Dietary Influences on Multiple Sclerosis

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Introduction

Multiple sclerosis, MS, is a chronic, autoimmune disease of the central nervous system characterized by inflammation and degradation of oligodendrocytes on neuronal axons. Over time, multiple sclerosis can cause damage to the underlying axons[1] and generate neuronal loss,[2] leading to muscle weakness, numbness, and an eventual inability to walk independently.[3] Multiple sclerosis affects 947,000 people[4] in the United States and almost 2.3 million people[4] worldwide. The neurological disease disproportionately affects women and individuals living at higher latitudes.[5] In the United States, multiple sclerosis is almost three times more likely in females than in males, and in 2012, the female to male ratio for the disease was 3.13:1.[5]

Etiology

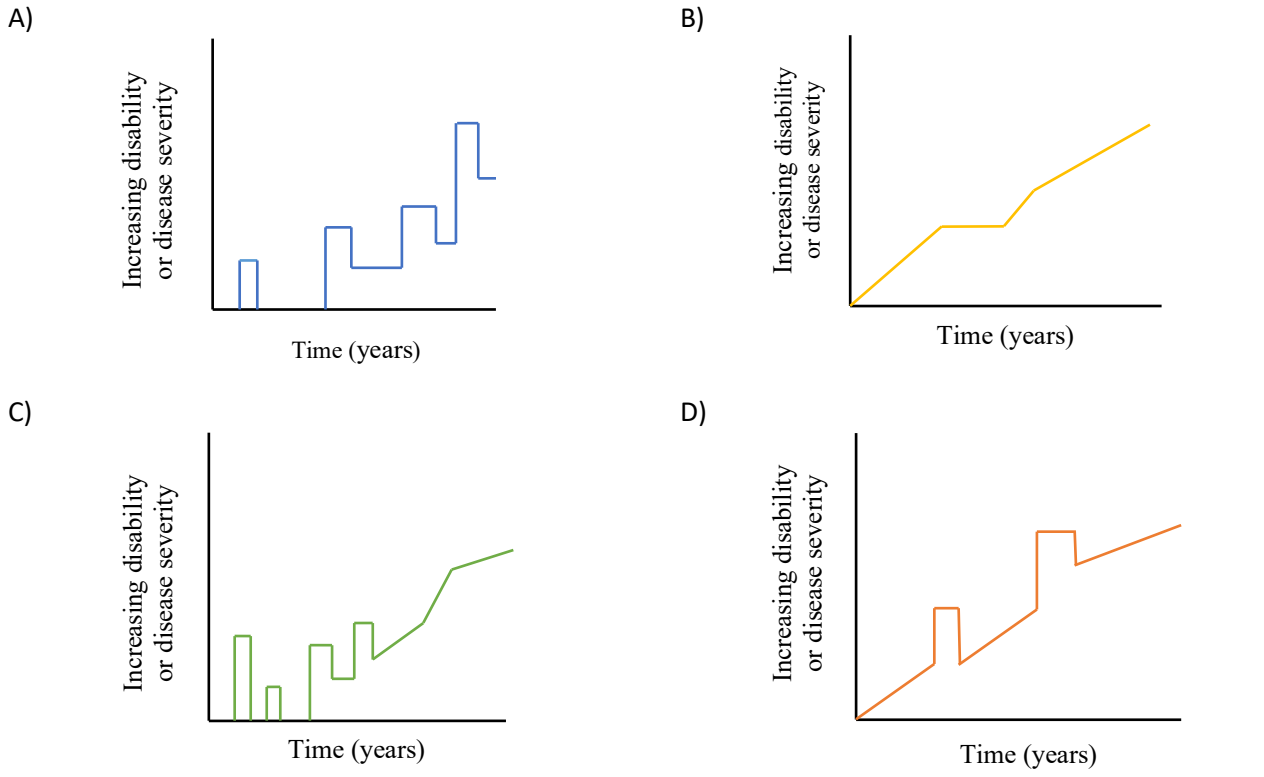
The complexity of MS development and progression makes the identification of causes quite difficult. To date, the exact cause of multiple sclerosis remains unknown. Although the etiology of multiple sclerosis remains unexplained, many genetic, environmental, and physiological risk factors have been pinpointed, including lower sunlight exposure[6] and cigarette smoking.[7] The connection between latitude and multiple sclerosis prevalence appears to be modulated by sunlight exposure, as those individuals at higher latitudes have decreased exposure to ultraviolet radiation from the sun, while individuals at lower latitudes have increased exposure.[8]

Symptomatic Progression and Sub-types

Multiple sclerosis moves through a few distinct stages of progression. Early in the disease, acute inflammatory events are common.[9] The acute inflammation often alternates with periods of remission and low inflammation.[9,10] From this stage, the disease typically moves into a period of secondary progression characterized by a more constant, chronic inflammation.[9]

The precise progression of the disease varies from one individual to the next and depends on the exact diagnosis of one of the four sub-types: relapsing remitting, secondary progressive, primary progressive, or progressive relapsing.[11] Relapsing remitting multiple sclerosis characterizes over 85 percent[11] of all cases, and most treatment options target this subset of the disease. In an attempt to standardize MS terminology and diagnosis, Dr. Fred Lublin, MD, and Dr. Stephen Reingold, PhD, defined the clinical course of multiple sclerosis for all four subtypes.[10] Relapsing remitting MS receives its name from its distinctive, cyclical progression pattern: repetitive inflammation and symptomatic flare ups followed by periods of stability (Figure 1A). Primary progressive MS, contrastingly, is defined by a gradual and continually worsening baseline for symptoms. With occasional plateaus and very rare periods of remission, primary progressive MS advances quicker compared to its relapsing remitting counterpart (Figure 1B). Secondary progressive MS initially follows an initial relapsing remitting course but evolves into a progression more similar to primary progressive MS, with constantly increasing severity of symptoms and inflammation (Figure 1C). Progressive relapsing MS develops the most rapidly of any sub-type. This sub-type has clearly defined acute relapses, like relapsing remitting MS. Periods in between the relapses are not characterized by remission, however, and instead show continual progression of symptoms, as seen in primary or secondary progressive MS (Figure 1D).

