients with MS and 1635 control subjects (never smoked population)	Retrospective evaluation on the risk of MS in persons exposed to tobacco	The risk of developing MS was increased among never smokers who had been exposed to passive smoking
Hedström et al.[19]	2013	Sweden	7883 patients with MS, 9437 control subjects	Retrospective assessment of snuff use and the risk of MS	Snuff-takers have a decreased risk of developing MS compared with those who have never used moist snuff
Ramagopalan et al.[20]	2013	United Kingdom	3157 MS cases and 756 spouse controls	Questionnaires on active and passive smoking history	Ever-smoking is associated with increased MS risk in males
Manouchehrinia et al.[21]	2013	United Kingdom	895 patients with MS (49% were smokers at disease onset)	Retrospective assessment of disability progression	Regular smoking was associated with faster disability progression
Asadollahi et al.[22]	2013	Iran	662 patients with MS	Risk of developing MS in ever-smokers	When compared with never smokers, the risk of MS was 2.91 for men and 1.69 for women
Hedström et al.[23]	2013	Sweden	7883 patients with MS 9264 control subjects	Follow-up epidemiological data on large databases	Duration and intensity of smoking contributed independently to the increased risk of MS
Manouchehrinia et al.[24]	2014	United Kingdom	1032 patients with MS	Follow-up epidemiological data on large databases	Tobacco smoking is a risk determinant for MS-related death
Briggs et al.[25]	2014	Sweden	1588 patients with MS and control subjects from the United States; 988 patients with MS and control subjects from Sweden	Follow-up epidemiological data on large databases	Genetic predisposition regarding smoke exposure in MS susceptibility
Ozcan et al.[26]	2014	Turkey	20 heavy smokers with MS 24 nonsmokers with MS	Cognitive assessment	Heavy smoking was associated with cognitive impairment (compared with nonsmoking).
Gustavsen et al.[27]	2014	Norway	756 patients with MS, 1090 control subjects	Cross-sectional study with a questionnaire	More patients with MS than controls reported smoking Fewer patients with MS reported snuff use

Continued

TABLE 23.2 Studies Assessing the Use of Tobacco in Patients With MS—cont'd

Author	Year	Country	Population	Assessment	Main results
Ramanujam et al.[28]	2015	Sweden	728 patients with MS	Cross-sectional study of patients who smoked at MS onset	Each additional year of smoking after diagnosis of MS accelerated the time to conversion to secondary progression by 4.7%
Munger et al.[29]	2015	Germany	648 patients with CIS	Prospective longitudinal study (5-year follow-up)	Tobacco use (assessed by plasma levels of cotinine) was not associated to the conversion of CIS into MS
Hedström et al.[30]	2015	Sweden	2455 patients with MS, 5336 control subjects	Follow-up epidemiological data on large databases	Both smoking and exposure to passive smoking contributed to MS risk in a dose-dependent manner
Fragoso et al.[17]	2015	Brazil	168 patients with MS, 168 control subjects	Cross-sectional study, individual interviews	Patients with MS and control subjects had similar history of smoking (24.5% and 23.3%, respectively)

MS, multiple sclerosis; *CIS*, clinically isolated syndrome.

with smoking.[30] Interestingly, nicotine snuff seemed to be associated with a lower risk of developing MS and it was suggested that the potential antiinflammatory effect of nicotine might have led to these results.[20,28]

DISCUSSION AND CONCLUSION

Attitudes toward alcohol, tobacco, illicit drugs, and reckless behavior may differ among different populations. Even if the findings are not uniform in all articles, there seems to be a potential risk of development of MS and worse outcomes from this disease among patients who smoke and/or drink alcohol. It is important to study the role of modifiable risk factors in MS development and progression. We hope that this review will serve as a basis for further work on the subject, as well as for health care measures to modify the risks.

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CHAPTER

24

Herbal Oil Supplement With Hot-Nature Diet for Multiple Sclerosis

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OUTLINE

OVERVIEW

Multiple sclerosis (MS) is a relatively common disease with unknown etiology and no cure, which results in neurological disability in young adults. This condition affects millions of people worldwide[1] and over 60,000 individuals in Iran.[2] Many of the current treatments are costly, limited in efficacy, and are associated with unpleasant side effects.[3] Although the exact etiology of developing MS depends on both genetic and environmental factors,[4] pathological events such as impairment of T helpers (Th) are involved.[5] The major types of Th cells are Th1 cells, which produce interleukin (IL)-2, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ, and Th2 cells, which produce IL-4, IL-5, IL-10, and IL-13,[6,7] and Th1/Th2 balance has been considered one of the risk factors in MS etiology. In addition, Th17 (new T-cell subset) produces IL-17 cytokine (a key player in MS pathogenesis) and cytokines derived from Th1 cells (IFN-γ) and Th2 cells (IL-4) are shown to repress the development of Th17 cells.[8–10] Altered cytokine profiles have been documented within CNS tissue[11] and peripheral blood mononuclear cells (PBMCs) derived from patients with MS.[12] Many of the cytokines altered in MS

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include IFN-γ,[13] TNF-α,[14] IL-8,[15] IL-6,[16] IL-4,[14] IL-10,[17] and IL-17.[18] In the majority of cases, several Th1 cytokines increase in MS, whereas Th2 cytokines decrease. Several Th2 cytokines have been implicated in the survival of neurons and oligodendrocytes, and may explain why therapies promoting a Th1 to Th2 cytokine shift are beneficial in MS.[12] In summary, cytokine profiling in MS has not only provided insights into disease pathogenesis, but also led to the development of potentially novel therapeutic targets, and to validating the mechanisms of approved therapies.[12] For example, IFN-β treatment shifts the immune response from the Th1 to Th2 pattern by enhancing the production of antiinflammatory Th2 cytokines (e.g., IL-4) and decreasing the production of proinflammatory Th1 cytokines (e.g., IFN-γ). IFN-β1a enhances the production of antiinflammatory cytokines IL-4 and IL-10 and IFN-β1b decreases the production of the proinflammatory cytokine IFN-γ.[19] Copaxone or glatiramer acetate (GA) is another Food and Drug Administration–approved therapy for treating relapsing remitting multiple sclerosis (RRMS). GA-reactive T cells secrete Th2 cytokines, such as IL-4, IL-5, IL-6, and IL-10, but failed to produce IFN-γ and TNF-α.[20] These drugs have a variety of different mechanisms of action, all of which appear to possess immunomodulatory and antiinflammatory roles by inhibiting, sequestering, or depleting lymphocytes.[21]

In this regard, Cold and Hot natures (*Mizadj*) are believed to exist in Traditional Iranian Medicine (TIM) and in many other traditional medical theories.[22,23] The study of Shahabi et al.,[24] on IL-4/IFN-γ ratios showed that the tendency of the Hot-nature people was to deviate toward Th2-like immune responses to a greater extent than the Cold-nature people. Hence, consumption of Hot-nature foods in a person suffering from an autoimmune disease with a deviation toward Th1 immune responses (such as MS) may be useful because they can accelerate warmth of nature and deviation toward Th2 immune responses.[25] However, these therapies were commonly accompanied by serious complications and multiple adverse side effects.[26]

On the other hand, epidemiological studies have demonstrated a relation between MS mortality and dietary fat.[27] Lipids serve important functions as membrane phospholipid constituents.[28] There is evidence that omega-3-polyunsaturated fatty acids (ω3-PUFAs) can suppress IFN-γ production in patients with MS.[29] Hence, Rezapour-Firouzi et al.,[30–34] designed a study to investigate the effects of a 9:1 combination of hempseed oil (HSO) with evening primrose oil (EPO) as a supplement to a Hot-nature diet in comparison with the 9:1 combination of HSO with EPO without a special diet and olive oil in the third group in patients with RRMS. HSO, as well as EPO, contains substances with antioxidative properties, and the combination of these oils as a dietary supplement has a potential to reduce proinflammatory cytokines and targets this key mechanism of disease and works like approved treatments. It contains over 80% PUFAs, with the ω6/ω3 ratio between 2:1 and 3:1, which is considered optimal for human health.[35]

ROLE OF LIPIDS IN MS

Dietary Fatty Acids in the Etiology of MS

Dietary fat has been implicated in the etiology of MS since the early 1950s.[36] In the CNS, a multilayered membrane layer known as the myelin sheath enwraps axons, and is required for optimal salutatory signal conductance. The sheath develops from membrane processes that extend from the plasma membrane of oligodendrocytes and displays a unique lipid and protein composition, and the sheath is highly enriched in lipids (Table 24.1).[37]

The lipid component has a relatively high turnover rate, in contrast to the protein component that is especially stable.[39,40] There are several clinical observations that suggest that abnormalities of PUFA synthesis may be involved in MS. An increased risk for MS was found to be associated with high-energy and animal food intake (Table 24.2).[41]

Epidemiological studies have demonstrated a relation between MS mortality and dietary fat.[27] Saturated fatty acids (SFAs), animal fat, and animal fat without

TABLE 24.1 Lipids Are Major Components in Brain Structure[38]

Component	Myelin (A Membrane With Little Activity)	Erythrocyte (A Membrane With Several Enzymatic Activities)	Mitochondrion (Inner Membrane With a Wide Variety of Enzymatic Activities)
Lipid:Protein (mass ratio)	3:1	1:2	1:3
Phospholipids (% of total lipid)	43	61	90

TABLE 24.2 Structure of Myelin Phospholipids

Structure of myelin phospholipids	Percentage	Turnover
Total phospholipids	43	
Lecithin	11.2	2–4 months
Sphingomyelin	7.9	1 year
Phosphatidylserine	4.8	2–4 months
Phosphatidylinositol	0.6	5 weeks
Phosphatidylethanolamine	1 year	Phosphatidylethanolamine

fish fat are correlated positively with MS mortality.[42] Increase in SFAs of lysolecithin has been demonstrated. A definite increase (77%) in plasma lysolecithin levels has been reported in patients affected by MS in its active stages as compared with normal subjects.[43] Analysis of values between normal subjects and patients with MS of SFAs/unsaturated fatty acids (USFAs) ratios showed significant increase ($p < .01$) in patients with MS. In addition, the increase in concentration of lysolecithin and its SFAs may explain the well-known changes in platelet behavior in patients with MS.[44,45] Human frontal cortex is composed of SFAs [~36% of total brain fatty acids (FAs)]; monounsaturated fatty acids (MUFAs), predominantly the ω9-oleic acid (~20% of total brain FAs); and PUFAs (~28% of total brain FA composition).[46] Table 24.3 shows through a process of desaturation, linoleic acid (LA) converts into gamma-linolenic acid (GLA), dihomo-gamma-linolenic acid (DGLA), and arachidonic acid (AA), whereas α-linoleic acid (ALA) converts into eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Low levels of both ω3 and ω6 PUFAs in both plasma and red cells are found in depression. The ω3 depletion is consistently greater than the ω6 depletion leading to elevations of the ω6/ω3, AA/EPA, and AA/DHA ratios. These abnormalities are associated with substantial elevations of the formation of prostaglandin (PG) E2.[47] The ideal ratio between ω6 and ω3 fatty acids should be 1:2.3; this ratio needs to be reached because these two groups of essential fatty acids (EFAs) perform distinct and complementary functions.[48] Because of this, it has been suggested that ω3 and ω6 EFAs be given together.[49]

Role of Delta-6-Desaturase in the PUFA Biosynthetic Pathway

Delta-6-desaturase (D6D; FADS2) is the rate-limiting step in the PUFA biosynthetic pathway. FADS2 deletion may prevent the conversion of ALA into very-long-chain PUFAs.[50] There is evidence that D6D activity was either very low or lacking. LA and ALA levels were very high, suggesting a block at the D6D level of desaturation. Supporting this view, metabolites such as GLA were so low as to register zero. They showed the same evidence

TABLE 24.3 Metabolic Pathways of Polyunsaturated Fatty Acids

SUMMARY OF OMEGA 9 PATHWAY:	SUMMARY OF OMEGA 3 PATHWAY:	SUMMARY OF OMEGA 6 PATHWAY:
18:0 Stearic acid	**Linolenic Acid/Omega 3 (LNA)**	**Linoleic Acid/Omega 6 (LA)**
⇩ **D9Desaturase**	⇩ **D6 Desaturase (D6D)** vitB6,Zn,Mg	⇩ **D6 Desaturase (D6D)**
18:1 n-9 oleic acid	**Stearidonic Acid**	**Gamma Linolenic Acid (GLA)**
⇩ **D6Desaturase**	⇩ **Elongase** **Eicosatetraenoic Acid** ⇩ **D5Desaturase (D5D)** VitC,B3,Zn	⇩ **Elongase** **Dihomo-gamma-linolenic Acid (DGLA)** ⇨ Anti-inflam: PG Series I
18:2 n-9	**Eicosapentaneoic Acid (EPA)** ⇨ Lipoxygenase converts EPA to: Anti-inflam: Leukotrienes	⇩ **D5Desaturase (D5D)**
⇩ **Elongase**	⇩ **Elongase** (COX 1) converts **EPA** to: Anti-inflam PG Series 3	**Arachidonic Acid (AA)** (COX 2) converts **AA** to: Pro-inflam PG Series II
20:2 n-9	**Docosapentaenoic Acid**	
⇩ **D5Desaturase**	⇩ **D4Desaturase**	
20:3 n-9	**Docosahexaenoic Acid (DHA)**	
eicosatrienoic acid		

of D6D inactivity, and EPA deficiency as well. EPA levels fluctuated radically between the pre-GLA and post-GLA assays.[51] In mammals, the conversion of LA to GLA is slow, especially during stress, aging, or diseases (hypertension, diabetes, etc.). If the conversion from LA to GLA by the enzyme D6D is disturbed, a dietary supplementation with GLA can help to improve the situation.[52,53] Moreover, excessive consumption of GLA metabolites: high rates of cell division, inflammatory and antiviral reaction and trauma. This is why the key deficits are observed in GLA, DGLA, and PGE1.[53]

GLA Metabolism and Role of Antiinflammatory Activities

GLA (*cis*-6, *cis*-9, *cis*-12-octadecatrienoic acid) is a *ω*6 FA and over the past four decades, human and animal studies have confirmed its antiinflammatory properties. The oils of evening primrose (*Oenothera biennis* L.; 8–12% GLA) and hempseed (*Cannabis sativa* L.; 6–8% GLA) are used for treating inflammatory conditions. GLA acts in several ways to exert its effects, including the modulation of eicosanoids (PGs, LTs) and cytokines, and by regulating genes that affect apoptosis and cell growth. GLA is functionally EFA because it can correct the symptoms of EFA deficiency.[54,55] It is produced endogenously in humans and animals as the first product of metabolism of LA, an EFA of the ω6 series. The enzyme D6D, which is the slowest and rate-limiting step in the metabolic pathway to GLA, catalyzes this reaction. Once synthesized, GLA is rapidly elongated to DGLA by the enzyme elongase, and DGLA is acetylated and incorporated into cell-membrane phospholipids. A small amount can be converted into AA. DGLA competes with AA for cyclooxygenase (COX) and lipoxygenase (LOX) enzymes, and the metabolites of DGLA and AA that are produced by these two types of enzymes, the PGs and LTs, respectively, have actions that oppose each other. The result of all of the foregoing factors is diminished endogenous synthesis of GLA, with a functional deficit of DGLA that leads to an imbalance in PG/LT production in which inflammatory PGs derived from AA are produced in excess. Under such circumstances, supplementation with GLA restores balance to the system of inflammatory cytokines. Like PGE3, PGE1 is an antiinflammatory that inhibits TNF-α, IL-1β, and IL-6.[56]

DGLA has also been shown to reduce proinflammatory eicosanoids such as LTs: B4 and C4 that are formed by AA.[57] Following an inflammatory stimulus, the enzyme phospholipase A2 (PLA2) releases DGLA from the cell membrane, and this released DGLA competes with AA for metabolism by COX and LOX enzymes. Metabolism of DGLA by COX enzymes produces antiinflammatory effects.[58] Besides exerting an antiinflammatory effect by inhibiting COX and LOX pathways that generate mediators of inflammation, GLA also counteracts inflammation by affecting pathways of cytokine synthesis.[59]

Stearidonic Acid Metabolism and Role of Antiinflammatory Activities

D6D is the rate-limiting step in the conversion of ALA to EPA and stearidonic acid (SDA or STA) has a biochemical advantage over ALA in elevating the levels of long-chain ω3 PUFA in tissues.[60] One reason for the limited impact of ALA may be that its conversion to the longer-chain active derivatives EPA and DHA is limited in human subjects.[61,62] This could be due to the low activity of D6D[63,64] and/or the inhibitory effect of a high intake of LA on ALA conversion. The lack of efficiency of conversion of ALA may relate to impaired D6D activity. SDA was almost two times more effective than ALA in increasing cellular EPA concentration, and SDA may be a potential alternative to EPA (and perhaps DHA) for people who do not consume fish or other sources of ω3 long-chain (LC)-PUFAs.[60] HSO contains a significant amount of GLA (7%) and SDA (2.5%). Consumption of GLA (2 g/day) in the absence of SDA or EPA increased DGLA content in PBMCs. SDA in combination with GLA increased the proportion of EPA in PBMCs, without an increase in AA content.[65] SDA was more potent than ALA in suppressing the transcription of the COX-2 gene, which may be indicative of a unique action imposed by SDA at the gene level.[60] The immunological effects of SDA are largely unknown, although one animal feeding study showed that dietary SDA could decrease TNF-α production as the same amount of dietary ALA or EPA.[66]

Essential Fatty Acids, the Blood–Brain Barrier, and the Brain

EFA determines the fluidity of the neuronal membrane and controls the physiological functions of the brain. EFA is also involved in the synthesis and functions of brain neurotransmitters, and of the molecules of the immune system. Since they must be supplied from the diet, a decreased bioavailability is bound to induce major disturbances. Although the brain needs a continuous supply of EFAs during the lifespan, there are two particularly sensitive periods, infancy and aging. EFA deficiency during infancy delays brain development and in aging will accelerate deterioration of brain functions. In discussing the role of EFA, two issues must be considered (1) the blood–brain barrier (BBB), which determines the bioavailability, and (2) the myelination process, which determines the efficiency of brain and retinal functions.[67] However, our knowledge of functional changes is quite limited the immature infant brain's ability to convert the LA and ALA fatty acids that are ingested from diet into

longer chain FAs. However, a majority of studies agree that even the infant brain has such a capacity.[39] The brain cannot distinguish among longer chain FAs that have been synthesized in the brain, and those same FAs that have been obtained from the diet and crossed the BBB.[67]

Myelin and Cell Membrane Fluidity

Cell membrane fluidity (CMF) is a parameter describing the freedom of movement of protein and lipid constituents within the cell membrane. CMF appears to influence several cellular processes including the activity of membrane-associated enzymes.[68,69] CMF may also be implicated in the changes associated with the aging process. Age-associated lowering of D6D activity will decrease PGE1 synthesis,[70] which would be expected to increase CMF.[71] The activity of membrane-associated enzymes increases in fluid membranes.[68] Among the significant components of cell membranes are the phospholipids that contain FAs. Phospholipids made from SFAs have a different structure and are less fluid than those that incorporate an EFA. LA and ALA have an effect on the neuronal CMF. They are able to decrease the cholesterol level in the neuronal membrane, which would decrease membrane fluidity, which in turn would make it difficult for the cell to carry out its normal functions and increase the cell's susceptibility to injury and death.[67] Higher membrane USFA levels are associated with increased CMF.[72]