Figure 1. Graphs of relapsing remitting, primary progressive, secondary progressive, and progressive relapsing multiple sclerosis. **(A)** Relapsing remitting MS. Acute periods of increased disease severity alternating with periods of remission and recovery. Shown with both complete recovery back to baseline and incomplete recovery **(B)** Primary progressive MS. Constantly increasing disease severity and very few plateaus or periods of remission. The plateau shown in this diagram is not always present in primary progressive MS. **(C)** Secondary progressive MS. Initial relapsing remitting progression and development into more constant increase in disease severity. **(D)** Progressive relapsing MS. Acute relapses and constant increase in disease severity in between relapses. Relapses can result in complete recovery or incomplete recovery.



Molecular Progression

The underlying source of the symptomatic progression in MS comes from the activity and movement of immune cells. The molecular progression of MS includes a few key immune cells: T-cells, macrophages, and cytokines. T-cells are small leukocytes, or white blood cells, that aid in the immune system's identification of invading pathogens.[12] Cytokines are messenger molecules released from leukocytes to signal the identification of a pathogen; their release initiates the migration of macrophages to the site of the pathogen.[12] Macrophages, larger leukocytes, migrate to the pathogen

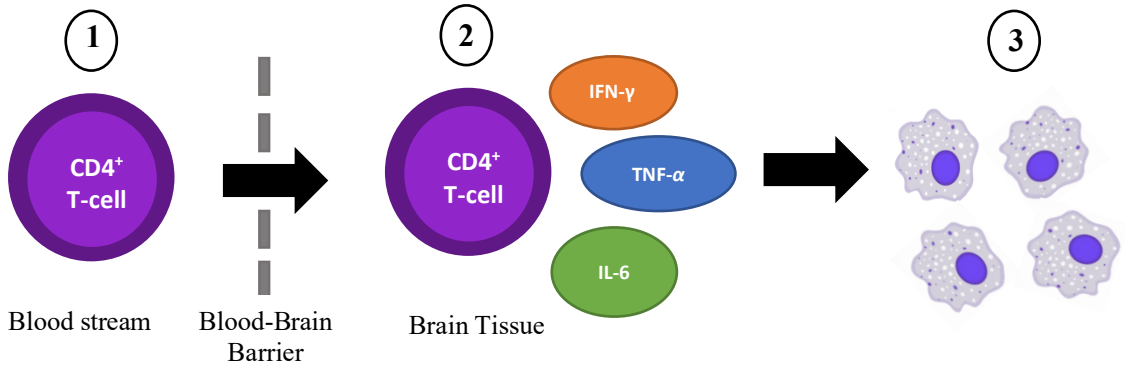
after the release of cytokines and proceed to phagocytose the pathogens labeled for destruction by T-cells. Macrophages can also release the cytokine messengers as a signal of pathogen identification.[12] Using these immune cells, multiple sclerosis progression proceeds through a few main stages: blood-brain barrier disintegration, leukocyte invasion, cytokine release and macrophage attack on myelin sheath (Figure 2).[13]

Multiple sclerosis begins with a breakdown of the blood-brain barrier, a highly selective barrier that separates the brain from the vasculature of the rest of the body.[13] The damage to the blood brain barrier starts when leukocytes adhere to the endothelial cells of the barrier.[13] After adherence, the leukocytes degrade surrounding vasculature and venules, and the blood-brain barrier partially disintegrates.[14] The damaged areas of the blood-brain barrier have an increased permeability to nearby leukocytes, which under normal circumstances would remain in the vasculature, isolated from the brain tissue.[13] A variety of leukocytes enter brain tissue, including a large percentage oligoclonal $CD4^+$ T-cells.[15] Oligoclonal $CD4^+$ T-cells, a subcategory of T-cells, then identify the myelin sheath on the axonal neurons as pathogenic and release inflammatory cytokines to initiate the degradation process.[13] In response to the cytokine release, macrophages flood the region and attack the oligodendrocytes.[13] The oligodendrocyte degradation generates massive encephalomyelitis,[13] inflammation in the brain and spinal cord. Oligodendrocyte degradation and associated encephalomyelitis are characteristic symptoms in all four sub-types of MS.[10]

After the initial leukocyte influx and attack, the disease continues progressing via a cyclical process between leukocyte degradation of myelin and cytokine release. The proliferation of T-cells and macrophage activation in the brain first provokes an increased secretion of inflammatory cytokines.[13,16] Then, the cascade continues as these inflammatory cytokines signal migration of even more leukocytes to the region.[13]

Inflammatory cytokines released in multiple sclerosis commonly include interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF- α), and a variety of interleukins, a class of glycoproteins used for immune responses (Figure 2).[16] These cytokines are particularly important in our understanding of MS progression because their levels can be easily measured to quantify the disease's progression.