The EFAs are important in the active phase of the myelin synthesis. If EFAs are not available in this phase or are metabolically blocked, amyelination, dysmyelination, or demyelination may occur.[73,74] If EFA deficiency occurs during the postnatal period, a major delay in the myelination process will occur, accompanied by impaired learning and motor, vision, and auditory abnormalities.[75] The rate of myelin lipid turnover is age dependent, and with a very slow turnover rate during aging, and the rate of repairing damaged sections of myelin is correspondingly slower.[76] Diets deficient in EFAs tend to be associated with the CMF-influenced diseases; EFA deficiency has been associated with MS.[77]

PUFA Turnover in CNS Membrane Phospholipids

Deacylation–Reacylation Cycle

Bioactive lipids are generated by hydrolysis from membrane lipids mainly by phospholipases giving rise to FAs and lysoPLs that either directly exert their function or are further converted to active mediators and thus regulate many fundamental cell responses.[28] Phospholipids in CNS membranes are enriched in PUFAs.[78] PLA2 can release AA, DGLA, and EPA from the *sn*-2 position of membrane phospholipids, but with vastly differing consequences: DGLA and AA, as well as EPA, can be transformed into PGs and TOXs of the 1-, 2-, and 3-class, respectively. The 2-class is highly proinflammatory, and the 1-class has intermediate properties, whereas the 3-class is antiinflammatory.[79] PLA2 belongs to a family of enzymes that catalyze the cleavage of fatty acids from the *sn*-2 position of phospholipids, and seems to prefer hydrolysis of AA from phosphatidylcholine.[78]

PLA2 enzymes, which are widely expressed in many types of mammalian cells, not only play a role in the maintenance of cell membrane phospholipids, but are also actively involved in the production of AA, the precursor for proinflammatory prostanoids.[80] Toxicity of AA was associated with increased lipid peroxidation and mitochondrial damage.[81] The AA liberated is converted to PGE2, possibly by COX-2, which is induced by inflammatory stimuli.[82] Literature review has shown the neurobiological and neuropathological consequences of AA metabolism via the COX-2 pathway and the potential therapeutic benefit of COX-2 inhibition in the setting of neurological disease. PLA2s have an important role in cellular death that occurs via necrosis or apoptosis. PLA2 activity increases, producing accelerated membrane phospholipid hydrolysis and, in turn, increased plasma membrane permeability and cell lysis.[81] One strategy for controlling inflammatory lipid mediator production is to protect the cell membrane from sPLA2 and incorporates into the cell membrane, interferes with sPLA2 action at the cell membrane.[83] To assess PLA2-COX coupling leading to PGE2 generation, sPLA2 was induced after cytokine stimulation, with IFN-γ exhibiting a more potent effect than IL-1β or TNF-α.[84]

Reactive Oxygen Species Targets PLA2

Reactive oxygen species (ROS) and reactive nitrogen species play an important role during the pathogenesis of MS. ROS are produced primarily by mitochondria as a by-product of normal cell metabolism during conversion of molecular oxygen.[85] ROS are implicated as mediators of demyelination and axonal damage in MS.[86–88] ROS can damage lipids, proteins, and nucleic acids of cells, resulting in disturbed mitochondrial function, which may induce cell death.[89,90] ROS may also damage the myelin sheath by promoting its attack by macrophages.[88] Furthermore, oxidative stress is involved in the cascade of events leading to neuronal cell death.[91] Oxidative stress is defined as the imbalance between biochemical processes leading to production of ROS and those responsible for the removal of ROS, the so-called cellular antioxidant cascade. Many lines of evidence suggest that oxidative stress resulting in ROS generation and inflammation play a pivotal role in the age-associated cognitive decline and neuronal loss in neurodegenerative diseases.[92] The brain's high oxygen

consumption can generate ROS and damages, which increase with age, in brain mitochondrial DNA, while neuronal membranes rich in PUFA-phospholipids.[93] The brain possesses modest antioxidant defenses for keeping oxidative stress as low as possible with reliable physiological functions.[94] Numerous studies now indicate that ROS themselves can increase/induce cellular COX-2 expression.[95–99] Free radical scavengers in numerous cell types including neurons can prevent this oxidative stress–induced COX-2 expression.[95,98,100] Several studies demonstrate that excitotoxicity and oxidant stress are specific and important inducers of COX-2 gene expression. Finally, the formation of ROS is important for the activation of cellular PLA2.[101] Druzhyna et al. and Cross et al. showed proinflammatory cytokines TNF-α and IFN-γ stimulate oligodendrocytes, macrophages, and microglia to express inducible nitric oxide synthase that produces nitric oxide (NO).[87,102] These findings support a role of ROS in phospholipase-mediated cell signaling.[103] It is thus reasonable to hypothesize that increased oxidative stress may be one of the mechanisms responsible for the reduction of membrane PUFAs. Increasing evidence has shown that the production of proinflammatory cytokines such as IL-1, IL-6, and IFN-γ may be affected by psychological stress. Maes et al. showed that the in vitro production of proinflammatory cytokines (IL-6, TNF-α, and IFN-γ) and IL-10 were increased significantly by stress.[104] Antioxidant status is defined as the balance between antioxidants and prooxidants in living organisms.[105] The brain, which is rich in PUFAs, is particularly vulnerable to free radical–mediated damage.[106] The brain is enriched in the more easily peroxidizable fatty acids and is not particularly enriched in antioxidant defenses.[94] Clinical trials of several neurodegenerative diseases have increasingly targeted the evaluation of the effectiveness of various antioxidants, for example, tea green flavonoids: catechins.[107] However, the limited regenerative nature of the CNS and the fact that diagnosis often does not occur until late in disease progression suggest that the ideal antioxidant should be a prophylactic given, continuously, before aging.[94] It is not clear at present if antioxidant deficiency in MS is constitutive or due to excessive antioxidant consumption to cope with oxidative stress. Antioxidant deficiencies may develop during the course of MS because of chronic inflammation that is accompanied by increased oxidative stress. Levels of the antioxidants α-tocopherol, β-carotene, retinol, and ascorbic acid were decreased in the sera of patients with MS during an attack.[108] This was also illustrated by increased oxidative burden, as reflected by lipid peroxidation. Another study showed lipoprotein oxidation being an important sign of oxidative stress during the course of MS.[109] CSF vitamin E levels were not significantly different between patients with MS during exacerbations and controls, whereas serum levels of vitamin E indeed were lower in patients with MS.[110] In MS plaques, levels of glutathione and vitamin E were significantly decreased as compared with adjacent and distant white matter.[111] Therefore antioxidant therapy may be beneficial in restoring these deficiencies and supporting the antioxidant defense capacity of patients with MS.[42]

Subcellular Localization of PLA2

The intracellular organelle plays central roles in the energy metabolism and homeostasis of the cell. Mitochondria are subcellular centers of energy metabolisms and key regulators of cell survival and death. Through these functions, mitochondria are involved in the development of lesions and define the fate of effector immune cells in the CNS.[112] The severity of mitochondrial changes secondary to inflammation correlates with the severity of neurodegeneration and the clinical disease course (Fig. 24.1). An acquired mitochondrial impairment may cause oligodendrocytopathy and demyelination.[112] In addition, the mitochondrion is a cytoplasmic organelle surrounded by a double membrane.[113]

The evidence suggests a link between inflammation, demyelination, and neurodegeneration via an acquired mitochondrial dysfunction. Inflammation-induced impairment of the mitochondrial energy metabolism may contribute to both acute forms of apoptosis and delayed inflammation-induced tissue degeneration in MS.[112] On the other hand, the subcellular localization of cPLA2γ also differs from those of other cPLA2s. The reports are not entirely consistent but cPLA2γ has been found in various membrane fractions, including endoplasmic reticulum and mitochondria cPLA2γ is a key enzyme in eicosanoid production. cPLA2γ was

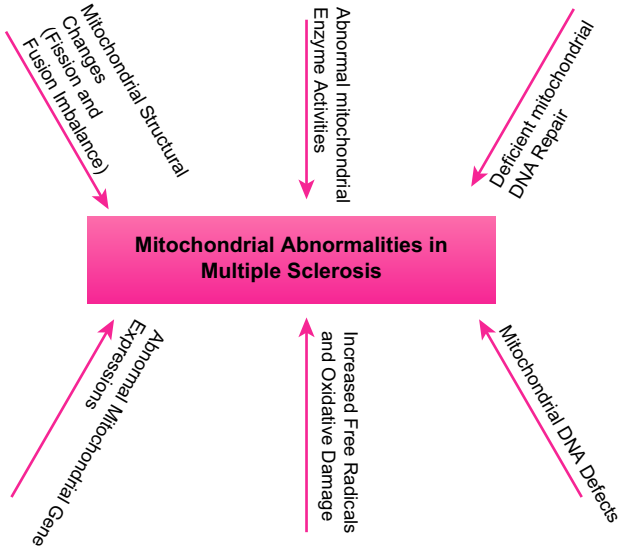

FIGURE 24.1 Mitochondrial abnormalities in patients with MS.[1]

reported to be located in endoplasmic reticulum and mitochondria and to have lysophospholipase activity beside PLA2 activity. cPLA2γ does have PLA2 activity, and some transacylation of FA from the *sn*-2 position of PC or PE to choline or ethanolamine lysophospholipids was also observed.[114]

Therefore, with note to evidences, disturbance in structure of phospholipids and dysregulation of lipid metabolism in myelin and subcellular membrane such as inner mitochondrial membrane, with a wide variety of enzymatic activities, may contribute to the acute oligodendroglial and neuronal apoptosis and chronic neurodegeneration in MS. Based on Fig. 24.1, increase in free radicals and oxidative damage may elevate cPLA2γ activity and play a predominant role in the inflammatory cascade, and finally in mitochondrial damage and necrosis.

Role of PLA2 in Pathophysiology of MS

Neurological and Inflammatory Disorders and Control of PLA2 for the Treatment

Cytosolic PLA2 plays a key role in the pathogenesis of MS-like and production of proinflammatory mediators, namely, the AA-derived eicosanoids, lysophospholipids, and platelet activating factor, and indirectly influences the generation of cytokines, NO, and free radicals.[115] Extracellular PLA2 inhibitors suppress CNS inflammation.[116] PLA2 appears to play a fundamental role in cell injury in the CNS. The AA liberated is converted to PGE2, possibly by COX-2, which is induced by inflammatory stimuli.[82] In the CNS, sPLA2 mRNA is expressed in response to the proinflammatory cytokines TNF-α, IL-1β, and IFN-γ.[78] It has been hypothesized that a highly reactive PLA2 is found in various psychiatric disorders. When coupled with high ω6-PUFA content in the cell membrane, it would thus lead to aggravated inflammatory conditions. This would be limited by the presence of sufficient ω3 fatty acids in the membrane. PUFAs can modulate many of the signal transduction mechanisms operating in neuronal membranes.[79] PLA2s have implicated in the pathology of a number of neurodegenerative diseases.[78] Increased activities of PLA2 and generation of lipid mediators play a central role in oxidative stress and neuroinflammation associated with neurological disorders such as ischemia, spinal cord injury, AD, MS, prion diseases, and epilepsy.[117] Hence, PLA2 inhibitors (PLIs) can be used as neuroprotectants and antiinflammatory agents against neurodegenerative processes in human disorders.[117] A number of putative mechanisms have been identified to explain the decreased PUFA levels in schizophrenia.[118] Increased cytoplasmic PLA2 activity has been found in serum of drug-free schizophrenic patients.[119,120] Moses et al. showed that secretory PLA2 is a new inflammatory factor for AD and sPLA2 upregulation in AD brain may facilitate the development of novel therapeutic strategies to inhibit the inflammatory responses and to retard the progression of the disease.[80]

Depression is a major feature of MS. Both depression and bipolar disorder are much more common in patients with MS than in the general population and in at least some the psychiatric disorder precedes the diagnosis of MS and so cannot be a reaction to it.[121,122] High levels of sPLA2 have found in serum and exudates from patients suffering from inflammatory diseases, like rheumatoid arthritis or acute pancreatitis and trauma.[123,124] For a range of diseases including rheumatoid and osteoarthritis, asthma, acute pancreatitis, and septic shock, recent research has focused on the role of PLIs as possible antiinflammatory agents.[125] PLA2s are present in the brain and spinal cord and are implicated in several neurological disorders. PLA2 activity increases following traumatic spinal cord injury and injection of secretory PLA2 demyelinates spinal cord axons.[126] Therefore the central role of PLA2 in inflammation makes this enzyme a potential therapeutic target.[116] cPLA2 enzyme and products influence several aspects of the cellular and cytokine involvement in the inflammatory response. Patients with MS also showed elevations in sPLA2 enzyme activity.[127] However, there are no effective sPLA2 or cPLA2 inhibitors available for clinical use.[128] Hence, inhibition of specific PLA2 and elevated levels of inflammatory cytokines TH1 may represent novel therapeutic strategies against these diseases.

HEMPSEED AND EVENING PRIMROSE WITH HOT-NATURE DIET FOR MS

Hempseed Oil as a Nutritional Resource

Hempseed (HS) or *C. sativa* L has been an important source of nutrition for thousands of years in Old World cultures.[129–132] HS typically contains over 30% oil and about 25% protein, with considerable amounts of dietary fiber, vitamins, and minerals. HS has been used to treat various disorders for thousands of years in traditional oriental medicine. Clinical trials have identified HSO as a functional food.[133] HSO has been used as a food/medicine in China for at least 3000 years.[131] HSO is over 80% in PUFAs, and is an exceptionally rich source of the two EFAs (LA and ALA). The ω6/ω3 ratio in HSO is normally between 2:1 and 3:1, which is considered optimal for human health.[35] The ω6/ω3 ratio in most commercial HSOs is typically near 2.5:1.[134,135] The presence of both GLA and SDA in HSO, typically at a favorable ω6/ω3 ratio of 2:1, allows this enzymatic step with D6D to be efficiently bypassed (Table 24.4).[136]

TABLE 24.4 Fatty Acid Profiles (%) of Hempseed and Evening Primrose Oils

Seed Oil	Palmitic Acid	Stearic Acid	Oleic Acid	Linoleic	ALA	GLA	SDA	% PUFA	n-6/n-3 Ratio
Virgin hempseed	5	2	7–16	56	22	7	2.5	84	2.5
Evening primrose	6	1	8	76	0	9	0	85	>100

From a nutritional point of view, up to 7% GLA and 2.5% SDA are very interesting. Due to the high amount of USFAs, HSO is very susceptible to oxidative deterioration, which results in a fast impairment of the oil during storage. The result is a product with an intensive green color, because of the high amounts of chlorophyll coextracted with the oil. Virgin HSO is characterized by a nutty taste with a slightly bitter aftertaste. The use of virgin HSO is recommended during mild processing of food without heat.[137] One published report has described the application of HS porridge, from folk medicine, in the treatment of tuberculosis without antibiotics.[138] The FA profile of HSO is remarkably similar to that of black currant seed oil, which also seems to have a beneficial impact on immunological vigor.[139,140]

Tocopherols, Phytosterols, and Terpenes in Hempseed Oil

Vitamin E is an important antioxidant that can interrupt the propagation of free radical chain reactions. The total amount of tocopherols in virgin HSO is high between 80 and 110 mg/100 g, with γ-tocopherol as the main tocopherol (85%). The ratio of the tocopherols in HSO is 5/2/90/3 for α-, β-, γ-, δ-tocopherol, respectively.[137,141] Investigating the health benefits of phenolic compounds is an enormous challenge to modern medicine.[142] Phytosterols are phenolic compounds that not only exhibit potent antioxidative properties for scavenging free radicals, but may also act on specific signaling pathways for regulating inflammatory responses. Investigation revealed that HSO contains 3.6–6.7 g phytosterols/kg oil (total phytosterols, 3922–6719 mg/kg oil), with β-sitosterol as the main component (70% of the total phytosterol content). Other phytosterols with some importance are campesterol, D5-avenasterol, and stigmasterol.[137] Several findings suggest that β-sitosterol is responsible for radical scavenging and antioxidant activities for preventive effects on the development of diseases due to ROS. Investigating the health benefits of phenolic compounds is an enormous challenge to modern medicine.[142,143] Moreover, Yoshida and Niki showed the antioxidant effects of the phytosterols β-sitosterol, stigmasterol, and campesterol, against lipid peroxidation.[144] The efficacy of β-sitosterol in reducing hypercholesterolemia, and additional antiviral, antifungal, and antiinflammatory properties have been studied and observed.[145] Within the intestinal lumen, phytosterols reduce cholesterol absorption. In addition, competition exists between the sterols and cholesterol for uptake into the intestinal mucosa.[146] The presence of several terpenes was confirmed in HSO, sesquiterpenes in very low concentrations, with the exception of β-caryophyllene and α-humulene that included 13% and 5% of total compounds, respectively. Myrcene, α-pinene, and β-pinene were the main compounds among the monoterpenes.[147] The most abundant β-caryophyllene and myrcene that were found were at 740 and 160 mg/L, respectively. β-Caryophyllene would include antiinflammatory and cytoprotective activities[148] and it has been reported that myrcene exhibits antioxidant properties.[149]

Evening Primrose Oil as a Disease-Modifying Agent

O. biennis L or evening primrose contains oils rich in ω6-GLA, precursors of eicosanoids, which are constituents of cell membranes. The biochemical pathway for metabolism of dietary GLA eventually leads to PGE1, which has potent antiinflammatory activity and is often recommended for inflammatory and autoimmune conditions.[150] EPO is being used in increasing amounts in nutritional and pharmaceutical preparations, and there are claims that it may alleviate various chronic disease states.[53,151,152] The EPO content of 9% GLA is the single most important parameter that is metabolized into DGLA, the natural precursor of PGE. β-Carotene is a pro-vitamin A and gives a characteristic color to EPO.[153] Horrobin showed that preliminary results of the use of EPO and colchicine combined therapy in patients with MS suggest that it may be of considerable value.[154] Phospholipids comprised only 0.05% of the oil, with the composition: phosphatidylcholine (31.9%), phosphatidylinositol (27.1%), phosphatidylethanolamine (17.6%), phosphatidylglycerol (16.7%), phosphatidic acid (6.7%).[155]

Ratio of Hempseed/Evening Primrose Oils

Thereby limitation of budget, the HSO/EPO ratio in this study is 9:1(8:2 ratio is better, which is considered to be optimal for human health), because, the therapeutic dosage of EPO in clinical trials Pruthi and Jantti was 3 g/day and 10 mL twice daily, respectively.[156,157] The

recommended daily dosage of EPO for adults does not exceed 4 g (containing ~300–360 mg GLA). In some cases, such as atopic eczema, the dosage could temporarily be higher, 4–8 g/day. For children, the advisable dose is 2–4 g/day.

Description of Diet With Hot Nature

Dietary consumption of Cold-nature foods and drinks were different. Several factors have been identified that could contribute a potential for the treatment properties which could be increased by diet of Hot nature. This includes:

1. Consumption of foods with Hot nature in diet can be very helpful.
2. Low intake of cholesterol.
3. Reducing the intake of saturated fats (particularly from fried foods).
4. Low intake of hydrogenated or trans fatty acids (the artificial fats found in most margarines and processed foods).
5. Eating plenty of fresh fruit and vegetables with Hot nature, nuts and seeds without additives, fish and seafood, and unrefined carbohydrates.
6. Drinking plenty of water (and avoiding too much drink that contains artificial additives and sweeteners or other stimulants).
7. Cutting down on sugar and refined starch (i.e., non–whole meal bread, cakes, pastries, biscuits, sweets, and soft drinks, which often have a high artificial additives content and sweeteners, which may also be best avoided).
8. Consumption of dairy products with honey or date.
9. Removing foods with Cold nature from the diet can be very helpful.
10. Avoid consumption of alcohol and smoking.