Figure 2. Leukocytes entering the brain. Step One: $CD4^+$ T-cells pass through a deteriorated blood brain barrier. Step Two: $CD4^+$ T-cells produce inflammatory cytokines, including interferon gamma (IFN- γ), tumor necrosis factor alpha (TNF- α), and interleukin six (IL-6). Step Three: Other immune cells, including macrophages, are recruited to the location to initiate the oligodendrocyte breakdown.



Clinical Markers

Clinical markers for MS can vary widely in its the early stages and depend heavily on the location of the brain lesions. Nonetheless, early markers of multiple sclerosis include fatigue, weakness, walking difficulties, numbness, tingling, and cognitive problems.[3] The physical symptoms of disability are often assessed on the Extended Disability Status Scale, or EDSS. The EDSS measures changes in disability over time by quantifying disability symptoms on a scale from 1 to 10, where 10 represents the highest level of disability.[17]

Changes in the anatomy of the brain can also be indicative of disease progression in multiple sclerosis. The presence of gadolinium-enhancing lesions in MRI scans, for example, is a common clinical measure for MS disease progression. [18] Gadolinium-enhancing MRI scans work via the use of

gadolinium, an element that marks regions with increased blood brain barrier permeability.[18] Increased permeability is correlated with an increased influx of leukocytes and degradation of oligodendrocytes, making gadolinium-enhancing lesions an excellent standardized, quantifiable measure of MS disease progression.[18]

Biomarkers for Multiple Sclerosis

In addition to gadolinium-enhancing lesions and EDSS scores, inflammatory cytokine levels are also assessed as markers of MS progression. Common biomarkers measured in MS include: matrix metalloproteinase-9 (MMP-9),[19] interleukin-17 (IL-17),[20] tumor necrosis factor-alpha (TNF-α),[21] interferon-gamma (IFN-γ),[22] interleukin-1 beta (IL-1β)[23] and interleukin-6 (IL-6).[21] Each of these biomarkers has its own distinct function related to multiple sclerosis. Matrix metalloproteinases describe a family of endopeptidases that degrade extracellular matrices and increase the permeability of the blood brain barrier, allowing non-neuroimmune cells like $CD4^+$ T-cells and macrophages to penetrate the normally tightly sealed endothelial cells.[19] IL-17 cytokines are produced by T-cells and stimulate the production of other inflammatory cytokines that contribute to the oxidative stress, inflammation, and encephalomyelitis seen in multiple sclerosis patients.[20] TNF-α promotes macrophage digestion and demyelination of the central nervous system axons and is present in high levels of many demyelinating diseases.[21] Interferon-gamma[22] and interferon-1 beta[23] are both involved in various inflammation activation pathways. IL-6 aids in antibody synthesis in leukocytes and contributes to the recruitment of other immune cells.[21] This list of inflammatory cytokines and their functions is not exhaustive, and there are many other signaling molecules and interleukins that are also measured as biomarkers of MS. The presence of any MS biomarkers suggests the progression of the disease, and changes in the levels of these biomarkers are often used as measurements for the effectiveness of multiple sclerosis treatments.

Diagnosis

There is no one imaging technique, genetic analysis, or lab test that can definitively diagnose an individual with multiple sclerosis. Official diagnosis requires that a physician identify two separate regions of the central nervous system with damage.[24] The diagnosis process is labor-intensive, costly, and time-consuming. The International Panel on the Diagnosis of Multiple Sclerosis recently published updates to their McDonald Criteria for diagnosis.[24] These updated criteria form the basis on which most diagnoses are now conducted. Identification and diagnosis of multiple sclerosis is most often generated from MRI imaging techniques via the identification of gadolinium-enhancing lesions in the white matter.[25]

In addition to MRI scans, cerebrospinal fluid analysis is also a historically relevant diagnostic tool for multiple sclerosis. The presence of oligoclonal bands in cerebrospinal fluid analysis helps identify the invasion of oligoclonal immune cells, like $CD4^+$ T-cells, in the central nervous system.[26] Oligoclonal bands are found in protein gels run on cerebrospinal fluid of patients with neurological diseases like MS. The bands consist of proteins produced by immune cells and are in much higher quantities in individuals with a deteriorated blood-brain barrier, making them a useful marker for MS diagnosis.[26] Cerebrospinal fluid analysis has lost some weight in the McDonald Criteria,[24] but many researchers and physicians still argue for its validity and relevance in diagnostic processes and it is recommended that cerebrospinal fluid analysis should still be conducted before a final diagnosis is presented to the patient.[26] The diagnosis process for MS is expensive and time-consuming, and cerebrospinal fluid analysis and MRI scans are only a few of the myriad of tests performed in the exhaustive process.[24]

Traditional Treatment Options

After diagnosis, patients are provided with an assortment of treatment options. As of now, there are two main categories of treatment for multiple sclerosis patients: acute relapse management