Evaluation of *Mizadj* or "Hot and Cold Natures"

The elaboration of the theory of "Hot and Cold natures" finds its origin in ancient Greece, by Hippocrates (Greek physician, 460–375 BC) and Galen (199–129 BC).[158,159] Hippocrates says let our diet be our medicine, and Avicenna said that for each person there is specific foods for himself. When attempting to determine a person's *Mizadj* (*Mizadj*: degree of Warmth/Coldness or degree of Th2/Th1 or degree of IL-4/IFN-γ), it is observed that intermediate forms or combinations of two or more temperaments are the rule rather than the exception. Therefore most people are under the influence of both the Hot and the Cold elements[160] and we can evaluate the severity of each nature in a person that can be shown as Warmth/Coldness ratio. In a person with a very Hot nature, the severity of the Warmth element is high and that of Coldness element is low. Therefore in such a person, the Warmth/Coldness ratio is high (such as allergic patients with tendency to Th2-like responses). In a person with a very Cold nature, the severity of the Warmth element is low and that of the Coldness element is high. Thus in such a person the Warmth/Coldness ratio is low (such as patients with MS with tendency to Th1-like responses).

The nature of a person can also be important in connection with allergy. It seems that an allergen can induce allergic reaction in Hot-nature persons with a higher probability than in Cold-nature persons, because the former have a greater tendency to Th2 responses than the latter, and, as mentioned earlier, Th2 responses to an allergen are necessary for the development of an allergic reaction to the allergen.[161] Increasing evidence indicates that Th2 cytokines dominate immune responses during infancy and early childhood, but the shifting toward Th2 pattern decreases with age.[162,163] This is in agreement with TIM's belief that the nature is dominated by Warmth at birth but its Warmth decreases with age.[22]

Hence, an allergen can induce allergic reaction in child more than in adults. Shahabi showed the persons of a Hot nature had more deviation of the immune system toward Th2 immune responses than the persons of a Cold nature, and in concordance with TIM practitioners' view that MS, which is a Th1-mediated autoimmune disease, is more prevalent in Cold-nature persons than in Hot-nature persons.[24] According to TIM, mean *Mizadj*, indicated that in supplement oil groups have a higher rate of deviation of the immune system toward Th2 responses and the intensity of Warmth/Coldness of nature is more, and were healthier in comparison with olive oil group,[31] while a hallmark in the pathogenesis of MS is a shift in the ratio of Th cells toward cells of the Th1 phenotype, which is accompanied by abnormal cytokine production.[164,165] Warmth/Coldness effects on supplement oil groups patients with MS, it is highly possible that Hot-nature substances have such an effect as well, because Hot-nature substances such as cosupplemented oils and Hot-nature diet accelerate Warmth of nature, and Cold-nature substances accelerate Coldness of nature in patients. Therefore paying due attention to the nature of the diet of patients may be important for the treatment of their diseases and to prevent their acceleration. For example, in a person suffering from an autoimmune disease with a deviation toward Th1 immune responses (such as MS), consumption of Hot-nature foods may be useful because they can accelerate warmth of nature and deviation toward Th2 immune responses in supplement oil groups, whereas consumption of Cold-nature foods aggravates their disease. This would mean less deviation toward Th1 immune responses and may lead to a reduction in disease severity in the supplement oil groups. In addition, women are dominated twice more than men by

Cold nature and this confirms autoimmune diseases are mostly common in women than in men (such as MS).[25] In Iran, the female to male ratio exceeds 3:1.[2]

Treatment of Patients With Multiple Sclerosis

The Rezapour-Firouzi studies compared the effects of dietary interventions on Expanded Disability Status Scale (EDSS) measures in patients with MS receiving a steady disease-modifying therapy. The results suggest that the cosupplemented oils with or without Hot-nature diet used might have a therapeutic effect toward MS.[31] At present, no pharmaceutical or other therapy exists that can confer prolonged remission in MS and therapeutic agents are only partially effective. Their long-term beneficial effects are uncertain and often-detrimental side effects have been reported.[166,167] In MS treatment, strategies can be either acute or long term. During the relapse, the goal of the treatment is to reverse neurological disability, delay further neurological dysfunction, and restore normal function. This is in contrast to the goal of long-term treatment, which is to decrease relapses (severity and frequency) and stop disability progression.[168] The major difference detected in this trial was the greater reduction rate of clinical relapses in cosupplemented oils groups. As a result, three diets might have the potential to improve patient's perception of physical and emotional disease burden in patients with MS, although both the case diets effects emerged as a more efficient intervention. In the Rezapour-Firouzi study, immunological assay confirmed the results of clinical examinations.[31] A hallmark in the pathogenesis of MS is a shift in the ratio of Th cells toward cells of the Th1 phenotype, which is accompanied by abnormal cytokine production.[164,165] The Th17 axis was also examined in this study. The current literature suggests Th17 immunity plays an important role in autoimmune diseases such as MS and blocking this cytokine network protects against autoimmune disease.[8–10] Rezapour-Firouzi evaluated multiple immunological parameters and significant differences were seen in the IL-4, IFN-γ, and IL-17 cytokine concentrations in the three trial groups, although certain trends for a stronger antiinflammatory effect was seen in cosupplemented oils groups, and revealed that cosupplemented HSO and EPOs and Hot-nature diet had an effect of reduction on the Th1 cytokine (IFN-γ) and Th17 cytokine (IL-17), but increased the Th2 cytokine (IL-4), and targets this key mechanism of disease and works like approved treatments.[31]

Shahabi showed that allergy is characterized by an imbalance toward the Th2 response[24]; similarly, the Rezapour-Firouzi results suggest that altering cytokine profiles is a potential mechanism that may influence allergic responses and alter the profile of cytokines known to participate in inflammatory and autoimmune responses.[31] It may therefore explain why therapies that promote a Th1 to Th2 cytokine shift are beneficial in MS. All approved therapies, in addition to many of those under investigation, appear to possess immunomodulatory and antiinflammatory roles as the main mechanism of action.

The study of Rezapour-Firouzi indicated an association between EDSS and IL-17 and IFN-γ concentrations, and a significant inverse correlation between EDSS and IL-4 in patients, as well as increased expression of IL-4 in patients with RRMS.[30] This would mean less deviation toward Th1 immune responses and may lead to a reduction in disease severity in the patients of cosupplemented oils with diet group. Consistent with the increase of cytokines of Th2/Th1 ratio (*Mizadj*), EDSS parameters were significantly better in the cosupplemented oils with diet group, and indicated greater reduction in relapses rate in cosupplemented oils with diet group.[30] Current studies found decreased levels of both ω3 and ω6 PUFAs in red blood cells (RBCs), plasma, and adipose tissue of patients diagnosed with MS, and a shortage of dietary PUFAs may be a risk factor in MS.[169] In combination, changes in cytokine production may provide prolonged changes in inflammatory responses relative to the rapidly reequilibrating levels of PUFAs and their metabolites. This suggests that the effects of dietary PUFAs differentially affect inflammatory functions and cytokine production from mononuclear cells during the intervention. Because MS is associated with an activated inflammatory response, ω3-PUFAs can suppress IFN-γ, IL, and TNF production in MS subjects.[29] Very high levels of FAs and lipids can be found in two structural components, the neuronal membrane (about 50%) and the myelin sheath (about 70%), and a high proportion of lipids 70–85%.[38] In addition, the BBB is a key to the bioavailability of brain essential fatty acid (EFA) and PUFA.[67] Using chromatographic lipid profiling, Rezapour-Firouzi et al. confirmed the expected significant increase in RBC PUFAs rate in the cosupplemented oils groups, whereas in the olive oil group it was not significant during the study. In addition, the PUFAs rate increased with the EDSS and functional score benefits at the last visit.[32] This results likely due to remyelination that occurs during the early phases of disease, whereas this is rare at more progressed stages.[170] It is suggested that dietary compounds such as PUFAs may support this process.[171] Current estimates of the ω6/ω3 PUFAs ratio in developed countries are as low as 1:25 with recommendations to the public that it should be much higher.[172] In the Rezapour-Firouzi et al. study, with combined HSO and EPO formulation (HSO/EPO: ratio 9/1),[35] the ω6/ω3 PUFAs ratio reach 1:4 or higher, that is competitive inhibition of the conversion of DGLA to AA resulting in more antiinflammatory PGE1.[173] AA is a precursor of proinflammatory and proaggregator PGE2, whereas EPA is a precursor of antiinflammatory PGE3, and GLA and DGLA

are precursors of antiinflammatory PGE1. GLA is produced in the body from desaturation of LA by the reaction catalyzed by the enzyme D6D. The activity of D6D had become impaired by aging, viral infection, high alcohol intake, high cholesterol level, high blood pressure, radiation, stress-related hormones, nutritional factors (deficiencies of zinc, magnesium, biotin, vitamins C, B6, B3, and excessive level of trans fatty acid), diabetes,[52,53] and genetic deficiency [inactive delta-5 desaturase (D5D) and D6D enzymes].[51] GLA is rapidly elongated to DGLA by elongase enzyme. The reaction catalyzed by D6D enzyme is the slowest reaction in the metabolic pathway of LA and is considered as a rate-limiting step. Desaturases catalyze the synthesis of PUFAs that are incorporated into cell membranes, which thereby affect permeability, and functional properties of cells. D5D and D6D are two enzymes required for the synthesis of LC-PUFA in mammals.[174] The noticeable presence of both GLA and SDA in HSO and EPO, typically at a favorable ω6/ω3 ratio of 2:1 allows this enzymatic step with D6D to be efficiently bypassed.[136] In the Rezapour-Firouzi et al., study D6D concentration decreased significantly in cosupplemented oils groups, whereas the olive oil group showed a nonsignificant in concentration of D6D.[34]

In considering the inflammatory role of secretory PLA2 (sPLAs), phospholipids constitute approximately 40%, 60%, and 90% of the total lipids in myelin, erythrocyte, and mitochondria, respectively, that play a role in double biomembrane structure.[122] Metabolism of PUFAs in membrane phospholipids is stringently controlled by PLA2 and acyl-transferases known as the "deacylation–reacylation cycle."[30] There is ample evidence that sPLA2 is involved in diverse inflammatory conditions, implicating almost all of membranes in any organ of the body (such as myelin, erythrocyte, and mitochondria).[91] The PLA2 superfamily hydrolyzes phospholipids to release free fatty acids and lysophospholipids, some of which can mediate inflammation and demyelination, hallmarks of the CNS autoimmune disease MS.[31] Mean levels of sPLA2 were increased sixfold in the urine of patients with MS with active disease and fourfold in patients in remission, regardless of immunomodulating therapy.[32] Thus PLA2 could serve as a convergence point in the induction of MS pathology because it can be induced by a variety of chemokines and cytokines present in the CNS in the early stages of these diseases and because it is metabolic products mediate both inflammation and demyelination.[124] Hence, inhibition of specific PLA2 and elevated levels of inflammatory cytokines Th1 may represent novel therapeutic strategies against these diseases. We found that elevated serum level of PLA2 activity in the patients (baseline), which might be due to increase hydrolysis of membrane phospholipids by PLA2, is a well-known early response to tissue damage in all organ systems including myelin in the nervous system, erythrocyte, and mitochondria. In the Rezapour-Firouzi et al. study, sPLA2 concentration decreased significantly in the cosupplemented oils groups and estimated sPLA2 and D6D concentrations were both inversely correlated with PUFA levels, and the olive oil group showed a nonsignificant in concentrations of sPLA2.[34]

The Rezapour-Firouzi et al. study's findings imply reduction in clinical symptoms and signs, and the patients' general health and well-being improved, which may be due to evidence of higher PUFA in peripheral tissue (RBCs) and maybe in brain tissue and mitochondria, and support the hypothesis of EFA abnormalities in MS, and indicate that the problem could well be one of conversion of EFA to PUFA, as originally suggested.[32,34]

Supplementation with PUFAs may require additional vitamin E intake to prevent increased peroxidation of membrane lipids.[175] Although the total amount of tocopherols of HSO is high, between 80 and 110 mg/100 g, γ-tocopherol is the main tocopherol (85%) that exhibits potent antioxidative properties for scavenging free radicals.[137] Since USFAs are highly susceptible to peroxidation, an increased intake of these agents without antioxidant protection might produce the undesirable effect of decreasing CMF through peroxidative cross-linking reactions in the cell membrane.[176] The "free radical" theory of aging[177] accords well with the fact that lipid peroxidation leads to decreases in CMF.[178] Cosupplemented oils are foodstuffs and do not act as rapidly as most medications, so any effects will take time to appear. Most individuals who respond to supplementation usually report noticeable benefits within 1 or 2 months. The minimum trial period should be at least 6 months, as studies have shown that it takes 10–12 weeks for PUFA levels in brain cell membranes to return to normal after a long-standing deficiency.[179] CMF is a parameter crucial to the maintenance of cellular function. Alterations in CMF are seen in several disease processes and the normalization of CMF in these diseases may prove therapeutic,[68,69] with the availability of membrane receptors[180] and events occurring during the course of the cell cycle.[181] The activity of membrane-associated enzymes increases in almost fluid membranes.[68] The rate of myelin lipid turnover is age dependent, and with a very slow turnover rate during aging, and the rate of repairing damaged sections of myelin is correspondingly slower.[76] Finally, the membrane fluidity index or CMF is a common denominator for the various effects of the various PUFAs and ω6/ω3 ratios.[39,40] It is another proof for the importance of our claim for the importance of our dietary intervention that no MS disorder happen during primary part of life and subjects with MS develop this disease in the period of their life that accompanies with their dramatic changes in their diet. In the Rezapour-Firouzi et al.

study, analysis of RBC total FAs in cosupplemented oils groups showed an increase in EFAs and PUFAs in RBC membranes, whereas examining the FAs composition of erythrocyte membranes of patients with MS in the olive oil group showed a decrease in PUFA and an increase in SFA levels.[32] Increase of EFAs/UEFAs or PUFAs/SFAs ratio agrees with the decrease of EDSS that was significantly better in cosupplemented oils groups compared with the olive oil group. It is suggested that cosupplemented HSO and EPO have a healthful balance of ω6/ω3 (2:1) fatty acids. That intervention modulates overall membrane fatty acid composition, and may help reduce the risk of MS. Alterations in the proportions of various fatty acid classes in Rezapour-Firouzi et al. were shown in-group cosupplemented oils and diet of patients with MS had significantly better values in comparison with cosupplemented oils and olive oil groups.[32] Furthermore, increases in SFAs and/or MUFAs have reported to replace plasma and/or RBC membrane PUFA deficiencies,[169,182] and this fact is present completely in the Rezapour-Firouzi et al. trial.[33]

The liver is the organ responsible for plasma synthesis, drug detoxification, and digestion. A few studies exist in relation between MS and markers of liver. Liver enzymes were elevated with treatment of interferon-β1b,[183] because the liver is the organ responsible for plasma synthesis, drug detoxification, and digestion. Rezapour-Firouzi et al. found an inverse relation between dietary supplement and markers of systemic inflammation [aspartate-aminotransferase (AST), alanine-aminotransferase (ALT), and gamma-glutamyl transferase (GGT)], particularly in cosupplemented oils groups of patients with MS. This suggests that compositions of cosupplemented oils as a functional food, likely dietary antioxidants, could protect against inflammation by decreasing AST, ALT, and GGT concentrations, primarily in patients at risk.[33] In conclusion, qualitatively selecting food items based on their total antioxidant capacity (TAC) was a useful and effective approach to demonstrate that, in addition to the quantity, the quality of certain food groups may be crucial to decreasing hepatic and systemic inflammation in patients with MS, and diets with a high dietary TAC can modify oxidative stress, low-grade inflammation, or liver dysfunction, all of which are risk factors for patients with MS.

Studies of Rezapour-Firouzi et al. suggest that cosupplemented oils and Hot-nature diet appear to possess immunomodulatory and antiinflammatory aspects; regarding the beneficial properties of this intervention, it can have prophylactic and therapeutic properties in patients with MS, and affect membrane phospholipid fatty acid composition, increase RBC membrane PUFA concentrations, and likely repair of mitochondrial and myelin membranes. The cosupplemented oils might be given alone or during treatment with synthetic drugs to permit reduction of dose level of the latter, and can be administered orally. Hot nature dietary intervention with cosupplemented hempseed and EPOs cause an increase PUFAs in patients with MS and improvement in the erythrocyte membrane fatty acids composition and it could be an indication of restored plasma stores, and a reflection of disease severity reduction,[30–34] to a novel therapeutic and protective agent and correct diet based on TIM for patients with MS.

FUTURE DIRECTIONS

1. The Rezapour-Firouzi et al. study recommends prospective studies on the use of cosupplemented HSO and EPO (8:2 ratio is better, which is considered to be optimal for human health) either alone or in conjunction with different immunomodulating therapy synthetic drugs (which may have synergistic effects with each other) for longer periods in patients with MS.[31]
2. Results of Rezapour-Firouzi et al. likely suggest that the cosupplemented, and diet and cosupplemented oils, which contribute to interindividual variability in serum IL levels, may affect not only D6D but also D5D activity, positively, and showed benefit from replication with an influence of cosupplemented oils on FADS2 gene transcription of patients with RRMS.[32,34]
4. Consistent with data that have shown an influence of cosupplemented oils on FADS2 level, our results also provide a rationale for performing additional functional studies on the FADS1 and FADS2 genes in all groups of patients with MS and healthy adults.[34]
5. Supplementing the diet with EFAs from sources such as HSO and EPO may prevent several inflammatory diseases.
6. It is likely that there are correlations between MS and an elevated level of D6D as shown in serum of patients.
7. The loss of activity and expression of D6D stresses the importance of supplementing diets with cosupplemented HSO and EPO to avoid deficiency, metabolic complications, or diseases.
8. To enhance the benefits of cosupplemented HSO and EPO, a regimen of supplements, such as Mg, Zn, and vitamins B6, B3, and C, should be taken. These supplements are important regulating factors of the D6D enzyme.
9. It is likely that the changes in lipid biology identified in MS may be relevant to other psychiatric conditions and more generally to other neurodevelopmental and neurodegenerative disorders.

10. As well as clinical outcome measures, future studies should incorporate additional outcome measures related to lipid metabolism, such as the measurement of enzyme activities (e.g., phospholipases), and EFA analysis.
11. The Rezapour-Firouzi et al. studies also established the need for a study on the long-term safety and efficacy of this cosupplemented oil in a larger population. The effects observed on cytokine and enzyme (D6D, sPLA2) concentrations in the patients with RRMS also need to be examined in future studies by using carefully controlled diets in appropriately selected populations.
12. Results of the Rezapour-Firouzi et al. studies do not compare the changes seen with other available topical immunomodulating therapy agents, such as steroids; such direct comparisons may be worthwhile future studies, that clearly indicated to optimize dosing and formulations that are maximally effective.
14. Results of the Rezapour-Firouzi et al. studies indicate that cosupplemented HSO and EPO can protect the brain from demyelination and stimulate remyelination, and are, thus, promising new therapeutic tools for the clinical treatment of MS. Heretofore, all approved therapies of MS, in addition to many of those under investigation, appear to possess immunomodulatory and antiinflammatory roles as the main mechanism of action. Nevertheless, one important therapeutic goal during CNS injury from demyelinating diseases such as MS is to develop methods to promote remyelination.

ABBREVIATIONS

AA Arachidonic acid
ALA Alpha-linolenic acid
ALT (SGPT) Alanine-aminotransferase
AST (SGOT) Aspartate-aminotransferase
BBB Blood–brain barrier
CMF Cell membrane fluidity
CNS Central nervous system
COX Cyclooxygenase
cPLA2 Cytosolic PLA2
CSF Cerebrospinal fluid
D5D (FADS1) Delta-5-desaturase
D6D (FADS2) Delta-6-desaturase
DGLA Dihomo-gamma-linolenic acid
DHA Docosahexanoic acid (omega-3)
EDSS Extended Disability Status Score
EFAs Essential fatty acids
EPA Eicosapentanoic acid (omega-3)
EPO Evening primrose oil
GGT Gamma-glutamyl transferase
GLA Gamma-linolenic acid
HS Hempseed
HSO Hempseed oil
IFN Interferon (β1b-β1a-β)
IFN-γ Interferon-γ
IL Interleukin (IL-2-IL-4, IL-5, IL-6, IL-10, IL-17)
LA Linoleic acid (omega-6 Family)
LC-PUFA Long-chain polyunsaturated fatty acid
LOX Lypoxygenase
LTs Leukotrienes
LysoPCs Lysophosphatidylcholines
LysoPL Lysophospholipids
MS Multiple sclerosis
NO Nitric oxide
PBMC Peripheral blood mononuclear cell
PC Phosphatidylcholine
PE Phosphatidylethanolamine
PG Prostaglandin
PGE Prostaglandin E (PGE1, PGE2, PGE3)
PLA2 Phospholipase A2
PUFA Polyunsaturated fatty acid (ω3-PUFAs, ω6-PUFAs)
RBC Red blood cell
ROS Reactive oxygen species
RRMS Relapsing remitting MS
SDA (STA) Stearidonic acid
sPLA2 Secretory PLA2
Th T helper (1-2-17)
TIM Traditional Iranian Medicine
TNF-α Tumor necrosis factor-α
UEFAs Unessential fatty acids
USFAs Unsaturated fatty acids
ω3-PUFAs omega3-polyunsaturated fatty acids
ω6-PUFAs omega6-polyunsaturated fatty acids

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SECTION VI

FOODS IN MULTIPLE SCLEROSIS

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CHAPTER

25

The Role of Natural Products in the Prevention and Treatment of Multiple Sclerosis

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INTRODUCTION

The existing multiple sclerosis (MS) drugs aim to stop relapses and/or slow the progression of the disease. Different classes of medications including immunosuppressive, antiinflammatory, and immunomodulatory drugs have been used successfully for this purpose. However, long-term use of these drugs is usually concomitant with various adverse drug reactions that reduce the patients' compliance.[1] Furthermore, the high price of MS drugs may affect the quality of life of the affected patients. The use of herbal-based medicines for the treatment of various diseases has risen substantially. Herbal medicine as an alternative or complementary treatment of MS may be used to increase the efficacy of the current MS treatments or reduce the side effects of drugs. For example, it has been reported that an Oriental herbal medicine, *Erhuang*, improves clinical symptoms and neurological signs, and decreases the rate of relapse in animals and patients with MS.[2] By employing animal models of MS, such as experimental autoimmune encephalomyelitis (EAE), our knowledge about the effects of herbs or their compounds in the treatment of this disease has increased substantially over the past decade. The similarities between EAE and MS have made it a good and valid model for the investigation of new drugs either from laboratories or from nature.[3] In this chapter, we will review the latest findings concerning the effects of herbs and some natural products in the treatment of MS in animals and humans. This is an effort to collect and summarize the effects of the compounds of natural origin in the treatment of MS. Some of these compounds have presented good and satisfactory results in basic experimental studies. However, more studies are needed to evaluate the effects of these compounds or products before clinical use.

ACHILLEA MILLEFOLIUM

Achillea millefolium (yarrow) belongs to the Asteraceae family. In traditional medicine, yarrow has long been used as a treatment for several disorders such as wounds, infectious diseases, pain, and gastrointestinal complaints. In addition, it is reported that yarrow has anxiolytic-like and antiinflammatory properties in the nervous system.[4] Interestingly, it has no adverse effects on memory in normal mice.[5]

Animal and Clinical Studies

A recent study showed that oral administration of aqueous extract of *A. millefolium* attenuated disease severity, inflammatory responses, and demyelinating lesions in mice with EAE.[6] A few clinical studies evaluated the therapeutic effects of *A. millefolium* in different diseases. These disorders include dysmenorrhea, cancer, and chronic kidney disease. An ongoing clinical trial that assesses the effect of *A. millefolium* aqueous extract on patients with MS was designed by the authors of this chapter.

ANDROGRAPHOLIDE

Andrographolide is a bicyclic diterpenoid lactone derived from the extracts of *Andrographis paniculata*, a plant originating from Southeast Asian countries that has been used as an official medicinal herb in China. This herb has antiinflammatory and immune-modulating properties.[7]

Animal and Clinical Studies

It was reported that treatment with andrographolide reduced the behavioral deficits in mice with EAE by inhibiting T-cell and antibody responses directed to myelin.[8]

There are several clinical trials that demonstrate the positive effects of *A. paniculata* on infectious diseases, hypertriglyceridemia, and autoimmune disorders such as ulcerative colitis and rheumatoid arthritis. Two clinical trial studies are ongoing to determine the efficacy of andrographolide in patients with MS.[9]

APIGENIN

Apigenin, a natural flavonoid, is commonly found in various plants, fruits, vegetables, herbs, and spices. Apigenin is produced mainly from parsley and dried

flowers of chamomile. The antioxidant, antiinflammatory and anticarcinogenic effects of apigenin have been studied well. It is effective in the treatment of asthma, insomnia, Parkinson disease, neuralgia, and shingles.[10]

Animal and Clinical Studies

Oral and intraperitoneal administration of apigenin reduced the progression and relapse in two mouse models of MS through modulating the immune system.[11]

Despite the proven antioxidant and antiinflammatory effects of apigenin, there is no clinical trial report on patients with MS.

BEE VENOM

The venom of the honey bee (*Apis mellifera*) has different types of light and heavy chain peptides. It also consists of various proteins such as apamin, melittin, adolpin, and phospholipase A2. Bee venom has antiinflammatory and antinociceptive effects on inflammatory reactions.[12]

Animal and Clinical Studies

Injection of bee venom to rats with EAE improved the pathological changes and the glutamate level and increased the brain level of γ-aminobutyric acid. However, researchers did not report the effect of the bee venom on behavioral deficits following EAE.[13] In a recent study, exposure of phospholipase A2, from bee venom and other venoms, to isolated myelin produced a deleterious effect on this structure.[14]

Two clinical trial studies evaluated the effect of the bee venom on patients with MS. The results showed that the bee venom did not reduce the disease severity or disability or fatigue or quality of life of patients with MS.[15,16]

BERBERINE

Berberine is an isoquinoline alkaloid. It is isolated from various herbs including *Hydrastis canadensis* (goldenseal), *Cortex phellodendri* (Huang bai), and *Rhizoma coptidis* (Huanglian). It has a wide range of pharmacological properties and is considered to have antiinflammatory and neuroprotective effects.[17]

Animal and Clinical Studies

It was reported that oral administration of berberine in mice with EAE improved behavioral deficits, pathological parameters, and attenuated the permeability of blood–brain barrier.[18–20] Moreover, oral administration of berberine in the experimental autoimmune neuritis model in rats ameliorated experimental autoimmune neuritis. Accordingly, it also may have therapeutic effects for other autoimmune diseases in the peripheral nervous system.[21]

There has been no clinical trial for the effects of berberine on patients with MS. However, over 90 clinical trials have been published for this compound so far. Most of these studies focused on its antidyslipidemic effect.

β-ELEMENE

β-Elemene is the main constituent of *Rhizoma zedoariae* and *Pterodon emarginatus* as Chinese and Brazilian medicinal herbs, respectively. This compound has antiinflammatory and antitumor properties and is able to pass the blood–brain barrier.[22]

Animal and Clinical Studies

Zhang et al. reported that β-elemene ameliorated motor disability and reduced the optic nerve inflammation in mice with EAE. Furthermore, it was revealed that part of the effects of β-elemene on EAE is mediated through inhibition of differentiation and development of Th17 cells–mediated inflammation.[22,23] In addition, a recent study demonstrated that oral administration of essential oil from *P. emarginatus* decreased the neurological signs and demyelination in mice with EAE.[24]

A few clinical trials have evaluated the beneficial effect of β-elemene on lung cancer. However, no clinical trial has been performed to evaluate its effects on MS, so far.

BLUEBERRIES

Blueberries are flavonoid-rich fruits and have been suggested to limit neurodegeneration associated with neurodegenerative diseases. They are able to prevent age- and neural-damage-related cognitive function loss.[25]

Animal and Clinical Studies

In an interesting study, Xin et al. fed EAE mice with a diet containing 1% whole, freeze-dried Tifblue blueberries (*Vaccinium ashei*). They reported that blueberry-fed EAE mice had lower motor disability scores as well as greater myelin preservation in the lumbar spinal cord.[26]

There are clinical trials on diseases other than MS, showing memory enhancing, antioxidant, and antiinflammatory properties for blueberry, but none have been specifically studied in patients with MS.

CASTANOSPERMINE

Castanospermine is a compound derived from the Australian rainforest plant, *Castanospermum australe*. This compound inhibits glucosidases I and II, which are crucial in posttranslational processing of the complex N-linked oligosaccharides within the endoplasmic reticulum. It was reported that castanospermine can modulate some immunopathologies including transplantation rejection and arthritis.[27]

Animal and Clinical Studies

It was reported that castanospermine prevented behavioral deficits of EAE and inhibited inflammatory infiltrates of the central nervous system (CNS).[28,29] No clinical study has been performed on patients with MS so far.

CHRYSIN AND CAFFEIC ACID

It has been reported that honey and propolis have high levels of the flavonoids chrysin and caffeic acid. They are neuroprotective, improve cognitive decline, and are effective in neurodegenerative diseases such as Alzheimer and Parkinson diseases.[30,31]

Animal and Clinical Studies

Treatment of EAE rats with caffeic acid inhibited reactive oxygen species production induced by EAE and ameliorated behavioral deficits in rats.[32] Also, Zhang et al. reported that oral administration of chrysin for 3 days before the induction of EAE alleviated the behavioral deficits and suppressed dendritic and Th1 cells.[33]

There is no clinical trial for chrysin and caffeic acid in patients with MS. However, caffeic acid was effective in the treatment of cancer in previous clinical studies.

CURCUMIN

Curcumin is a natural polyphenolic phytochemical isolated from the rhizome of the *Curcuma longa*.[34] Traditionally, curcumin has been used for coloring and flavoring food products, treatment of inflammatory diseases, and wound healing.

Animal and Clinical Studies

There are studies reporting that treatment with curcumin reduced behavioral disability scores and decreased inflammatory reactions through the immune system in mice and rats with EAE.[35–37]

Up to 144 clinical studies have been conducted on potential therapeutic effects of curcumin, so far. Most of them focused on cancer prevention and treatment. One clinical trial is going on to determine the efficacy of curcumin in patients with MS,[9] but no results are available as of this writing.

EPIGALLOCATECHIN-3-GALLATE

Epicatechin-3-gallate is the most biologically active and most abundant catechin in green tea (accounting for 50–80% of the total tea catechins). Green tea or epicatechin-3-gallate can quench several different reactive oxygen species, and its health benefits have been partially attributed to its antioxidant properties.[38]

Animal and Clinical Studies

Feeding with a diet (30 days before induction of EAE) supplemented with epicatechin-3-gallate attenuated motor disability and pathological features (leukocyte infiltration and demyelination) in mice with EAE. Interestingly, dietary supplementation of epicatechin-3-gallate after EAE induction also effectively ameliorated motor disability.[39] Epicatechin-3-gallate treatment improved animals' recovery from EAE and protected them for long term.[40]

The results of a clinical trial showed that administration of epicatechin-3-gallate to patients with MS (over a 12-week period) improved muscle metabolism during moderate exercise. The improvement was greater in women than in men.[41] Five clinical trials are under progress to determine the efficacy of epicatechin-3-gallate in patients with MS.[9]

ERHUANGFANG

Erhuangfang (Bu Shen Yi Sui) is a Chinese remedy mainly composed of Shengdi (Radix Rehmanniae), leech (the dried body of *Whitmania pigra*), Zhe Bei Mu (Bulbus Fritillariae), scorpion (the dried body of *Buthus martensii*), and He Shou Wu (Radix Polygoni Multiflori).

Animal and Clinical Studies

Administration of erhuangfang decreased behavioral deficits and diminished inflammatory reaction and demyelination in CNS of animals with EAE.[42,43]

In 2012, Zhou and Fan, in a 1-year retrospective study, indicated that erhuangfang effectively reduced relapse rate in patients with MS.[44] After that, it was approved by the Beijing Food and Drug Administration as a hospital preparation (No. 10003). In 2015, in a 2-year, prospective,

randomized study, they showed that erhuangfang significantly reduced relapse rate and prevented progression of MS. Again, the researchers suggested this product as an effective therapy for relapsing MS.[45]

GENISTEIN

Genistein is a common form of phytoestrogens that are found in a variety of plants, especially in soy. Phytoestrogens are a group of plant substances that have a chemical structure similar to estrogen, exerting estrogenic and antiestrogenic effects.[46]

Animal and Clinical Studies

Administration of genistein ameliorated the behavioral deficits and modulated pro- and antiinflammatory cytokines in mice with EAE.[47] Lately, it was demonstrated that oral administration of 300 mg/kg genistein reduced EAE severity if started in early phases of the disease.[48]

There are many clinical trials for genistein in metabolic syndrome, prostate disorders, osteoporosis, and breast cancer. Up to the present, no clinical study has been performed on patients with MS.

GINGER

Ginger is the rhizome of *Zingiber officinale*, commonly used as a spice or food supplement. In Iranian traditional medicine, ginger is used for the treatment of memory deficit and digestive diseases.[49] Recent research has reported potent antiinflammatory effects for ginger and its derivatives.

Animal and Clinical Studies

Administration of hydroalcoholic extract of ginger reduced the behavioral deficits and modulated the immune functions in mice with EAE[50]; no clinical study has been performed on patients with MS.

HESPERIDIN

Hesperidin is a natural flavonoid that is abundant in citrus species such as lemon and orange. Various biological properties have been reported for hesperidin including anticancer, antiviral, and antiinflammatory activities.[51]

Animal and Clinical Studies

Recently, Ciftci et al. treated EAE mice with hesperidin. They reported that hesperidin prevented the oxidative stress and decreased behavioral deficits in these animals.[52]

The therapeutic effects of hesperidin in diseases other than MS have been evaluated extensively in clinical studies. However, there is no clinical trial on patients with MS.

HUPERZINE A

Huperzine A is a sesquiterpene alkaloid extracted from *Huperzia serrata* (club moss), a plant that is native to India and Southeast Asia. It has potential antiinflammatory properties with anticholinesterase effects. Huperzine A has been used for the treatment of certain neurodegenerative diseases such as Alzheimer disease.[53]

Animal and Clinical Studies

Wang et al. reported that administration of 0.2 mg/kg of huperzine A ameliorated EAE by suppressing autoimmune responses, inflammatory reactions, and by subsequent demyelination and axonal injury in the spinal cord.[54,55]

There are a few clinical trials for Huperzine A, and most of them were performed on patients with Alzheimer disease. Results indicated that huperzine A improved both cognitive functions and the quality of life.[56] However, there is no clinical trial on MS yet.

HYPERICUM PERFORATUM

Hypericum perforatum (St. John's wort) is a member of the Hypericaceae family. It has been used in folk remedies for the treatment of depression. Moreover, there are reports about the therapeutic effect of this plant and its derivative (hyperforin) in psychiatric and neurological disorders such as Alzheimer and Parkinson disease.[57]

Animal and Clinical Studies

It was reported that oral treatment of the EAE mice with hydroalcoholic extract of *H. perforatum* or with hyperforin attenuated EAE-induced behavioral deficits possibly via modulation of immune system function.[58] In line with these results, it was revealed that oral administration of MS14, an Iranian herbal-marine medicine that contained 90% *Penaeus latisculatus* (king prawn), 5% *Apium graveolens* (Umbelliferae), and 5% *H. perforatum*, decreased the motor disability and attenuated the inflammation in the CNS of the rats with EAE.[59–61]

There are many clinical trials for *H. perforatum*. Most of them are focused on the treatment of depression and anxiety. However, we did not find any relevant study on

patients with MS. There is one clinical trial study that investigated the effect of MS14 on patients with MS. The results demonstrated that MS14 may improve patients' mobility (lower limb) without serious adverse effects on vital signs and biochemical, hematological, liver, and kidney function tests.[62]

LIPOIC ACID

Lipoic acid is a natural antioxidant that exists in many foods. The main sources of the lipoic acid are liver, kidney, heart, spinach, broccoli, and yeast extract. It may be used as a dietary supplement. Studies showed that lipoic acid is effective in ischemia-reperfusion injury, diabetes, and neurodegeneration.[63]

Animal and Clinical Studies

The effect of lipoic acid on EAE has been evaluated in many studies. The results demonstrated that lipoic acid suppressed EAE possibly through modulation of the immune system and inflammatory responses.[64–69]

There are some clinical trials of this compound on patients with MS. First, Odinak et al. reported that administration of lipoic acid reduced relapse frequency and decreased corticosteroid consumption in patients with MS. However, the sample size was only 14. They also reported that administration of a combination of antioxidants to patients with MS was more effective than administration of lipoic acid alone.[70] Other studies revealed that consumption of 1200 mg lipoic acid per day decreased inflammatory cytokines in patients with MS.[71–75] There are even more ongoing clinical trials that aim to determine the efficacy of lipoic acid in patients with MS.[9] However, more studies with bigger sample sizes are required to make a clearer interpretation of the effects of lipoic acid on patients with MS.

LUTEOLIN

Luteolin is a common flavonoid abundantly present in several plant products, including broccoli, pepper, thyme, and celery. Studies have shown that luteolin possesses beneficial neuroprotective effects both in vitro and in vivo. It also has antioxidant and immunomodulatory properties.[76]

Animal and Clinical Studies

Hendriks and colleagues administered luteolin (50 mg/kg/day) to rats with EAE. They reported that both oral and intraperitoneal administration of luteolin suppressed behavioral deficits, prevented relapse, and reduced inflammation and axonal damage.[77] Furthermore, it was recently reported that exposure to a special mixture of palmitoylethanolamide/luteolin promoted the maturation of oligodendrocyte precursor cells that make myelin sheet around neurons.[78] Conversely, Verbeek et al. reported that oral administration of luteolin (10 mg/day) delayed the recovery of behavioral deficits rather than reducing disease severity.[79]

Some clinical trials show the effectiveness of luteolin on autism, diabetes mellitus type 2, and some kinds of cancers. However, there are no clinical trials on patients with MS.

MATRINE

Matrine and oxymatrine are two natural alkaloid components extracted from the herb Radix Sophorae flavescentes. Various pharmacological activities have been reported for matrine including antiinflammatory, antiallergic, and cardiovascular protective effects.[80]

Animal and Clinical Studies

A growing number of studies revealed that matrine (150, 200, and 250 mg/kg) reduced behavioral deficits, inflammatory cells, and blood–brain barrier leakage in rats with EAE.[81–86]

Many experimental reports demonstrated beneficial effects of this compound on EAE. In addition, matrine has long been used for the treatment of viral hepatitis, cardiac arrhythmia, and skin inflammation, without known side effects. So, it can be recommended to enter clinical trials on patients with MS.

N-ACETYLGLUCOSAMINE

N-acetylglucosamine is a simple sugar (monosaccharide derivative of glucose). This sugar is mainly derived from chitin, which is abundant in fungi. *N*-acetylglucosamine is a dietary supplement available in some countries. It has been used safely in humans by the oral route. Several biological effects have been reported for *N*-acetylglucosamine, including modulation of the immune system.[87]

Animal and Clinical Studies

Oral administration of *N*-acetylglucosamine enhanced *N*-glycosylation, suppressed inflammatory T-cell responses, and inhibited behavioral deficits in mice with EAE.[88]

No clinical trial has been performed on patients with MS.