and disease modifying treatments.[27] The first, acute relapse management, involves treatment options for individuals in the midst of severe inflammation. High doses of corticosteroids, like methylprednisolone, can help reduce the severity and duration of a relapse.[28] Corticosteroid treatment serves as a great temporary solution for acute inflammation, but it does not prevent future relapses or slow the disease progression. Resultantly, corticosteroids do not represent a long-term, curative treatment option for multiple sclerosis patients. The second, disease modifying treatment, is relatively newer and targets the earlier stages of the disease. Three common disease modifying drugs are beta interferons (interferon beta), glatiramer acetate, and natalizumab. Interferon beta and glatiramer acetate are considered moderately effective and decrease the yearly relapse rate by 30 to 50 percent while natalizumab is considered highly effective and decreases the yearly relapse rate by more than 50 percent.[27] Each drug acts via a different mechanism of action. Interferon beta works by controlling the transcriptional regulation of thousands of genes that alter dendritic cells and T-cells and has been shown to dramatically slow disease progression in multiple sclerosis patients.[29] Glatiramer acetate, on the other hand, is believed to activate T-cells that secrete anti-inflammatory cytokines and help decrease encephalomyelitis.[30] Natalizumab is considered the most effective treatment option of the three, yet it is also associated with severe risks. It is considered a monotherapy and should be taken in the absence of other multiple sclerosis pharmacological treatments. The risks associated with natalizumab are higher than with other treatments, as the disease is occasionally associated with progressive multifocal leukoencephalopathy (PML). PML is a rare but serious brain infection caused by the John Cunningham virus.[31] Natalizumab interferes with leukocyte adherence to the blood brain barrier and is believed to reduce healthy T-cell mediated immune responses in patients undergoing treatment, making them more susceptible to infection by the John Cunningham virus.[32,33]

Animal Model of Multiple Sclerosis: Experimental Allergic Encephalitis

Researchers continue to explore treatment options on both multiple sclerosis patients and animal models. No exact replica of multiple sclerosis exists in an animal model. In current research, experimental allergic encephalitis, commonly referred to as EAE, is the widely accepted animal model for multiple sclerosis because T-cell activation, demyelination, and symptomatic progression are very similar in multiple sclerosis and EAE.[34] The EAE animal model serves as a valid tool to inform our understanding of the disease, while prompting additional research in human subjects. The study of disease-modifying therapies and acute relapse management medications have been analyzed in both EAE animal models[35] and human multiple sclerosis patients.[32,33]

Diet and dietary supplements have recently come under more intensive investigation for their effects on the etiology and treatment of multiple sclerosis, but diet has been considered a potential influence in MS since the 1950s.[36] Diet, like traditional forms of treatment, has been studied in human patients and EAE animal models. Both representations of multiple sclerosis will be considered in the context of this review.

Review

Diet in Multiple Sclerosis

To date, our understanding of diet in multiple sclerosis remains relatively unexplored. Preliminary research, however, suggests that certain supplements and dietary patterns may influence the onset and progression of the disease. Omega-3 fatty acids and vitamin D_3 are two main supplements that continue to show promise for multiple sclerosis patients. More broadly, Western diets and Mediterranean diets have also been explored with regards to their influence in multiple sclerosis.

Omega-3 Fatty Acids

Increased intake of ω-3 fatty acids has been linked to a lower risk of multiple sclerosis development. In a retrospective study conducted by Kampman et al.,[37] researchers asked 152 multiple sclerosis patients and 402 controls in Norway about their past consumption of fish and cod-liver oil, foods high in ω-3 fatty acids. Those participants who claimed to consume fish three or more times per week had a significantly lower risk of having multiple sclerosis ($p = 0.024$). Hoare et al.[38] uncovered similar findings when examining the environmental risk factors for demyelination diseases in Australia. The researchers obtained data on the dietary intake of 267 people with multiple sclerosis and 517 controls and, after adjusting for age, education, and other variable factors, found a significant negative association between intake of the ω-3 fatty acids DHA ($p = 0.05$) and EPA ($p = 0.03$) and multiple sclerosis development.

Pre-clinical and clinical trials have also suggested the importance of ω-3 fatty acid supplementation in delaying MS progression (Table 1). In a pre-clinical intervention trial, Nordvik et al.[39] investigated the impact of daily fish oil supplementation (0.5g DHA, 0.4g EPA) and dietary advice from nutritionists on the progression of relapsing remitting multiple sclerosis in patients ($n = 16$) diagnosed within 12 months of the study. Clinical assessment of disease progression was completed at baseline, 12 months, and 24 months using the EDSS. After two years of supplementation, EDSS scores of patients significantly decreased compared to baseline ($p < 0.01$). Weinstock-Guttman et al.[40] found similar results in their year-long double-blind placebo-controlled clinical trial on daily ω-3 fatty acid supplementation. The participants that received a low-fat diet supplemented with ω-3 fatty acids (1.98g EPA and 1.32g DHA) ($n = 14$) showed a significant decrease in relapse rate ($p = 0.021$) and quality of life fatigue ratings ($p = 0.035$) compared to participants that received a higher fat diet, with placebo olive oil capsules ($n = 13$). Similarly, a double-blind, placebo-controlled clinical study conducted by Kouchaki et al.[41] found that omega-3 fatty acid supplementation in conjunction with vitamin D_3 reduced the clinical symptoms of

relapsing-remitting multiple sclerosis. Over the course of 12 weeks, participants who received two ω-3 fatty acid tablets daily (500mg DHA and 106 mg EPA per tablet) and vitamin D_3 (50,000 IU biweekly) (n = 26) had significantly lower EDSS scores (p = 0.01) than those participants that received sunflower oil placebo tablets. In contrast, Ramirez-Ramirez et al.[42] found no significant difference in EDSS scores (p = 0.73) or relapse rates (p = 0.79) for multiple sclerosis patients treated daily for 12 months with ω-3 fatty acid supplements (1.6 g DHA, 0.8g EPA) (n = 20) compared to patients given a placebo capsule (n = 19). Torkildsen et al.[43] likewise found that the daily administration of ω-3 fatty acids (0.85g EPA and 1.35g DHA) did not significantly impact EDSS scores between the placebo (n = 36) and treatment groups (n = 40) (p = 0.72) or differences in the number of gadolinium-enhancing lesions between groups (p = 0.17).