NIGELLA SATIVA

Nigella sativa or black cumin belongs to the botanical family Ranunculaceae. The seeds of this plant have been used in Middle Eastern folk medicine as a remedy for various diseases. Recent studies reported neuroprotective, antioxidant, and antiinflammatory effects for black cumin. Thymoquinone is the major bioactive component of this seed.[89]

Animal and Clinical Studies

Oral administration of *N. sativa* seeds, 2 weeks prior to EAE induction or after the appearance of first signs of the EAE, decreased behavioral deficits, suppressed inflammation, and enhanced remyelination.[90,91] No clinical trial has been performed on patients with MS.

OLEANOLIC ACID, ERYTHRODIOL, AND CELASTROL

Oleanolic acid, erythrodiol, and celastrol are natural pentacyclic triterpenes, which are widely found in a variety of plants including *Tripterygium wilfordii* hook (thunder of god vine). One of the sources of oleanolic acid is the leaves and fruits of *Olea europaea* (olive tree). There are reports of antiinflammatory, antitumor, and immunomodulatory effects of these triterpenes.[92,93]

Animal and Clinical Studies

It was reported that treatment with either oleanolic acid or erythrodiol (50 mg/kg), before or at the early phase of EAE, ameliorated neurological signs. Moreover, oleanolic acid treatment decreased the levels of anti-MOG antibodies, blood–brain barrier leakage, and infiltration of inflammatory cells within the CNS.[94,95] In line with these findings, oral administration of 80 mg/kg of olive leaf extract in rats with EAE reduced behavioral deficits, cellularity of the draining lymph nodes, and production of interferon-γ and interleukin-17.[96] It was also reported that celastrol (1 mg/kg/day) ameliorated the behavioral deficits and inhibited the relapse in rats with EAE.[97]

Oleanolic acid had beneficial effects in clinical trials on chronic kidney disease, diabetes mellitus type 2, and some inflammatory conditions such as arthritis. There are about 500 registered clinical trials regarding the therapeutic effects of olive.[9] However, none of them are related to patients with MS. Similarly, no clinical trial has been performed for oleanolic acid, erythrodiol, or celastrol on patients with MS.

PANAX GINSENG AND GINSAN

Ginsan is an acidic polysaccharide. It is extracted from the roots of *Panax ginseng*. *P. ginseng* is a medicinal herb of the family Araliaceae and has traditionally been used for over 2000 years in oriental countries as a medicinal preparation for various degenerative diseases. It is used for physical strength and vigor and prevention of aging as well.[98]

Animal and Clinical Studies

Hwang et al. studied the effect of pretreatment with ginsan (200 mg/day) on behavioral and inflammatory responses of mice with EAE. They reported that ginsan reduced behavioral scores of EAE and inhibited the proliferation of autoreactive T cells and the production of inflammatory cytokines.[99] In accordance, Bowie et al. also found that treatment with an aqueous ginseng extract (150 mg/kg), during the acute phase of EAE, decreased the severity of behavioral and pathological signs of EAE.[100] Recently, it was demonstrated that oral treatment with Korean red ginseng extract (20 and 100 mg/kg) caused attenuation of behavioral signs, loss of body weight, spinal demyelination, and glial activation in rats with EAE.[101]

There are a few clinical trials regarding the treatment of MS by ginseng or ginsan. Most of them have focused on the treatment of fatigue during MS.[102] According to these studies, it has been proposed that ginseng can reduce fatigue and has a significant positive effect on the quality of life of patients with MS.[103]

PROBIOTICS

Since hundreds of years, probiotics (live microorganisms) have been consumed by humans. Many studies have demonstrated the beneficial effects of probiotic bacteria on human health. Nowadays, probiotic preparations have become more common in the prevention and treatment of diseases such as gastrointestinal and autoimmune disorders.[104]

Animal and Clinical Studies

In a study, Kobayashi et al. reported that oral administration of two common probiotics (*Lactobacillus casei* and *Bifidobacterium breve*) improved neurological symptoms in EAE.[105] In accordance, it was reported that administration of a mixture of three lactobacilli strains suppressed the progression and reversed the behavioral and histological deficits in mice with EAE. Also, it was suggested that strain-specific differences influence the effect of probiotics in EAE.[106,107] No clinical trial was reported on patients with MS.

RESVERATROL

Resveratrol is a nonflavonoid polyphenol that is found in various food sources including white *Veratrum grandiflorum* (hellebore), grapes, berries, red wine, chocolate, and peanuts. It is a plant antibiotic compound produced as a part of a plant's defense system against fungal infection. Resveratrol also has anticancer, antioxidant, and antiinflammatory properties.[108]

Animal and Clinical Studies

Oral administration of resveratrol (100 and 250 mg/kg) reduced behavioral deficits and neuronal loss and demyelination in mice with EAE. However, its effect on the immune system is controversial as some reports demonstrated little effect on inflammation in the spinal cord or optic nerves, whereas others demonstrated an antiinflammatory effect in rats with EAE.[109,110] Conversely, it was also reported that resveratrol exacerbated demyelination and inflammation and behavioral deficits of EAE.[111] The source of these discrepancies is unknown.

SESAME OIL

Sesame seed (*Sesamum indicum*, Pedaliaceae) has long been categorized as a traditional healthy food in Asian countries. Sesame oil inhibits lipid peroxidation and is a potent inhibitor of proinflammatory mediators.[112]

Animal and Clinical Studies

We found two studies from a single research group that reported both oral administration[113] and intraperitoneal administration[114] of sesame oil decreased behavioral deficits and improved immune system function in mice with EAE.

Although several clinical trials evaluated the effects of sesame oil in various disorders such as hypertension, diabetes mellitus, allergy, and inflammation, no clinical trial has been conducted on patients with MS yet.

TRIPTERYGIUM WILFORDII HOOK F

T. wilfordii Hook F is a medicinal herb. It has been used in the traditional Chinese medicine for the treatment of rheumatoid arthritis. This plant has antiinflammatory and immunosuppressive properties. The major compounds with antiinflammatory and immunosuppressive properties isolated from this plant include triptolide, 5-hydroxytriptolide, and tripchlorolide.[115–117]

Animal and Clinical Studies

It was reported that *T. wilfordii* extracts had efficacy in guinea pigs with EAE.[118] In line, Fu et al. reported that 5-hydroxytriptolide as an analog of triptolide (1 mg/kg/day) prevented EAE. It also inhibited T-cell proliferation and activation[117]. In accordance, oral administration of triptolide (100 mg/kg) exhibited both preventive and therapeutic effects in mice with EAE.[119] Similar results were obtained for tripchlorolide (40 μg/kg) as well.[116]

There are about 50 clinical trials regarding the therapeutic effect of the *T. wilfordii* Hook F on thrombocytopenia, Crohn disease, and chronic primary glomerulopathy. However, the effect of this plant on patients with MS has not been evaluated yet.

VINDEBURNOL

Vindeburnol is a semisynthetic derivative of the plant alkaloid vincamine. Vincamine is a peripheral vasodilator isolated from the plant *Vinca minor*. Vindeburnol increases activation of the locus coeruleus neurons. The primary source of noradrenaline in the brain is the neurons located in the locus coeruleus. Endogenous noradrenaline directly affects neurons and reduces neurotoxicity elicited by inflammatory or excitotoxic stimuli, both in vitro and in vivo.[120,121]

Animal and Clinical Studies

Vindeburnol (20 mg/kg) reduced behavioral deficits and the number of demyelinated regions in the cerebellum. It also improved locus coeruleus physiology and function and increased noradrenaline levels in the spinal cord.[120] There are many reports demonstrating that noradrenaline has a role in MS pathogenesis (for review please see Ref. 122). No clinical trial has been performed on patients with MS.

WHITE GRAPE JUICE

White and red grapes are well known for their health-promoting and antioxidant activities. There are many bioactive compounds in grapes including flavonoids (quercetin, catechin, epicatechin, and procyanidins) and phenolic compounds (gallic acid, resveratrol, and ellagic acids). Many studies reported that grape juices have neuroprotective, antioxidant, and antiinflammatory properties.[123]

Animal and Clinical Studies

Oral administration of white grape juice extract (20 and 40 mg/kg/day) for 1 week before EAE induction

diminished behavioral deficits, lymphocytic infiltration, and demyelination of the neurons.[124] No clinical trial has been performed on patients with MS.

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CHAPTER

26

Effects of B Vitamins in Patients With Multiple Sclerosis

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OUTLINE

THE B VITAMINS

Although the main B vitamin studied for use in multiple sclerosis is vitamin B12, the other B vitamins have been shown to have some effect in general neurodegenerative diseases in animal studies. The B vitamins are thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxine (B6), biotin (B7), folate (B9), and cobalamin (B12). Of all of the B vitamins only four have been studied in mouse models due to their known involvement in neurological structures and functions. The four main vitamins studied in this regard are thiamine, pyridoxine, folate, and cobalamin.

Thiamine is mainly used in many metabolic processes, but has been shown to cause neurological issues. More specifically, Korsakoff syndrome, optic neuropathy, and peripheral nervous system diseases have been related to deficiencies in thiamine. The researchers studied the effects of neurodegeneration in thiamine-deficient mice and found that all mice that were deficient for thiamine had decreased cognitive function and significant developmental brain damage when compared with their controls. This hints at thiamine's role in proper neurocognitive function, but no further research has been attempted to discover thiamine deficiency in patients with multiple sclerosis.

There have been a couple of mouse studies done on the vitamin pyridoxine. One study used pyridoxine-deficient mice that did not gain any pyridoxine from their diet during the course of the study. The researchers found no consistent data regarding neurodegeneration in the absence of pyridoxine. There has been no further human research with pyridoxine because there seems to be no causal link between the function of pyridoxine and the brain. Pyridoxine is mainly used in amino acid, lipid, and glucose metabolism.

Folate deficiency has been discussed in many different neurological papers due to its prevalence in the brain. A mouse study was done to assess neurotransmitter histopathology and neurocognitive function in folate-deficient mice. The researchers examined the cerebrocortical microvascular wall in brain cross-sections of

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mice using electron microscopy. After eight weeks the folate-deficient mice showed significant damage to their cerebrocortical microvascular walls, profound motor dysfunction, increased number of falling incidents, approximately 20% loss of neurons, and amyloid-β peptide aggregation that is seen in elderly patients with Alzheimer disease. Even though there was no follow-up experiment on the mechanism of how folate deficiency causes brain damage, it is known that folate is used in many metabolic processes. Folate is used in the maintenance of cell division, amino acid production, and for conversion of key metabolites. Folate deficiency has also been linked to depression, cognitive function, and developmental defects of the neural tube, which hints at its importance in neurological health. Currently there have not been any correlations in human trials with folate supplementation in patients with multiple sclerosis, but more research is warranted on this subject to determine if folate deficiency can lead to multiple sclerosis or other neurodegenerative diseases.

Vitamin B12 has been the most clinically studied B vitamin in multiple sclerosis, even though there are not significant results in mouse models. In one study a group of researchers examined the effect of low-dose vitamin B12 supplementation in neurologically impaired mice. The researchers found that there was no significant improvement in movement or performance in these mice during the course of the experiment when compared with the controls. Even though the data set as a whole did not show significant results, there were a few outliers that showed significant improvement, which could show that some mice might have a positive effect based on genetics or some other underlying condition. Although nothing can be said for certain, researchers are continuing to analyze the function of vitamin B12 due to its role in the synthesis of important proteins in the brain.

PHYSIOLOGY OF VITAMIN B12

Vitamin B12 is utilized in many parts of the body and metabolism. Vitamin B12, also known as cobalamin, is absorbed by the body in the intestine. Once food containing vitamin B12 is in the intestine, it binds to the protein R-binder, which is a protein in phagocytes and in the plasma. R-binder protein then takes releases vitamin B12 to the intrinsic factor (IF), which is produced by the parietal cells of the stomach. Lastly, vitamin B12 is transferred to transcobalamin II, which is secreted into circulation.[1] Vitamin B12 is then used rapidly in the bone marrow, liver, and brain. Vitamin B12 deficiency can be caused at each one of these stages. First, a deficiency may occur if the protein R-binder is not be able to bind vitamin B12, which results in decreased vitamin B12 absorption and an increase in vitamin B12 excretion.[2] Second, if the parietal cells in the stomach are damaged or are not secreting the IF to bind vitamin B12 from the R-binder protein, then vitamin B12 will never reach circulation. Last, if there is a problem with *trans*-vitamin B12 II then vitamin B12 will not get to the organs that require it.

In addition to the problems in vitamin B12 uptake, some patients have pernicious anemia. Pernicious anemia occurs when the body cannot take up enough vitamin B12, which results in a decrease in the red blood cell count. Pernicious anemia is detected by the Schilling test, but in recent years it has been deemed inadequate.[2] Serologic testing for parietal cell and IF is used in vitamin B12 uptake, which is a much more effective method. It is still unclear whether pernicious anemia is a direct cause of multiple sclerosis, but further research into the processes of vitamin B12 transport might yield fruitful results.

NEUROLOGICAL PROBLEMS ASSOCIATED WITH VITAMIN B12

Vitamin B12 deficiency can cause multiple problems neurologically and systemically that are similar to symptoms of multiple sclerosis.[3] The deficiency if untreated for 3–6 years can result in demyelination, axonal degeneration/axonal death, autoimmune destruction of parietal cells, and gastric mucosa atrophy. This could be a potential precursor or cause of multiple sclerosis and other neuroinflammatory diseases.[3] More in-depth research must be done to better understand vitamin B12 pathways before we can link it to neuroinflammatory diseases.

VITAMIN B12 METABOLISM

To understand vitamin B12 metabolism and its effects on multiple sclerosis, there must be a good understanding of the myelin sheath, which is damaged in patients with multiple sclerosis. The myelin sheath is an insulator of the axons that connect neurons throughout the brain. These axons are the ones that transmit electrochemical signals across relatively large spatial distances in the brain to create a communication network. For the electrochemical information to be transmitted properly, these axons need to be insulated to prevent the loss of the signal and increase the speed of conduction. The axons are insulated by the myelin sheath. In multiple sclerosis the myelin sheath is attacked by the immune system, resulting in demyelination of the axons and thus in weak signal transmission.[4] This demyelination directly effects the patient's motor movements and reaction time, resulting in the diagnosis of multiple sclerosis.

Vitamin B12 is an important vitamin that acts as a cofactor for many of the enzymes in the body. One key enzyme, methionine synthetase, which has some

prevalence in multiple sclerosis, may be affected. This enzyme is responsible for the conversion of homocysteine (tHcy) to methionine.[4] If the body does not have enough vitamin B12, this enzyme will not be able to function correctly, resulting in the buildup of tHcy and lack of methionine. Lack of methionine can lead to defects in DNA synthesis and has been linked to defective production of choline-containing phospholipids, which is responsible for neurological complications in the formation of myelin around the axon.[4] However, an excess amount of tHcy in the brain has been theorized to cause an inflammatory response, which could exacerbate multiple sclerosis. Methylmalonyl CoA synthase requires vitamin B12 as a cofactor and is used to convert methylmalonyl CoA to succinyl CoA.[5] In this case, deficiency of vitamin B12 will cause buildup of methylmalonyl CoA, which leads to the formation and incorporation of nonphysiologic fatty acids into neuronal lipids, which in turn interferes with myelin formation.[4] This is caused by the nonphysiologic fatty acid methylation of myelin basic protein, a major component of the myelin structure of the central nervous system. The incorrect formation of these myelin sheaths could be lead to triggering the autoimmune response, which causes multiple sclerosis. Much of this evidence comes from inborn errors of vitamin B12 metabolism and inborn errors in myelination or demyelination.

DIAGNOSIS OF LOW VITAMIN B12

There are a couple different assays that look at the concentration of vitamin B12 in the cerebrospinal fluid (CSF) and serum. One diagnostic tool involves measuring the tHcy serum levels, which are increased early in vitamin B12 deficiency. This is caused by Le Chatelier's principle, which drives the equilibrium of reactants and products.[1] tHcy is converted to methionine in the presence of methionine synthetase, which requires vitamin B12 as a cofactor. If methionine synthetase is not functioning correctly due to deficiency of vitamin B12, there will be more tHcy in the blood. Another diagnostic tool is the Schilling test for the detection of pernicious anemia.[1] Pernicious anemia is caused by deficiency of vitamin B12, which results in less red blood cell count, which can be quantified using this test. Lastly, the most effective tool is the serologic test for parietal cell and IF, which was stated earlier.

IMMUNOREGULATORY EFFECTS OF VITAMIN B12

Vitamin B12 has many immunoregulatory effects such as modulation of cytokines (tumor necrosis factor-α) and aberrant activation and proliferation of immunocompetent cells.[2] Both these immune responses may actually exacerbate multiple sclerosis by worsening the inflammatory response and the myelin repair process. An increase in proliferation of immunocompetent cells requires an increase in vitamin B12. If the cycle of myelination and demyelination continues, then there will be a greater need for vitamin B12, which could result in a deficiency. It causes other neurological problems associated with vitamin B12 deficiency resulting in exacerbated symptoms in multiple sclerosis.

ROLE OF OXIDATIVE STRESS IN NEURODEGENERATION

The brain and nervous system maintain an extremely precise regulation of all metabolites, but when the brain is deficient in B vitamins oxidative stress can occur. Oxidative stress occurs when the body cannot capture free radicals produced by reactions using vitamins. These free radicals have been known to cause all sorts of problems including DNA damage and apoptosis, which generally lead to neurodegeneration and other tissue degeneration if untreated. A decrease in folate concentration and an increase in tHcy levels have been shown to cause oxidative stress. This oxidative stress caused by vitamins B9 and B12 could potentially be an early-onset cause of neurodegeneration and multiple sclerosis.

AGE PROMINENCE

It is hard to draw a clear causation between vitamin B12 and multiple sclerosis. This is because vitamin B12 deficiency is rare in most people younger than 40 years, whereas multiple sclerosis is relatively common in people aged from 30 to 50 years with a median age of 32 years.[2] Although many of the younger patients may not have vitamin B12 deficiency, it might be a cause related to older-onset multiple sclerosis.

VENERABILITY TO VITAMIN B12 DEFICIENCY/DAILY REQUIREMENTS

Vitamin B12 must be acquired through meat or dairy products. The minimum daily requirement is 2.5 μg, which is a relatively small amount. People who are most at risk for vitamin B12 deficiency are those with malabsorption, defective release of vitamin B12 from food (which may be a genetic problem), vegetarians who do not have much dairy in their daily diet, and inadequate production as a result of pernicious anemia, as described earlier.

Little is known about the requirements of the central nervous system for vitamin B12, and also about the

mechanisms by which it is transported across the blood–brain barrier.[2] This information may prove useful in determining whether vitamin B12 is in fact a precursor to multiple sclerosis. Even though we can quantify the CSF and serum concentrations of vitamin B12, we still do not know if there could be a concentration problem directly in the brain.

CLINICAL TRIALS INVOLVING VITAMIN B12 THERAPY

The following study was done to determine whether the serum and CSF concentrations of vitamin B12 and folate affected patients with multiple sclerosis and dementia. There were 293 patients studied, all being diagnosed with either Alzheimer dementia or multiple sclerosis. The vitamin B12 and folate levels were obtained via blood sample and lumbar puncture between 8 and 9 a.m.[5] The samples were then measured by Quantaphase and affinity-purified porcine IF. The results showed that the concentrations between sexes did not deviate significantly. Although there was a positive correlation between serum and CSF vitamin B12 when compared with a control group, the difference between the two was not significant enough to warrant causation. The slight changes in concentration that are observed in patients with multiple sclerosis are hypothesized to be a potential cause of the disorder, but further testing must be done to analyze the causal effects of vitamin B12 deficiency in the development of multiple sclerosis.