Omega-3 fatty acid supplementation has also been found to significantly reduce multiple sclerosis biomarkers in patients. In a clinical trial conducted by Shinto et al.,[44] researchers assessed the impact of daily ω-3 fatty acid supplementation (2.9g EPA and 1.9g DHA) for three months on MMP-9 secretions from peripheral blood mononuclear cells. Levels of MMP-9 in mononuclear cell secretions significantly decreased after 3 months of supplementation (p = 0.002) and stayed significantly lower after a 3 month wash out period with no further supplementation (p = 0.01). In further analysis from Shinto et. al.,[45] researchers applied ω-3 fatty acids directly to a line of human T-cells (Jurkat cells) and found significant decreases in MMP-9 secretions in cells treated with 50 μg/mL DHA (p = 0.05), 25 μg/mL EPA (p = 0.04), and 50 μg/mL EPA (p = 0.01). The migration of the T-cells past a pseudo-blood brain barrier was also significantly decreased by EPA (10μg/mL, 30μg/mL 100μg/mL) and DHA (30μg/mL, 100μg/mL) application (p < 0.05 for all). Gallai et al.[46] also found a reduction in multiple sclerosis biomarkers after treatment with ω-3 fatty acids. Multiple sclerosis patients (n = 20) and control individuals (n = 15) were given daily supplements of ω-3 fatty acids (0.51g EPA, 0.31g DHA). After three months of treatment, multiple sclerosis patients saw a significant difference in their serum levels of IFN-γ (p < 0.002), TNF-α (p < 0.02), and IL-1β (p < 0.01). Similar reductions were found in control patients

too, with significant decreases in the levels of IFN-γ ($p < 0.03$), TNF-α ($p < 0.03$), and IL-1β ($p < 0.04$). Ramirez-Ramirez et al.,[42] also found a significant reduction in oxidative stress and multiple sclerosis biomarkers in their double-blind, placebo-controlled study of ω-3 fatty acid supplementation in multiple sclerosis patients (1.6 g DHA, 0.8g EPA). Measures at 6, 9, and 12 months of consecutive supplementation showed significant decreases from baseline of serum TNF-α levels ($p < 0.001$), IL-1β levels ($p < 0.001$), and IL-6 levels ($p < 0.001$). Research conducted by Weinstock-Guttman et al.,[40] however, found no significant differences in inflammatory cytokine levels for multiple sclerosis patients on a year-long low-fat diet with ω-3 fatty acid supplements (1.32g DHA, 1.98g EPA) compared to patients on a higher fat diet with no supplements ($p > 0.05$ for all).

A considerable amount of research with ω-3 fatty acids has been conducted in the main animal model of multiple sclerosis: EAE. Mancera et al.[47] found comparable results to Gallai et al.[46] in an EAE model, as their female mice given supplements of DHA (50mg/kg - 250mg/kg) had significantly lower levels of cytokine biomarkers TNF-α and IL-6 ($p < 0.05$ for 50mg/kg and $p < 0.01$ for 250mg/kg for both). Adkins et al.[48] conducted a comparable study with female mice ($n = 35$), in which they determined that a supplementation of DHA (0.48mg/kg body weight; $n = 13$ and 1.6 mg/kg body weight; $n = 16$) significantly decreased EAE symptoms ($p = 0.028$) compared to the mice that received a control diet. Moreover, the supplementation of DHA delayed the onset of the disease, as significantly fewer mice fed the DHA diet displayed symptoms 12 days after EAE induction ($p = 0.049$) compared to control mice. Both Adkins et al.[48] and Mancera et al.[47] looked at DHA in specific, but EPA has also been shown to generate similar findings. Salvati et al.[49] found that dietary intake of EPA delayed the appearance of EAE symptoms in female rats 10 days after EAE induction (0.2% EPA: $p < 0.01$ and 0.4% $p < 0.01$).

Table 1. Clinical trials with Omega-3 Fatty Acids supplementation in multiple sclerosis patients organized by duration of the trial.

Researchers	Daily Dosage	Duration	Concurrent Treatments	Results
Kouchaki et al.	1.00g DHA, 0.21 EPA	12 weeks	Vitamin D_3 (required)	EDSS scores lower than control ($p = 0.01$)
Shinto et al.	2.90g DHA, 1.90g EPA	3 months	Interferon Beta or Glatiramer Acetate (permitted)	Decrease in MMP-9 levels ($p = 0.002$)
Gallai et al.	0.31g DHA, 0.51g EPA	6 months	Adrenocorticotropic Hormone or corticosteroids (permitted)	Decrease in IFN-γ levels ($p < 0.002$), TNF-α levels ($p < 0.02$), and IL-1β levels ($p < 0.01$)
Ramirez-Ramirez et al.	1.60g DHA, 0.8g EPA	1 year	Interferon Beta (required)	No change in relapse rates ($p = 0.79$) and no change in EDSS scores ($p = 0.73$) Decrease in TNF-α levels ($p < 0.001$), IL-1β levels ($p < 0.001$), and IL-6 levels ($p < 0.001$)
Weinstock-Guttman et al.	1.98g DHA, 1.32g EPA	1 year	Glatiramer Acetate or Interferon Beta (permitted)	Relapse rate decreased ($p = 0.021$) and fatigue ratings decreased ($p = 0.035$)
Nordvik et al.	0.5g DHA, 0.4g EPA	2 years	None	EDSS scores decreased ($p < 0.01$)
Torkildsen et al.	1.35g DHA, 0.85g EPA	2 years	Interferon Beta (required)	No change in lesions ($p = 0.17$) and no change in EDSS scores ($p = 0.72$)

Vitamin D_3

Vitamin D_3 intake has also been studied in the development and progression of multiple sclerosis. In addition to dietary intake, exposure to UV radiation increases the formation of vitamin D_3 in the skin cells.[50] Therefore, vitamin D_3 and its potential impact on multiple sclerosis is studied both via its acquisition from sunlight and from the diet.

Increased Vitamin D_3 levels have been associated with a decreased risk of MS development. In a study performed by Kampman et al.,[37] researchers analyzed the role of UV radiation in the development of multiple sclerosis through a retrospective analysis of 152 multiple sclerosis patients' and 402 control patients' habits. Participants who reported spending more time outdoors in the sunlight between the ages of 16 and 20 had a significantly reduced risk of developing multiple sclerosis ($p = 0.001$). Munger et

al.[51] performed a prospective case-control study and analyzed serum samples stored at the United States Department of Defense from more than 7 million American military personnel. Risk of multiple sclerosis development in white individuals was significantly lower in individuals with higher serum levels of hydroxyvitamin D, and for every 50nmol/L increase in hydroxyvitamin D levels, there was a 41% lower risk that an individual would develop multiple sclerosis ($p = 0.04$). Hydroxyvitamin D is a pre-hormone produced by the liver during the breakdown of vitamin D_3; increased levels of hydroxyvitamin D indicate increased intake of vitamin D_3.[51] Fetal vitamin D_3 exposure is also a risk factor for multiple sclerosis development according to a retrospective study performed by Mirzaei et al.[52] Mothers of women who participated in the Nurses' Health Study II ($n = 35,794$) filled out a questionnaire regarding their diet during pregnancy, and researchers discovered a significant negative association between milk and vitamin D_3 consumption and the development of multiple sclerosis in the fetus ($n = 199$) after adjusting for the mother's age ($p = 0.012$). Ascherio et al.[53] investigated the role of vitamin D_3 in multiple sclerosis disease progression and discovered a similar protective role of vitamin D_3 in individuals already clinically diagnosed with multiple sclerosis ($n = 468$). Baseline serum levels of hydroxyvitamin D were significant predictors of MS activity and progression over a 60 month period, as those individuals with higher serum levels (at least above 50nmol/L) had a significantly lower appearance of new lesions ($p = 0.002$), lower volume accumulation in pre-existing lesions ($p < 0.001$), and smaller changes in EDSS scores ($p = 0.004$).

Vitamin D_3 supplementation has also been studied in the context of multiple sclerosis biomarkers. Smolders et al.[54] gave relapsing remitting multiple sclerosis patients ($n = 15$) vitamin D_3 supplements (20,000 IU/day) for 12 weeks and found a significant decrease in the balance of inflammatory cytokines (IFN-γ) to anti-inflammatory cytokines (IL-4) ($p = 0.035$). In animal model research conducted by Farias et al.,[55] rats induced with EAE and treated with vitamin D_3 (15μg/Kg/day) had significant reductions in the levels of IL-17A ($p < 0.05$), IFN-γ ($p < 0.05$), and TNF-α ($p < 0.01$). Using

different dosages, Mahon et al.[56] contrastingly found that supplementation of vitamin D_3 (1,000 IU/day) and calcium (800mg/day) for 6 months in multiple sclerosis patients (n = 17) did not significantly change the levels of biomarkers for IFN-γ and TNF-α ($p > 0.05$). Burton et al.[57] looked at a wide variety of markers for multiple sclerosis in a clinical trial and found that supplementation of titrating doses (28,000 IU/week to 280,000 IU/week to 28,000 IU/week) of vitamin D_3 and calcium supplementation (1,200 mg/day) in multiple sclerosis patients (n = 24) did not significantly change TNF-α, MMP-9, IFN-γ, or any other major components of the participants' serum cytokine profile ($p > 0.05$).

Vitamin D_3 supplementation has been utilized as a treatment option for multiple sclerosis patients in multiple clinical trials (Table 2). In their unblinded, placebo-controlled clinical trial Burton et al.[57] found that 28 weeks of titrating doses of vitamin D_3 supplementation (28,000 IU/week to 280,000 IU/week to 28,000 IU/week) and calcium supplementation (1,200 mg/day) created a significant difference in the number of participants with increased EDSS scores compared to the control group ($p = 0.019$). In another clinical trial, Kimball et al. 2007 found similar results. Researchers investigated the impact of increasing vitamin D_3 doses (700μg per week to 7000μg per week) on the lesions and symptomatic progression in 12 relapsing remitting multiple sclerosis patients. After 28 weeks of treatment (dosage increase every 6 weeks), all 12 participants had significantly lower number of gadolinium lesions ($p = 0.03$). In contrast, in a double-blind, placebo-controlled trial by Kampman et al.,[58] researchers found that there was no significant difference in the percentage of patients without a relapse in the group treated for 96 weeks with vitamin D_3 supplements (20,000 IU/week) and calcium (500mg/day) (n = 35) than those patients that received the placebo pill (n = 33) ($p = 0.56$). Moreover, there was no significant difference between the EDSS scores ($p = 0.22$) or the annual relapse rates ($p = 0.42$) of the groups after 96 weeks of supplementation.