This next study examined the effects of vitamin B12 deficiency in patients with multiple sclerosis. Investigators measured both serum and CSF levels of vitamin B12, folate, methylmalonic acid (MMA), and tHcy. There were 72 patients with multiple sclerosis studied and 23 healthy control group participants.[6] The results showed that the mean plasma tHcy levels were elevated in patients with multiple sclerosis when compared with the control group. As previously discussed, tHcy is a binder of vitamin B12, so increased levels of tHcy mean that there is more unbound tHcy present in patients with multiple sclerosis. None of the patients showed significant elevated MMA, folate, or vitamin B12 levels in both serum and CSF.[6] Even though there were elevated levels of tHcy found in patients with multiple sclerosis, there was no correlation found between tHcy and vitamin B12 levels in multiple sclerosis. Although no clear link was found between vitamin B12 and multiple sclerosis, the researchers could not dismiss the possibility of a genetically induced dysfunction of tHcy metabolism in the development of neuroinflammation.

In Japan, a massive-dose methyl vitamin B12 therapy was used to treat six patients with severe multiple sclerosis. In addition, this study observed the vitamin B12 levels of 18 other patients with multiple sclerosis to look for a correlation between low vitamin B12 levels and exacerbation of multiple sclerosis. The patients' serum vitamin B12 levels were measured using radioimmunoassays and serum unsaturated vitamin B12 binding capacities.[7] Their cognitive function was also examined using the expanded disability status scale (EDSS), which is further broken down into multimodality evoked potentials (MEP), visual evoked potentials (VEP), brainstem auditory evoked potentials (BAEP), and short-latency somatosensory evoker potentials (SEP).[7] Patients who were treated with massive-dose methyl vitamin B12 therapy had no improvement in motor disability; however, the MEP abnormalities in the demyelinating lesions in the afferent pathways improved more frequently after the pretreatment period.[7] There was also a small improvement in VEP and BAEP, but not in SEP, which shows that the treatment slightly benefited the patients' visual and auditory systems. Although the researchers recorded small improvements in the treated patients, there is no evidence to support the wide-scale use of this therapeutic treatment.

Another therapeutic study done by researchers examined combination therapy. The objective of this study was to determine whether the combination therapy of vitamin B12 with lofepramine and L-phenylalanine would benefit patients with multiple sclerosis. In recent years, 75% of people with multiple sclerosis show a reduction of noradrenaline levels. Lofepramine, a tricyclic antidepressant limits the uptake of noradrenaline at the synapses resulting in more available noradrenaline in the synaptic cleft to act on its receptors.[3] L-Phenylalanine is a known precursor of noradrenaline, which may promote the synthesis of more noradrenaline when present. The combination of these two medications has been shown to increase the noradrenaline levels in the brain in previous studies. The study was a placebo-controlled, double-blind, randomized study carried out in five UK centers on outpatients with multiple sclerosis. The patients were assessed using the Guys Neurological Disability Scale (GNDS) and the Kurtzke extended disability status scale (EDSS).[3] Disability for this experiment was defined as a score of more than 2 on GNDS and a score of 3 or more on the Kurtzke extended disability status scale. There were 138 patients enrolled in the study. All patients received intramuscular injection of vitamin B12. Half of the patients received 70 mg lofepramine and 500 mg L-Phenylalanine and the other half of the patients received a placebo.[3] The results showed that both groups over the course of 24 weeks showed a 2-point reduction in the mean GNDS and little to no change in the Kurtzke extended disability status scale.[3] There was a reduction of 0.6 in the GNDS for patients taking the combination therapy when compared with the control group.

Although taking the combination therapy showed improvement, there were adverse effects reported by a majority of the patients. This is most certainly due to the symptoms expected from the use of lofepramine. The researchers concluded that the combination therapy of vitamin B12 with lofepramine and L-phenylalanine should not be considered as a widespread treatment option for patients with multiple sclerosis.

The first two clinical trials looking into vitamin B12 and tHcy levels show that there is a correlative imbalance of vitamin B12 in patients with multiple sclerosis. Currently we are unsure if there is a clear link between multiple sclerosis and vitamin B12 deficiency. Vitamin B12 deficiency could be a potential cause of late-onset multiple sclerosis because vitamin B12 deficiency is relatively rare at a young age. Although it does not seem to be directly related, it could be indirectly associated to multiple sclerosis though abnormal tHcy levels found in these patients. In the massive dose of vitamin B12 study it seems that there was very little benefit to the patient. Visual and auditory functions did slightly improve, which means that vitamin B12 could be helpful as a supplement or in a combination therapy as we saw in the last study. The final study used a combination therapy of vitamin B12 with lofepramine and L-phenylalanine. Since approximately 75% of patients with multiple patients have low norepinephrine blood levels, this combination therapy looked to combine the small positive effects of vitamin B12 with a possible fix to low norepinephrine levels. The study did show a small benefit, but many of the patients had the side effects of lofepramine. It seems that clinical supplementation of vitamin B12 could prove useful to slightly boost cognitive functions in patients with multiple sclerosis but that it is clearly does not cure the underlying condition.

CONCLUSION

We have only scratched the surface of the complicated metabolism of the B vitamins and their possible hypothesized effects in neurodegeneration. Vitamin B1 has been shown to be involved in an intricate framework in cognitive development and maintenance. Vitamin B1-deficient mice showed significant impaired motor function and brain damage. The mechanism of action of vitamin B1 could be further studied to determine a possible causal role between deficiency and neurodegeneration. It has also not been further explored as a possible therapeutic or supplement in trials of patients with multiple sclerosis patient. Vitamin B9 deficiency has been shown to cause neurodegeneration, depression, and cognitive impairment. In a mouse model researchers found that vitamin B9-deficient mice had significant cognitive decline, 20% axonal death, and general neurodegeneration. Although there have not been any significant results from clinical trials, there could be a causal role discovered if more research is done to understand the intricate pathway of vitamin B9 in neurodegeneration. Vitamin B12 has been shown to be needed to create the myelin sheaths that insulate the axons in the brain—the same structures that are attacked in patients with multiple sclerosis. Vitamin B12 deficiency has also been linked to neurodegeneration and inflammation in patients without multiple sclerosis. With that said, at this time there is no accurate correlation between vitamin B12 deficiency and multiple sclerosis. The Japanese research team concluded that massive-dose methyl vitamin B12 therapy provided some therapeutic benefit to motor function related to vision and hearing. The combination therapy of vitamin B12 with lofepramine, and L-phenylalanine also showed small therapeutic benefit in motor function. This shows that it is possible that vitamin B12 therapy could be used as a supplemental therapeutic in combination with other treatments to give patients with multiple sclerosis the best possible outcome. Although the other two research teams discussed did not find any correlation between vitamin B12 and multiple sclerosis, they proposed the idea of tHcy metabolism related to neuroinflammation. There is still much research to be done to assess the potential benefits of vitamin B12 therapy before a conclusion can be made, but at this time vitamin B12 therapy alone is not considered an effective therapy for multiple sclerosis.

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CHAPTER

27

Eicosapentaenoic Acid in Myelinogenesis: Prospective in Multiple Sclerosis Treatment

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OUTLINE

INTRODUCTION

Multiple sclerosis (MS) is considered as one of the major neurological illness of young adults. It is a chronic neurodegenerative demyelinating disease, reflected by the presence of widespread oligodendrocyte (OL) damage and axonal demyelination. The axonopathy and demyelination lead to deficits in impulse conduction, which is expressed in multiple neurological symptoms. The clinical course of MS is unpredictable and varies individually, with some patients experiencing minimal impairment. The majority of patients initially have a form of MS referred to as relapsing-remitting MS (RR-MS) characterized by periods of clinical stability that are interrupted by relapses.[1,2] MS is thought to be an autoimmune disease characterized by inflammatory lesions in the brain and spinal cord. Fortunately, the central nervous system (CNS) has a remarkable capacity to regenerate, and in healthy individuals as well as early in the disease course of MS, remyelination occurs readily following a demyelinating event.[3] However, with time and recurrent attacks of demyelination, remyelination eventually fails, leading to significant clinical disability in affected patients.[4,5] On the other hand, OL development from an oligodendrocyte precursor cell (OPC) to a mature myelinating OL, is controlled by a number of both inhibitory and inductive factors. In areas of white matter injury in human disease OPCs have been found in an arrested state seemingly unable to fully differentiate into myelinating OLs.[6–10] These data suggest either a failure of the mechanisms that promote myelination or the presence of strong inhibitory molecular signals that act to suppress OPC differentiation and myelination.

Currently, there is no cure for MS and therapeutic options mainly target the inflammatory component of the disease failing the principal goal of MS therapy to promote remyelination. The approved treatments include immunomodulatory [interferon (IFN)-β, glatiramer acetate] and immunosuppressive agents (fingolimod, azathioprine, mitoxantrone) or antibody-mediated (natalizumab) approaches.[11–13] Although the efficacy of all these drugs has been proved in large-scale clinical trials, some major issues still remain unresolved including partial efficacy, considerable variation in patient response, inconvenience of parenteral application, safety and tolerability aspects, insufficient neuroprotective capacity, and last but not the

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least, considerable treatment costs. Very often patients chose to follow a healthy lifestyle such as peculiar diets containing beneficial nutrients to ameliorate symptoms. Among them n-3 polyunsaturated fatty acids (n-PUFAs) or fish oils have received attention for many years. In this chapter we would like to recapitulate personal and literature data concerning this issue.

n-3 FATTY ACIDS

The first indication of a beneficial effect of n-3 PUFAs was suggested by Swank et al.[14] Indeed, epidemiological studies showed a lower incidence of MS among coastal communities with a high consumption of fish rich in these fatty acids (FAs), than in areas with a high consumption of animal fat rich in saturated FAs.

n-3 FAs are a family of PUFAs that contain a common carbon–carbon double bond at the third carbon from the terminal methyl end of the molecule. The parent n-3 FA is α-linolenic acid (ALA; 18:3 n-3). It is an essential fatty acid (EFA) and cannot be synthesized in humans and therefore must be supplied in the diet. Eicosapentaenoic acid (EPA; 20:5 n-3) and docosahexaenoic acid (DHA; 22:6 n-3) are synthesized from ALA through a series of enzymatic steps, although limited conversion of ALA to the longer chain FAs EPA and DHA can occur, particularly in men. The same set of enzymes converts another EFA, linoleic acid (LA; 18:2 n-6), to arachidonic acid (AA; 20:4 n-6). Therefore, AA, DHA, and EPA availability depends on the precursor concentrations as they compete for the same conversion enzymes.

MECHANISMS OF ACTION

The FA composition of neuronal membranes influences cellular function through direct effects on membrane biophysical properties, by providing a precursor pool for signaling molecules and lipid-derived mediators and also via neurotransmission.

The brain has particularly high levels of n-3 and n-6 PUFAs, especially DHA and AA, and it is sensitive to alterations in dietary intake.[15,16] It has been demonstrated that in mammals, DHA, in free form, rapidly crosses the blood–brain barrier (BBB), similar to freely diffusible lipophilic drugs.[17] The first transport mechanisms of n-3 PUFAs into the cell was described as a rapid flip-flop diffusion across cell membranes.[18] Others suggest a protein-mediated uptake via FA transport proteins, FA-binding proteins, FA translocases, long-chain fatty acyl-CoA synthetases, or long-chain fatty acyl-CoA-binding proteins.[19–22] DHA is an essential component in phospholipids because it maintains membrane fluidity, which can be defined as the optimum transition point between gel and liquid crystal where the neuronal lipid bilayer can exist. High concentrations of DHA within the lipid bilayer provide neuronal membranes with the flexibility/fluidity that is required to function properly during axonal and synaptic growth and improve functioning of ion channels and receptors through better transmission.[23–26]

The second major function of PUFAs is that n-3 FA produces widespread effects on gene expression.[27] The genes affected are involved in a diverse array of functions, in a variety of cellular locations, such as lipid metabolism, signal transduction, energy metabolism, receptors, regulatory kinases, synaptic proteins, and membrane proteins. For example, EPA and DHA inhibit in vitro adenylyl cyclase–dependent kinase, protein kinase C, and Ca^{2+}/calmodulin-dependent protein kinase and also inhibit the 5-hydroxytryptamine-induced activation of mitogen-activated protein kinase (MAPK) in the hippocampus.[28,29]

A putative target involved in the mediation of the n-3 PUFA control of gene expression is the steroid/thyroid/retinoid receptor superfamily. This superfamily of receptors are nuclear receptors that function as ligand-activated transcription factors, and include the peroxisome proliferator–activated receptors (PPARs) and the retinoid X receptors (RXRs).[30] PPARs are nuclear receptor proteins, functioning as transcription factors that regulate gene expression in metabolic processes relating to cellular differentiation and development.[31] They are widely expressed throughout the brain, especially in areas concerning learning and memory, such as the hippocampus.[32] Retinoid signaling pathways have been implicated in regulating synaptic plasticity and learning and memory in rodents, and also in the pathophysiology of Alzheimer disease, schizophrenia, and depression.[33] DHA and EPA have been shown to act as endogenous ligands of RXR[34,35] and PPAR[36] and may thus work at a fundamental level of cell regulation.

The third domain of action of PUFAs is that they are precursors of a range of molecules engaged in inflammatory processes. In this capacity, n-6 and n-3 PUFAs have divergent functions: n-6 PUFAs are proinflammatory precursors, whereas n-3 PUFAs are antiinflammatory precursors. Mechanisms underlying the antiinflammatory actions of n-3 FAs include altered cell membrane phospholipid FA composition, disruption of lipid rafts, inhibition of activation of the proinflammatory transcription factor nuclear factor-κB, activation of the antiinflammatory transcription factor PPARγ, and binding to the G protein–coupled receptor GPR120. These mechanisms are interlinked, although the full extent of this is not yet elucidated.[37] When released from animal cells upon activation through response to phospholipase activation, mainly phospholipases A_2, PUFAs become the precursors of numerous oxygenated derivatives with specific

biological activities. Many of these mediators, including the "classic" eicosanoids, prostanoids, and leukotrienes, derive from AA and EPA, while a number of other oxygenated products extend the eicosanoid family to octadecanoids and docosanoids. These metabolites are produced by two principal dioxygenase enzymes, namely, cyclooxygenases and lipoxygenases, as well as monooxygenases belonging to the cytochrome P_{450} family.

Oxygenated metabolites can also be formed when oxygen reactive species react with PUFA, notably in esterified forms, in a nonenzymatic manner, followed by their release. Under free radical reactions DHA forms neuroprostanes[38,39] and EPA forms isoprostanes.[40] Once produced, all metabolites may undergo a number of metabolic transformations related predominantly to their alcohol groups leading to their degradation or production of further species with different biological activities.[41] Thus, some studies have showed that isoprostanoids derived from n-3 PUFA are new actors to be considered suggesting that the bioactive role of oxygenated n-3 PUFA is not limited to those released through enzymatic pathway. For example, it has been demonstrated that preincubation of endothelial cells with oxidized EPA and DHA reduced the adhesion of monocyte cells to endothelial cells, whereas the native EPA and DHA had no effect.[42] Others studies clearly demonstrated that the biological properties of n-3 PUFA depended on their peroxidation,[43,44] but the exact nature of the bioactive molecules was not elucidated.

Furthermore, in vitro experiments have demonstrated that both EPA and DHA can inhibit T lymphocyte (T-cell) proliferation and decrease the production of the key Th1 cytokine interleukin (IL)-2.[45,46] As known, the major families of T cells express either CD4 or CD8 on their surface, and different immunologic stimuli trigger differentiation of naive $CD4^+$ T cells along different developmental pathways resulting in different T-cell phenotypes emerging. Initially these were described as T-helper 1 (Th1) and T-helper 2 (Th2) cells. Afterwards, other subclasses of T cells have been described including T-helper 17 (Th17) cells and regulatory T cells; both these cell types have a role in host defense and inflammatory disease.[47,48]

Animal feeding studies with fairly high amounts of fish oil, or of EPA or DHA, also report reduced T-cell proliferative responses, alterations in Th1 cytokine gene expression, and modified production of Th1 and Th2 cytokines.[49–51] The functional effects of n-3 FAs on T cells have been linked with changes in membrane order, altered patterns of eicosanoid production, and modification of early signal transduction events, including reduced generation of diacylglycerol and inhibition of the activation of specific isoforms of protein kinase C and MAPKs.[52,53] It has also been observed[54] that EPA induces regulatory T cells and elevates the number and percentage of $CD4^+CD25^+$ and $CD4^+CD25^+FoxP3^+$ cells. $CD4^+CD25^+$ regulatory T cells (Treg) appear to be critical in regulating immune responses to self-antigens, thus Tregs deficiency is associated with several human autoimmune diseases.

Due to their important biological activity, n-3 PUFA deficiency has been linked to a variety of diseases including, but not limited to, cancer,[55] cardiovascular disease,[56] rheumatoid arthritis,[57] asthma,[58] depression,[59] schizophrenia,[60] attention-deficit/hyperactivity disorder,[61] and Alzheimer disease.[62] The evidence for altered FA levels in tissues in patients with MS is currently unclear, with decreases in plasma n-3 PUFA levels shown by some,[63] whereas unaltered erythrocyte levels found by others.[64]

n-3 POLYUNSATURATED FATTY ACIDS AND MYELIN

There is growing evidence that omega-3 PUFAs are able to directly and indirectly modulate neurological activity on many different levels, operating through a multitude of overlapping mechanisms. In our in vitro experimental model developed to identify the FAs involved in myelinogenesis process, we showed that EPA regulates myelin gene proteins. The role of exogenous FAs in the regulation of proteolipid protein (PLP) gene expression was investigated using a model culture system: C6 glioma cells expressing the green fluorescent protein driven by different segments of PLP promoter. EPA, but not AA, induced a significant increase in medium fluorescence intensity determined by fluorescence-activated cell sorting. The induction of PLP promoter was time and dose dependent showing maximal activity between 24 and 48 h after EPA exposure with maximum activation at 200 μM. Furthermore, this treatment increased cyclic adenosine monophosphate (cAMP) levels and activated MAPK in C6 cells. PLP promoter activity was inhibited by pretreatment with H89 (protein kinase A inhibitor), but not with PD98059 (MAPK inhibitor), suggesting that EPA stimulates the expression of PLP via cAMP-mediated pathways.[65]

Afterward, to verify whether the n-3 PUFAs have an in vivo role on the myelinogenesis process, a single dose of either EPA or DHA was injected intracerebroventricularly into 2-day-old rats, which were then killed 3 days postinjection. Total RNA was isolated from the medulla, cerebellum, and cortex, and the expression of myelin-specific messenger RNAs (mRNAs) was analyzed by real-time PCR. The levels of PLP, myelin basic protein, and myelin OL protein mRNAs increased in nearly all brain regions of DHA- and EPA-treated animals, but the effect was more pronounced in EPA-treated rats. The enhancement in PLP transcript levels was followed by an increase in PLP translation in EPA-treated rats.