Table 2. Clinical trials with Vitamin D_3 supplementation in multiple sclerosis patients organized by duration of the trial.

Researchers	Dosage	Duration	Concurrent Treatments	Results
Smolders et al.	20,000 IU per day	12 weeks	Interferon Beta and Multivitamins (permitted)	Decrease in inflammatory to anti-inflammatory cytokine ratio ($p = 0.035$)
Kimball et al.	Increasing 700µg per week to 7000µg per week	28 weeks	Interferon Beta or Glatiramer Acetate (permitted)	Decreased lesions ($p = 0.03$)
Burton et al.	Titrating 28,000 IU/week to 280,000 IU/week to 28,000 IU/week	28 weeks	Interferon Beta and Glatiramer Acetate (permitted), Calcium (required)	Decreased number of patients with increased EDSS scores compared to control group No change in TNF-α, MMP-9, IFN-γ, or serum cytokine profile ($p > 0.05$)
Mahon et al.	1,000 IU per day	6 months	Calcium (required)	No change in IFN-γ levels and TNF-α levels ($p > 0.05$)
Kampman et al.	20,000 IU per week	96 weeks	Interferon Beta, Glatiramer Acetate, or Natalizumab (permitted), Calcium (required)	No change in relapse rates ($p = 0.42$) or EDSS scores ($p = 0.22$)

Western Diet and Mediterranean Diet

In addition to individual nutrient analyses, investigations looking at individuals' diets on a larger scale can inform our understanding of the risks, development, and progression of multiple sclerosis. Two main diets have come under more intense examination: the Mediterranean Diet and the Western Diet (sometimes referred to as the Standard American Diet).

The Western diet, a diet consisting of heavy consumption of saturated fats, simple sugars, refined grains, alcohol, salt, and high fructose corn syrup, results in high BMI scores, high cholesterol levels, and lower health composite scores.[59] Investigations of cholesterol and saturated fat are common in analyses of the effects of "Western-like" diets on multiple sclerosis and are an indication of how general dietary patterns may impact the disease. Tettey et al.,[60] for example, performed a cohort study with multiple sclerosis patients (n = 282) to analyze the relationship between BMI, triglyceride levels, and the progression of multiple sclerosis over the course of 5 years. The researchers discovered a

significant positive correlation between BMI and relapse hazard rates ($p = 0.012$), and for every increase in BMI of 5 kg/m^2, there was a 25% increase in the likelihood that the patient would have a relapse during the 5 years. Similarly, the researchers identified a positive correlation between triglyceride serum level and relapse hazard rate ($p = 0.012$), and for every 1mmol/L increase in triglyceride levels there was a 22% increase in the risk of relapse. Weinstock-Guttman et al.[61] performed a similar prospective cohort study in which they investigated the lipid profile of 135 high-risk multiple sclerosis patients (individuals with one episode of inflammation but no clinical diagnosis of multiple sclerosis before the start of the study) and found that the number of lesions was positively correlated with the total cholesterol levels ($p = 0.001$) and low-density lipoprotein cholesterol levels ($p = 0.006$). In contradiction, the researchers in the prospective study by Tettey et al.[60] did not find any significant relationship between the lipid profiles (total cholesterol, high density lipoprotein, and low density lipoprotein) and the risk for relapse in MS patients.

The Mediterranean diet, in contrast to the Western diet, consists of higher consumption of fruits, vegetables, and fish and a lower consumption of red meat, dairy, and saturated fats. A minimal amount of research has been conducted on the Mediterranean diet and multiple sclerosis, but some support has been garnered for its protective effects. In a retrospective study conducted by Sedaghat et al.,[62] researchers analyzed the role of diet on the risk of multiple sclerosis development in control ($n = 140$) and multiple sclerosis patients ($n = 69$). They analyzed the diet in terms of 9 separate components (vegetables, fruits, ratio of saturated to unsaturated fats, legumes, nuts, fish, refined grains, dairy products, and ratio of red and processed meats to white meats) and found a significant positive association between the consumption of foods in the Mediterranean diet (fish, vegetables, fruits, etc.) and decreased likelihood of multiple sclerosis development ($p = 0.04$).

Overall diet quality and its impacts on multiple sclerosis has also been analyzed in recent research and integrates aspects of multiple diets, including the Western diet and Mediterranean diet.

Rotstein et al.[63] conducted a massive prospective cohort study (n = 185,000) of women in the United States, using participants from both the Nurses' Health Study and the Nurses' Health Study II. Women participating filled out food frequency questionnaires once every four years. Dietary analysis asked about 130 different foods and involved three separate scales: Alternative Eating Index 2010, Alternate Mediterranean Index, and Dietary Approaches to Stop Hypertension Index. The Alternate Mediterranean Index in particular focused on ratio of unsaturated fatty acids to saturated fatty acids and the intake of vegetables, legumes, fruit, nuts, whole grains, and fish. The researchers discovered that all dietary measures and scales, including those reflective of a Western diet and Mediterranean diet had no significant correlation with multiple sclerosis development (n = 480) ($p > 0.05$). In other words, major dietary patterns and risk of multiple sclerosis were not significantly linked.