A further indicator of accelerated myelination was the increase in 20, 30-cyclic nucleotide 30-phosphodiesterase protein levels. In EPA-treated rats, the increased expression of myelin genes coincided with a decrease of cAMP response element–binding protein (CREB) in the cerebellum and cortex. After 16 hours, this effect was still present in the same cerebral regions, even though the decrease in EPA-treated rats was less pronounced than in controls. The downregulation of CREB activity was due to a decrease in the levels of CREB phosphorylation.[66]

EICOSAPENTAENOIC ACID AND REMYELINATION

The experimental autoimmune encephalomyelitis (EAE) induced in dark Agouti rats is a well-established animal model for testing potential therapies for MS.[67] In this model, animals experience pathology similar to those of patients with MS with T cells, macrophages, glial cells, and autoantibody involved in demyelination and axonal loss spreading beyond the spinal cord into the cerebellum. In most of the animals the condition also relapses. We used this model to evaluate the EPA effect in myelin repair. Diets supplemented either with 0.2% or 0.4% of ethyl EPA were administrated daily from the day of EAE induction until the end of experiment. One group of rats started 0.2% EPA diet 10 days before the EAE induction. The control group (immunized rats) was fed with chow diet. The animals were analyzed at two different stages of the disease: during the acute phase and during the recovery phase. When compared with EAE rats fed standard diet, all groups of EPA-fed rats showed delayed onset of clinical course and reduced severity of disease. The beneficial and protective effects of EPA were mediated, at least in part, through a stimulation of myelin repair process as well as through restraining immune inflammatory responses within the CNS. Indeed, increased expression of myelin proteins and improved integrity of the myelin sheath as well as upregulation of expression of the forkhead transcription factor (Foxp3, the most specific marker for Treg cells) were observed in the CNS of EPA-feeding rats.[68]

We also evaluated the effects of EPA in an MS model characterized by toxic demyelination induced by cuprizone (CPZ). CPZ feeding, with mechanisms not yet well established, induces OL degeneration, disruption of myelin sheath, and demyelination mainly localized in the medial corpus callosum. After ending CPZ exposure, remyelination occurs fast and spontaneously.[69] Since in the EAE model EPA pretreatment ameliorated the clinical score more than EPA treatment from the induction day, EPA was daily administered by gavage to newborn rats for 21 days, and then they were fed CPZ diet for 9 days. The results showed that EPA was adsorbed and metabolized, and it was able to partially protect the brain against damage induced by CPZ intoxication.[70]

n-3 POLYUNSATURATED FATTY ACIDS AND MULTIPLE SCLEROSIS

To date, only a limited number of human studies investigating the effects of omega-3 PUFAs in MS are available. The largest study, with over 300 patients, used 10 g fish oil supplementation or olive oil as placebo.[71] After 2 years, no statistically significant differences were observed, although there was a trend favoring the fish oil group. There was an absolute risk reduction of 10% and a relative risk reduction of 18% of progressing one point on the Disability Status Scale. This treatment effect is similar to that reported for standard medical therapies.

An open-label study[72] showed that n-3 PUFA supplementation (6 g/day of fish oil; 86% EPA + DHA) for 6 months in patients with MS and healthy controls decreases the levels of IL-1β, tumor necrosis factor-α, IL-2, and IFN-γ produced from unstimulated and stimulated peripheral blood mononuclear cells as well as reduces the secretion of the inflammatory eicosanoids, prostaglandin E2, and leukotrienes LTB4, which are known to be increased in patients with MS.

In a preliminary open label study, 16 newly diagnosed patients were given dietary advice and were supplemented with 0.9 g/day of long-chain omega-3 PUFA and vitamins.[73] The patients were followed for 2 years with respect to dietary habits, blood parameters, and neurological assessment, including exacerbation rate. There was a significant reduction in the mean annual exacerbation rate and the mean Expanded Disability Status Scale when compared with prestudy values. The plasma total phospholipid omega-3 PUFA increased and omega-6 PUFA decreased significantly. Overall, the study suggested that omega-3 PUFA supplementation given together with vitamins and dietary advice improved clinical outcome in patients with newly diagnosed MS.

Another trial involved 31 patients with RR-MS who had been using disease-modifying therapies for at least 2 months prior to the trial.[74] 15 patients received a very low-fat diet (<15% calories from fat) supplemented with omega-3 PUFA (6 capsules per day containing 1.98 g EPA and 1.32 g DHA) and 16 a low-fat diet (<30% calories from fat) and olive oil as a placebo. At the end of 1 year there was a nonsignificant trend in favor of the n-3 PUFA-treated group on the Physical Component Scale. The Expanded Disability Status Scale scores weakly worsened in the placebo group, whereas they improved in the n-3 PUFA-treated group.

Swank and Goodwin[75] conducted a 35-year long, nonrandomized, retrospective study in patients with MS and found that a diet with very low saturated fat content

(average saturated fat 15–17 g/day) supplemented with cod liver or vegetable oils (10–40 g/day) provided long-term benefits on mortality, relapse severity, and disability, particularly if initiated during the earliest stages of MS. This study, although it is a unique long-term follow-up of an intervention for MS, has been criticized for lacking a control group for comparison for scientific validation.

In a multicenter, randomized, placebo-controlled trial, in which 92 patients with RR-MS received either fish oil (5 g/day; 60% EPA + DHA) or placebo, no significant differences were found in either the number of lesions on gadolinium-enhanced magnetic resonance imaging (MRI) at 6 months or the relapse rate at 6 or 24 months.[76] Furthermore, no differences were detected in disability progression, fatigue, or quality of life scores. However, no dietary restrictions or recommendations were given to participants, which might have attenuated any benefit of the FA supplementation.

In 2013, Pantzaris et al.[77] showed in a randomized, double-blind, placebo-controlled clinical trial that the administration of n-3 and n-6 FAs with vitamins reduce the annualized relapse rate in RR-MS. However, in this study it is not possible to discriminate the effects of n-3 and n-6 FAs.

These available data are not sufficient to assess the real effect of these compounds in MS. The conflicting results reported in the literature could be due to the different experimental protocols. The PUFA composition of enriched and control diets, the source and dose of PUFA, and the time of exposure to treatment are highly variable and could lead to heterogeneous results. In particular, the different concentrations of EPA and DHA present in the various sources of fish oils administered to patients could affect the results.[78]

From our data, compared with DHA, EPA has the most potent effect on inflammatory processes and on myelin repair mechanisms so that further studies should be designed to evaluate the effectiveness of EPA both as monotherapy and in combination with disease-modifying drugs. In this view we are setting up a multicenter, randomized, double-bind, placebo-controlled, phase 2 clinical trial to investigate whether ethyl EPA in combination with IFN-β treatment reduces disease activity in patients with RR-MS. One hundred patients will be randomized in two cohorts (1:1): the first will be treated with IFN-β and ethyl EPA of high purity 2 g/day and the second with IFN-β and placebo. Treatments will last for 12 months. The primary efficacy end point is MRI disease activity as measured by the presence of combined unique activity (presence of new T2 lesions or Gd^+ lesions). Other end points include evaluation of lesion progression by conventional and nonconventional MRI measures, relapse rate, recovery from relapses, disability progression, quality of life, and immunological markers.

CONCLUSIONS

In conclusion, data from the literature and from our in vitro and in vivo studies indicate that nutritional intervention in people with MS can positively affect their wellness. When compared with a generic mixture of n-3 PUFA, EPA treatment seems to be able to promote remyelination, which represents the principal goal of MS therapy.

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CHAPTER

28

Biomarkers of Multiple Sclerosis and Their Modulation by Natural Products

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OUTLINE

INTRODUCTION

Multiple sclerosis (MS) is one of the leading causes of neurological disabilities. It is a chronic, inflammatory and autoimmune disease related to the central nervous system (CNS). MS is an enigmatic disease having complex pathophysiology that progresses through various clinical pathways. The clinical manifestation of MS differs for each patient depending on the involvement of different mechanisms, viz., demyelination, neurodegeneration, gliosis, remyelination, and various combinations thereof. A personalized and rational regimen can be defined for each patient by understanding which mechanism is prominent in each case.[1]

A "biomarker" is defined as a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention.[2] Biomarkers can play important roles in defining and understanding the involvement of various

mechanisms that are specific for the different mechanisms. The success of the therapeutic treatment can be measured by evaluating the biomarkers that can serve as a "surrogate end point" of a clinical outcome, provided that it is fully capable of representing it. However, none of the existing biomarkers can fully replicate the extent of pathogenic mechanisms of MS. The applicability of biomarkers involving MS can be determined by the use of certain criteria, which can be listed as extent of the correlation of the biomarker with pathogenic mechanism; accuracy in representing clinical status; predictability of initiation, reactivation, or progression of disease; differentiation of other demyelinating diseases; sensitivity and specificity; reproducibility of a result; practicality of the method in use for the measurement; and correlation with therapeutic outcome.[3,4]

There are various ways to differentiate or classify the biomarkers of MS depending on their nature, site of occurrence, and involvement in pathogenesis of disease. In the following, we will present the classification of biomarkers in MS suggested by Bielekova and Martin.[3] This classification is based on the pathophysiological processes involved in the progression of disease. Examples of these classes are shown in Fig. 28.1.

Immune System Biomarkers

Immune system biomarkers are useful in studying the diversity of a disease. Although these biomarkers cannot be used as surrogate end points, they can be effectively used in the development of new therapies. These biomarkers include cytokines and related receptors, chemokines and their receptors, antibodies, complement-related biomarkers, adhesion molecules, antigen-processing and antigen-presentation biomarkers, cell

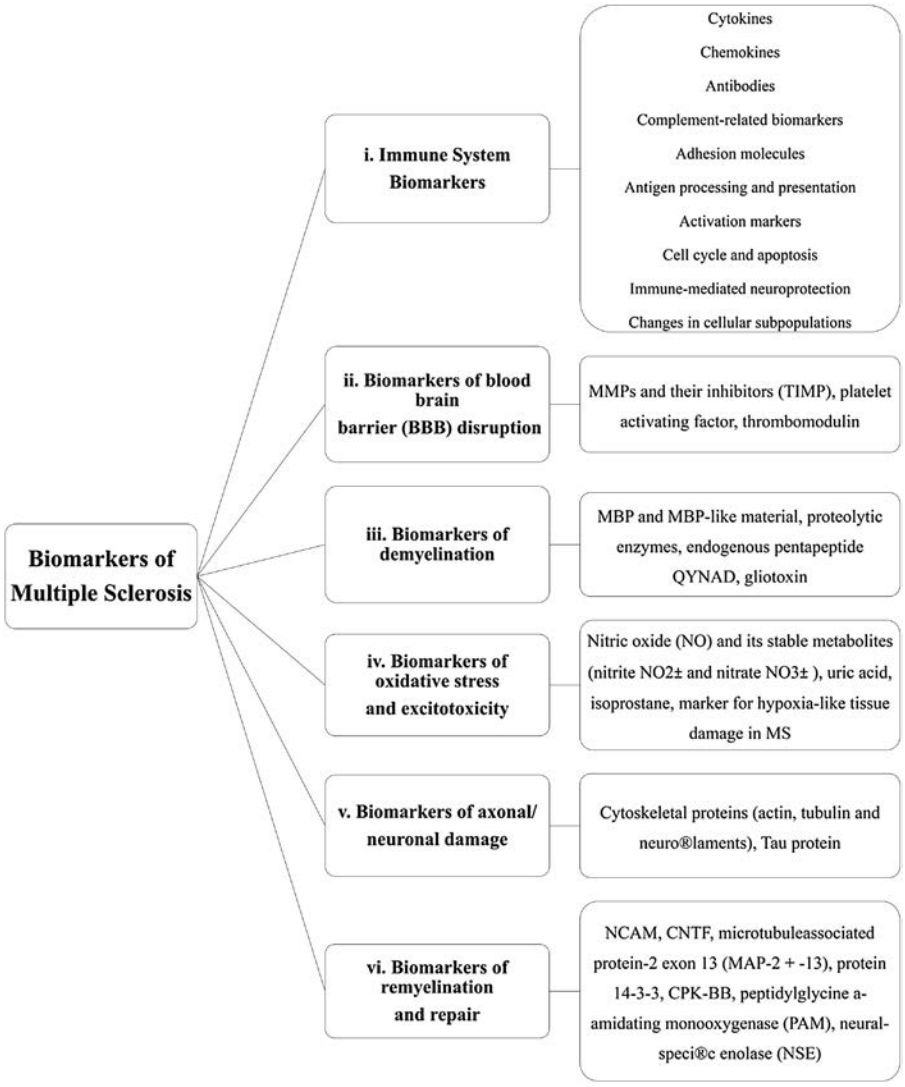


FIGURE 28.1 Classification of biomarkers. *NCAM*, Neural cell adhesion molecule; *CNTF*, ciliary neurotrophic factor; *MAP*, microtubule-associated protein; *CK-BB*, creatine kinase-BB.

cycle–related and apoptosis-related biomarkers, and markers reflecting immune-mediated neuroprotection changes in cellular subpopulations.

Cytokines and related receptors are the most widely studied biomarkers in MS. They can be used for differentiation between different stages of MS. These cytokines include interleukin (IL)-1, IL-2, IL-6, IL-10, IL-12, IL-18, tumor necrosis factor (TNF)-α, lymphotoxin (LT)-α/β, transforming growth factor-β, cluster of differentiation (CD)-25, etc.

Chemokines and their receptors may also help in studying disease diversity. C–C chemokine receptor (CCR) type 5, chemokine (C-X-C motif) receptor (CXCR) 3, chemokine (C-X-C motif) ligand (CXCL) 10, and CCR2/CCL2 are studied in MS. Specifically, CCR5 is suggested as a candidate biomarker of Th1 T cells, whereas CXCR3/CXCL10 are markers of activated T cells.

Antibodies are one of the well-studied biomarkers for MS. These biomarkers require systematic development and standardization as they have sparked interest among researchers regarding their relevance for use in the diagnosis of MS. Antibodies like anti-myelin basic protein Ab, anti-myelin oligodendrocyte glycoprotein Ab, and cerebrospinal fluid (CSF) IgG index as well as k light chains and oligoclonal bands are being utilized.

Complement-related biomarkers can help in the assessment of disease heterogeneity based on pathological classification of MS lesions. They include C3, C4, activated neo-C9, regulators of complement activation (CD35, CD59), etc.

Secondary to magnetic resonance imaging (MRI)–based markers, adhesion molecules can prove useful for deciding the blood–brain barrier (BBB) dysfunction. Until now E-selectin, L-selectin, intercellular adhesion molecule-1, vascular cell adhesion molecule-1, CD31, surface expression of LFA-1, and VLA-4 were studied under this category.

Biomarkers related to antigen processing and presentation are an important category of biomarkers in MS. There is still a wide scope for the development of these biomarkers as very few studies have been conducted on them. These biomarkers include CD40/CD40L, CD80, CD86, heat shock proteins, etc.

Activation of the innate immune system is one of the mechanisms involved in the pathogenesis of the disease and the markers reflecting this activation, i.e., the activation markers can be used in selection and screening potential immunomodulatory agents. These activation markers include CD26, CD30, CD71, perforin, OX-40 (CD134), osteopontin, macrophage-related protein (MRP)-8 and MRP-16, neopterin, amyloid A protein, and somatostatin.

Cell cycle and apoptosis-related biomarkers are specific for defects in the regulation of immune cells and proapoptotic properties of CNS components. Fas (CD95) and Fas-L, FLIP, Bcl-2, and TRAIL represent the biomarkers in this category.

Biomarkers of immune-mediated neuroprotection are less studied markers for MS and present a wide scope for development of the appropriate biomarker for evaluation of new agents for the treatment of MS. Brain-derived neurotrophic factor expression is the one well-studied biomarker under this class.

Biomarkers reflecting changes in cellular subpopulations are widely focused upon; however, they need to be reassessed with recent techniques. Changes in cellular subpopulations have an important immunoregulatory role in animal studies as well as in human autoimmune disorders including MS. These biomarkers can be listed as natural killer (NK) cells, $Va24^+$ NK T cells, $CD4^+/CD25$bright and IL-10-producing immunoregulatory T cells, CSF cells, and $CD45RA\pm/RO^+/CD4^+$ (memory) T cells.

Blood–brain Barrier Disruption Biomarkers

Matrix metalloproteinases (MMPs) and their inhibitors [tissue inhibitor of metalloproteinase (TIMP)], platelet-activating factor, and thrombomodulin are useful markers of BBB dysfunction secondary to MRI-based markers.

Demyelination Biomarkers

These biomarkers can prove helpful in understanding the MRI/pathological correlations and can be used as partial surrogate markers.

Oxidative Stress and Excitotoxicity Biomarkers

These biomarkers are important from the perspective of disease diversity.

Axonal/Neuronal Damage Biomarkers

These markers have potential to become the surrogate markers in MS.

Remyelination and Repair Biomarkers

Development of remyelination and repair biomarkers is important as they can guide the development of repair-promoting strategies in the treatment of MS.

The various biomarkers of MS can be measured from various biological samples obtained from the patients. As pathological mechanisms of MS mainly occur in the peripheral blood and the CNS, key sample sources are peripheral blood and CSF. Along with these sources, urine and tears have also been investigated for the presence of MS-specific biomarkers.[3]

ROLE OF NATURAL PRODUCTS IN THE MODULATION OF MULTIPLE SCLEROSIS

Today it is universally accepted that drugs obtained from natural sources have an important role in the treatment and management of different diseases, and MS is not an exception. Various scientific studies were performed regarding minerals, vitamins, and natural products and their involvement in the modulation of pathogenesis of MS. The following information provides details of the use of natural products in the management of MS.

Role of Polyunsaturated Fatty Acids in the Modulation of Multiple Sclerosis

Polyunsaturated fatty acids (PUFAs) are reported to have neuroprotective activity. Few of the omega-3 PUFAs also possess antiinflammatory activity and have been found to reduce the production of proinflammatory cytokines like IL1 and IL2 and TNF-α.[5–7] Docosaexanoic acid (DHA) and eicosapentaenoic acid (EPA) have been studied for their usefulness in the treatment of MS and were found promising in its management. Both EPA and DHA are found in high quantity in fish oil. DHA is a common PUFA present in the brain, which becomes depleted in patients with MS. The food rich in DHA and EPA show remarkable antiinflammatory, antithrombotic, and immunomodulating activities, which are responsible for its activity in MS. EPA and DHA are reported to have large therapeutic value in the treatment of various neurological diseases. Mechanistic studies showed that these two PUFAs inhibit transcription factors nuclear factor kappa-light-chain-enhancer of activated B cells (NF)-kβ, Sterol regulatory element-binding protein-1c, and liver X receptor, and activate the nuclear receptor peroxisome proliferator-activated receptor (PPAR). EPA and DHA inhibit the formation of interferon (IFN)-γ involved in myelin breakdown. Treatment of experimental animals with EPA and DHA showed decreased plasma levels of IFN-γ and IL-17 as well as decrease in the number of $CD4^+$ cells in the spleen and CNS.[8,9] Along with this, DHA also elevates the levels of TIMP-1.

Lipoxins are known as inflammation-resolving molecules and are generated from the omega-6 fatty acid, arachidonic acid.[10] Lipoxins bind with lipoxin A4 receptor with high affinity, and this activity of lipoxins can be assigned to its inflammation-resolving properties.

Role of Vitamin in the Modulation of Multiple Sclerosis

Fat-Soluble Vitamins

Animal studies suggest that fat-soluble vitamins, viz. vitamin A, D, E, and K, have possible positive effects as modulators of disease activity in MS.[11] Vitamin A, retinol, retinal, retinoic acid, various provitamin A, and carotenoids have been studied for their use in MS. Of these β-carotene plays an important role in the modulation of MS.

Epidemiological studies have shown that variation in geographic prevalence of MS and increased risk of MS are correlated with low sunlight exposure,[12,13] which points toward the deficiency of vitamin D_3 as a risk factor.[14,15]

Vitamin D mediates its activity in MS via the vitamin D_3 receptor (VDR), which controls the functions of T-helper (Th) cells, and this mechanism is being focused on by researchers for the treatment of MS with vitamin D. Activation of VDR receptors leads to increased levels of antiinflammatory Th2 cytokines including IL-4, IL-10, and IL-13[15] and decreased proinflammatory Th1-, Th9-, and Th22-derived cytokines, which may modify the progression of the disease.

Water-Soluble Vitamins

Vitamin B_{12} deficiency is related to the neurodegeneration of sensory and motor neurons, but there are no reports of neuroprotective activity of vitamin B_{12}. It is suggested that vitamin B_{12} deficiency is also associated with MS,[16] but clinical studies focusing on this concern failed to provide sufficient evidence to support this suggestion.[16,17] Vitamin C, i.e., ascorbic acid is a potent antioxidant and radical scavenger that might protect against oxidative damage, but again studies failed to prove this hypothesis and did not show protective effects against neurodegeneration.[18]

Role of Minerals and Trace Elements in the Modulation of Multiple Sclerosis

Administration of trace elements like selenium and zinc in experimental animals showed protection in experimental autoimmune encephalomyelitis (EAE).[19] EAE is a well-studied experimental model for the evaluation of drugs in MS. One suggested possible mechanism includes selenium-mediated activation of glutathione peroxidases, which further increases its antioxidative effects. Zinc aspartate supplementation also showed reduction of Th1 and Th17 cell proliferation in EAE.[20] The activities of selenium and zinc suggest the possible application of these minerals in the positive modulation of MS.

Calcium and magnesium are important in the development, structure, and stability of myelin and hence play an important role in MS. Calcium also reinforces the effect of vitamin D_3. Calcium supplementation in the standard diet of experimental animals reduced the relapse of MS.[21] In contrast to the effect of calcium, high doses of sodium chloride (NaCl) through diet aggravated EAE in experimental animals.[22]

Role of Natural Compounds in the Modulation of Multiple Sclerosis

There are various mechanisms by which the naturally occurring compounds modulate the progression of MS. Many of the compounds, having antioxidant properties, inhibit the differentiation of Th17, which is triggered by IL-21 receptor–mediated and IL-23 receptor–mediated Janus kinase (Jak)–signal transducer and activator of transcription (STAT) signaling.[23,24] Hence specific Jak or STAT signaling pathways can act as better targets to reduce the autoimmune-mediated oligodendrocyte death and axonal damage caused by MS.[25]

Immune responses regulated by Toll-like receptors (TLRs) like TLR2, TLR4, TLR7, TLR8, and TLR9 take part in the development of MS.[26–28] TLR4 may also cause activation of the transcription factor, IFN regulatory factor 3, which are involved in the immune-mediated pathogenesis of MS.[28,29]

NF-kβ is a key regulator in innate and adaptive immune response and plays important role in the pathophysiology of MS.[30,31] Various extracellular stimuli like TNF-α, reactive oxygen species (ROS), viruses, or genotoxic stress activate the NF-kβ, which further contributes to the activation of T cells and increased expression of proinflammatory cytokines, chemokines, immune receptors, and adhesion molecules.[30,31] Targeting of the NF-kβ pathway was found to reduce symptoms in EAE models.

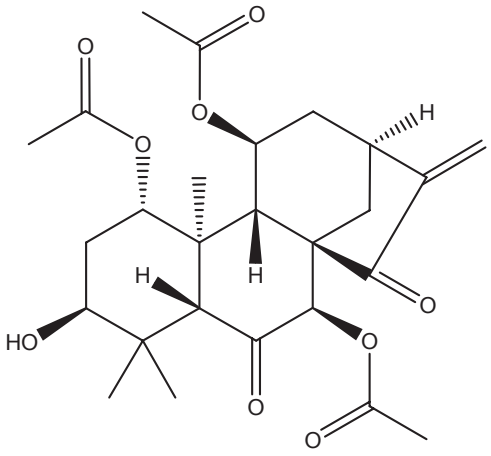
Breakdown of the BBB is one of the mechanisms involved in the pathogenesis of MS and its experimental model EAE. Breakdown of the BBB increases vascular permeability and extravasation of blood cells, extracellular fluid, and macromolecules into the CNS parenchyma. This leads to edema, inflammation, oligodendrocyte damage, and eventually, demyelination.[32]

The integrity of the BBB can be maintained by various ways. One is the inhibition of MMPs or upregulation of their inhibitors as excessive expression and activation of MMPs, specifically MMP2 and MMP9, causes protease-mediated BBB disruption and damage in EAE models.[33,34] MMPs break various proteins like myelin proteins, components of the basement membrane like collagen IV, and proteins of tight junctions.[35] This increases the access of autoreactive T cells and other leukocytes to the brain parenchyma. MMP inhibitors can be effectively deployed as BBB stabilizers for the treatment of MS.

Demyelination is another mechanism involved. It leads to increased exposure of para- and internodal neuronal K^+ channels causing higher K^+ outward current and slow action potential conduction.[36] Hence K^+ channel blockers can be used to lessen symptoms like paresthesia, palsy, vertigo, and bladder disturbances.

ROS and increased oxidative stress play an important role in the pathogenesis of MS, and this paves way for treatment with antioxidants, which can prove beneficial in cases of MS. Treatment with antioxidants might prevent tissue damage, BBB leakage, and CNS infiltration.[37] An alternative strategy is ROS scavenging, which has been unsuccessful because the scavengers do not sufficiently reach the CNS, or prevention of pathological ROS generation.[24]

Adenanthin



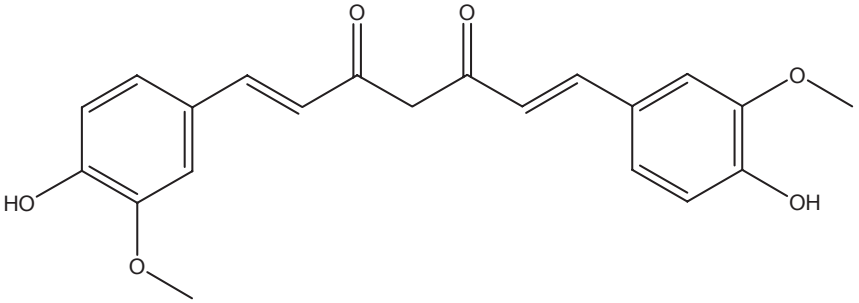
Adenanthin is a diterpenoid isolated from the leaves of *Isodon adenanthus*. Treatment with adenanthin reduced EAE scores in EAE model.[38] This activity is supposed to be mediated via the inhibition of NF-kβ through reduction of T-cell proliferation, decreased levels of Th1 and Th17 cells, and proinflammatory cytokines.[38]

Cannabinoids

Cannabinoids fall under the class of fatty acids and include around 60 natural phytocannabinoids derived from *Cannabis sativa* (Cannabaceae). Cannabinoids are being used in the treatment of painful symptoms of MS such as muscle spasms, central neuropathic pain, and headaches. It is increasingly recognized that cannabinoids may also positively change the course of the disease due to their effects on immune cells. Cannabinoids mediate their effects through cannabinoid receptor type (CB) 1 or CB2 and transient receptor potential channels like transient receptor potential cation channel subfamily V member 1[39]; nuclear receptors like PPARα, PPARγ and PPARδ[40]; and TREK potassium channels.[41] Cannabinoids are also found to stabilize the BBB in EAE.

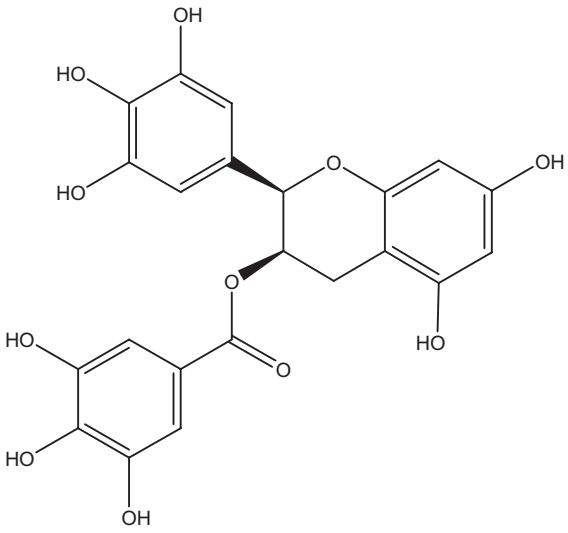
Curcumin

Curcumin is a polyphenol obtained from the rhizome of *Curcuma longa* belonging to the family Zingiberaceae ([42,43]).



Treatment with curcumin was found to diminish EAE and inhibit T-cell responses.[29,42,43] Studies proposed that the activity of curcumin can be due to the downregulation of Jak2, STAT3, and STAT4 phosphorylation leading to a decrease of IL-12 levels and abolishing Th1 and Th17 differentiation.[42,43] Curcumin treatment mainly affects Th17 by reducing IL-6, IL-21, STAT3, and retinoic acid receptor-related orphan receptor gamma t and phosphorylation of STAT3.

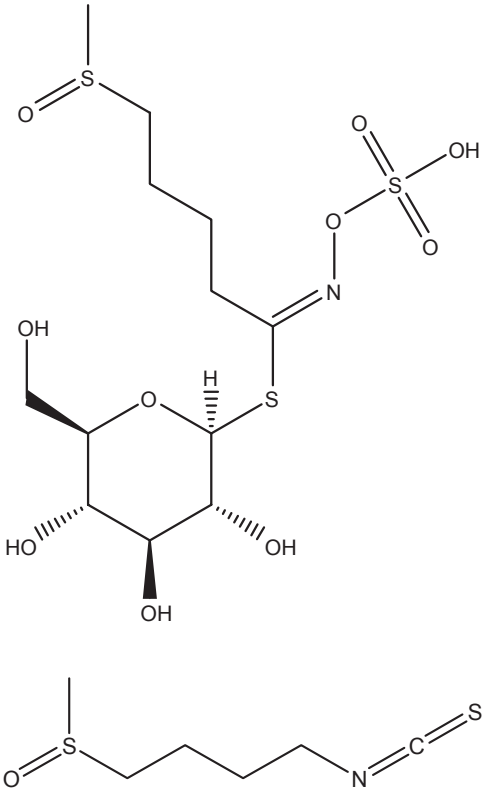
Epigallocatechin Gallate



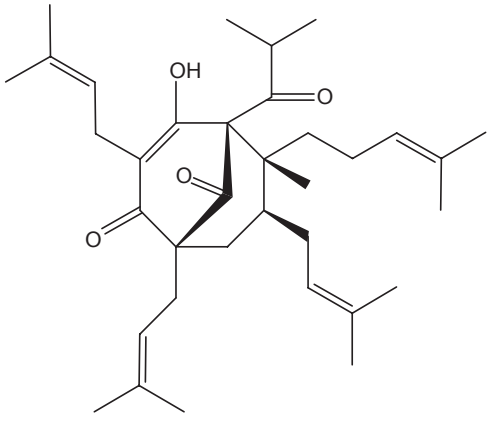
Epigallocatechin gallate (EGCG) is an ester of epigallocatechin and gallic acid. EGCG is reported to inhibit the phosphorylation of STAT1, STAT3, and STAT4 in EAE mice,[24] which leads to limiting the differentiation of Th1 and Th17. This mechanism can be explained by the possible inhibition of costimulatory effects of antigen-presenting cells and may be due to the antioxidant property of EGCG.[24,44]

Glucoraphanin

Glucoraphanin is a glucosinolate reported to exist in broccoli (*Brassica oleracea* var. italica) and cauliflower (*B. oleracea* var. botrytis) as well as sprouts.[45,46] It is also the precursor of sulforaphane. Treatment of EAE mice with glucoraphanin was found to reduce EAE scores.[47] This effect is linked with a reduction of proinflammatory cytokines like IL-1β due to inhibition. This inhibition of NF-kβ is mediated via decreased degradation of inhibitor of kappa B.[47] Sulforaphane also reduced the EAE-evoked loss of the tight junction proteins.[48]

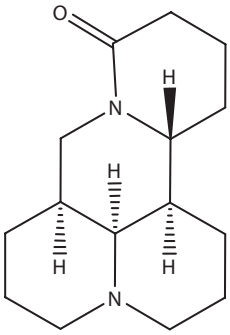


Hyperforin



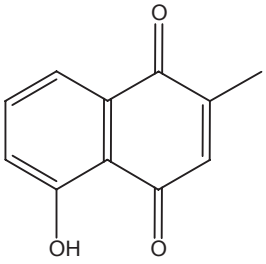
Hyperforin is the major phytoconstituent present in *Hypericum perforatum*. It is one of the most well-studied MMP inhibitors and is known for its antidepressant activity.[49] EAE rats treated with hyperforin reduced the severity of the disease symptoms. It was also found to reduce the IFN-γ levels.[50] Hyperforin-treated animals showed reduced expression of MMP9 and hence the migration of T cell across the BBB.[50]

Matrine



Matrine is a quinolizidine alkaloid obtained from *Radix sophorae* flave. It was found to inhibit the TLR4 signaling in the EAE model and can prove effective in the treatment of MS.[29,51] Matrine upregulates the glial glutamate transporters, which may reduce glutamate excitotoxicity.[52] Matrine was also reported to decrease MMP9 and MMP2 levels in matrine EAE rats.[53] It is suggested that matrine can decrease levels of some chemokines and adhesion molecules possibly via the inhibition of NF-kβ.[51] Treatment of EAE mice with matrine also reduced the leakage of the BBB,[53] which can contribute to its MMP inhibitory activity.

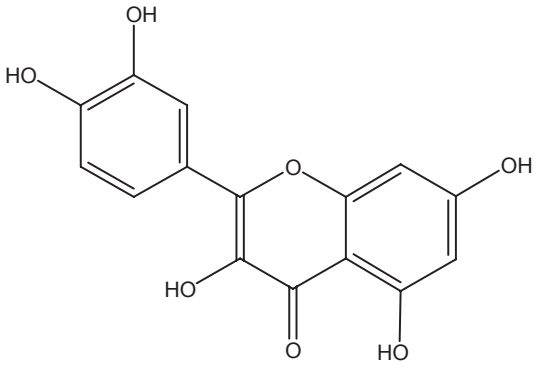
Plumbagin



Plumbagin is a phytochemical isolated from roots of plant *Plumbago zeylanica*. Plumbagin inhibits the NF-kβ-regulated gene transcription of proinflammatory cytokines and other related molecules like IFN-γ, IL-6, and inducible nitric oxide synthase. A study also suggests that the activity of plumbagin may also involve the inhibition of Jak/STAT signaling.[54]

Quercetin

Quercetin is a phytochemical belonging to the class of flavonoids. It is widespread in various fruits, vegetables, etc. Onion is one of the richest sources of quercetin.



In EAE, preventive treatment in mice with quercetin decreased demyelination as well as inflammation, which normally occurs via the inhibition of IL-12 production and Th1 differentiation.[55] Quercetin has also been observed to reduce the production of IL-12 and IFN-γ, which can be due to the reduction of Jak2, STAT3, and STAT4 phosphorylation.[55] It can also possibly due to downregulation of T-bet expression.[56]

Bowman–Birk Protease Inhibitor

Bowman–Birk inhibitor (BBI) is a serine protease inhibitor obtained from soy.[57] A BBI-enriched soy bean extract was found to reduce EAE symptoms.[58] It is suggested that the BBI effects may be mediated through the inhibition of MMPs, which stabilizes the BBB and prevents its breakdown.[57,59,60] This also reduces CNS infiltration with inflammatory cells.[61] In an EAE experiment, BBI reduced MMP2 and MMP9 activities, which decreased the severity of symptoms in EAE.[58]

Margatoxin, Agitoxin-2, and Kaliotoxin

These are the Kv1.3 channel blockers found in scorpion venom.[62,63] These compounds block the potassium pore of voltage-gated potassium channels.[64] Kaliotoxin treatment of EAE rats attenuated the symptoms of EAE, and it also suppressed the proliferation of T cells, reduced calcium influx, and inhibited IL-2 and TNF-α release by blocking Kv1.3.[63]

SUMMARY

Epidemiology of MS shows that it has become one of the major neurological disabilities. MS progresses through a very complex set of mechanisms, which are currently being explored to determine the best treatment strategies. Biomarkers of MS gained importance in this process of understanding, as they are expressed specifically in different mechanisms and are now providing the information about the diverse nature of MS. Biomarkers are helping researchers to discover various targets for designing new therapies for MS. Some of the biomarkers also provide confidence about

the success of the treatment as well as serve as surrogate end points for the clinical treatment of MS. Since these biomarkers are as important as the treatment itself, their development should be done in a more systematic and scientific way. Stringent criteria should be applied for selecting the biomarkers as surrogate end points.

Natural products are gaining universal acceptance for their usefulness in treating various disorders, and evidence-based scientific studies have encouraged people to use drugs of natural origin for the treatment of MS also. They are also gaining attention of researchers in the domain. Biomarkers have played a crucial role in providing scientific confirmations for use of natural drugs in MS treatment. As a result, a wide range of natural compounds are being explored for their possible efficacy in the treatment of MS. Some of these natural products are being tested clinically for the treatment of MS.

ABBREVIATIONS

APCs Antigen-presenting cell
BBB Blood–brain barrier
BBI Bowman–Birk inhibitor
CB Cannabinoid receptor type
CCR5 C–C chemokine receptor type 5
CD4 Cluster of differentiation 4
CNS Central nervous system
CSF Cerebrospinal fluid
CXCL10 Chemokine (C-X-C motif) ligand 10
CXCR3 Chemokine (C-X-C motif) receptor 3
DHA Docosaexanoic acid
EAE Encephalomyelitis
EGCG Epigallocatechin gallate
EPA Eicosapentaenoic acid
IFN-γ Interferon gamma
IL Interleukins
iNOS Inducible nitric oxide synthase
IκBα Inhibitor of kappa B
Jak Janus kinase
LXR Liver X receptor
MMP Matrix metalloproteinases
MRI Magnetic resonance imaging
MS Multiple sclerosis
NaCl Sodium chloride
NF-kB Nuclear factor kappa-light-chain-enhancer of activated B cells
PPAR peroxisome proliferator-activated receptors
PUFA Polyunsaturated fatty acids
RORγt Retinoic acid receptor-related orphan receptor gamma t
ROS Reactive oxygen species
SREBP-1c Sterol regulatory element-binding protein 1
STAT Signal transducer and activator of transcription
Th1 T cells Type 1 T helper cells
TIMP Tissue inhibitors of metalloproteinases
TLR Toll-like receptors
TNFα Tumor necrosis factor-α
TRPV1 Transient receptor potential cation channel subfamily V member 1
VDR Vitamin D receptor

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'*Note*: Page numbers followed by "f" indicate figures and "t" indicate tables.'

NUTRITION AND LIFESTYLE IN NEUROLOGICAL AUTOIMMUNE DISEASES: MULTIPLE SCLEROSIS

EDITED BY

RONALD ROSS WATSON AND **WILLIAM D. S. KILLGORE**

Multiple Sclerosis (MS) is a neuroinflammatory disease with no effective pharmaceutical therapy without major side effects. Consequently, patients and medical personnel often resort to their own therapies including lifestyle and dietary changes. Therapies include behavior modification, depression treatment and gait therapy, and there is growing interest in physical exercise and activities to reduce fatigue. In addition, new research is demonstrating the influence of nutrition, such as in vitamin D and food, as factors that can reduce symptoms.

Nutrition and Lifestyle in Neurological Autoimmune Diseases: Multiple Sclerosis discusses important discoveries relating to types and efficacy of nutritional and lifestyle responses to symptoms and reoccurrence of MS. Each chapter defines a new approach to use of foods, dietary supplements, exercise, behavior and/or lifestyle in health promotion and symptoms management for MS. This book discusses the role of non-pharmaceutical approaches and is essential reading for neurologists, physicians, nurses, nutritionists, dieticians, health care professionals, research scientists, biochemists, and general practitioners.

Ronald Ross Watson is a Professor of Health Promotion Sciences in the college of Public Health, Family and Community Medicine in the School of Medicine, and Adjunct Professor of Nutritional Sciences at the University of Arizona. He teaches a course each semester on drugs of abuse.

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