Discussion

Omega 3 Fatty Acids

Despite a few exceptions, the supplementation of DHA and EPA in the form of fish oil has shown to reduce the clinical symptoms and markers of multiple sclerosis in a majority of clinical trials and preclinical trials.[39–41] The dosages of ω-3 fatty acids and the ratio of EPA to DHA in the fish oil is similar across most of the trials, and the main difference lies in the other combined supplements and treatments. When patients enter a clinical trial for ω-3 fatty acid supplementation, they are often allowed to continue administration of other forms of treatment, like interferon-beta.[39,40,42,43] Consent for a study would likely decrease drastically if participation required termination of other courses of treatment, so this variability between studies is often unavoidable. In addition to clinical markers, the common biomarkers for multiple sclerosis: MMP-9, IL-17, IL-1β, TNF-α, IFN-γ, and IL-6 tended to decrease after dietary supplementation with ω-3 fatty acids in both human multiple sclerosis

patients[42,44,46] and the EAE induced rodent model.[47] Omega-3 fatty acid supplementation also has been linked to lower risk for multiple sclerosis development.[37,38]

The evidence from both clinical and pre-clinical studies strongly favors ω-3 fatty acid supplementation as a safe[42,43] and effective treatment option for multiple sclerosis patients. Very few side effects are recorded in the studies, and there are nearly no negative interactions with other multiple sclerosis treatments, meaning that the supplements can easily and safely be taken in conjunction with interferon beta,[42,43] glatiramer acetate,[40] and other dietary supplements like vitamin D_3.[39,40] Omega-3 fatty acids have demonstrated a protective effect in higher latitudes,[37,38] so individuals at higher latitudes or with a genetic predisposition for multiple sclerosis could also take fatty acid supplements as a preventative measure to decrease their likelihood of developing the disease. Taking the supplements poses no major health risks, so even if its addition to the diet does not provide any benefit, it will not harm the individual. Omega-3 fatty acid supplements appear to be a simple, safe, and potentially effective method for protection against multiple sclerosis and as a method to slow the symptomatic progression.

In future research, researchers could address the varying supplements and treatments combined with ω-3 fatty acid supplementation. As of now, the durations and doses of the trials vary too significantly to make many direct comparisons.

Vitamin D_3

In multiple cohort studies, low Vitamin D_3 levels have been shown to be predictive of multiple sclerosis development[51,58] in adults and prenatally for children in utero.[52] Moreover, low vitamin D_3 levels are predictive of multiple sclerosis disease progression after its diagnosis.[53] Vitamin D_3 supplementation has been shown to significantly decrease levels of IFN-γ,[54,55] TNF-α,[54,55] and IL-17A[55] in a few studies, yet it also has been shown to have no effect on MMP-9,[57] INF-γ,[56,57] and TNF-α levels[56,57]

in other studies. Mahon et al.[56] used a much smaller dose of vitamin D_3 (1,000IU/day compared to 20,000 IU/day), however, which is why they may have found no significant results. Likewise, contradictory research has shown that vitamin D_3 supplementation has significantly beneficial impacts on the EDSS scores[57] and brain lesions[64] in a few studies and no significant impact on relapse rates and EDSS scores[58] in a different study.

The study of vitamin D_3 has generated a more scrambled set of results than ω-3 fatty acids. Nonetheless, there is a compelling amount of evidence supporting the effectiveness of Vitamin D_3 as a predictor of reduced multiple sclerosis development[51,52,58] and progression,[53] and thus there is a substantial amount of evidence to support its usefulness in treating multiple sclerosis that has already been diagnosed.[57,64] Vitamin D_3 has also been shown to have minimal negative health effects at high doses (10,000 IU/day).[57] This means that taking Vitamin D_3 supplements at 10,000 IU per day could help improve symptoms of multiple sclerosis without generating unwanted hypercalcemia, excessive calcium in the bloodstream. According to the National Institute of Health (NIH), however, the tolerable upper limit for vitamin D_3 in adults is much lower, only 4,000 IU per day.[65] It's important to consider the time frame of the study in which the dosage of 10,000 IU was defined; participants only received doses of 10,000 IU for 12 weeks.[57] Long-term use of this dosage for more than 12 weeks, therefore, may not be safe. Vitamin D_3 supplementation is a simple addition to the diet, and does not pose any major health risks if taken at low enough doses (10,000 IU[57] per day according to Burton et al. or 4,000 IU[65] per day according to the NIH). More research is imperative in order to determine the maximal, safe dose. Overall, however, vitamin D_3 supplementation appears to be a valid and safe treatment method for MS and an accurate prediction tool for MS development.

In many of these studies, vitamin D_3 was taken in combination with other treatments and supplements including glatiramer acetate,[57,64] interferon-beta,[54,57,64] and calcium supplements.[56–58] The interactions and potential synergistic combinations of these treatments needs to be studied in greater

depth in order to determine the best combination of treatments for multiple sclerosis patients. Additionally, future research could work to define a general serum level of hydroxyvitamin D and corresponding dosage of vitamin D_3 that are most effective. Myriads of studies have been performed, all using different doses and different durations, and this information can be compiled to make an estimate, but a definitive dosage remains poorly characterized as of now in the literature.

Vitamin D_3 and Omega-3 Fatty Acids

Omega-3 fatty acid supplementation and vitamin D_3 supplementation have been studied separately in numerous studies, but the combination of the two nutrients has only been studied on a limited basis. Kouchaki et al.[41] investigated both nutrients and found that their concurrent administration significantly improved EDSS scores in MS patients compared to those patients that received placebo treatment. The combination of vitamin D_3 with ω-3 fatty acids may have a synergistic effect on MS progression, and more research is required in order to determine if their co-administration is a more effective course of treatment than taking either ω-3 fatty acids or vitamin D_3 supplements alone.

Cytokine Profile

A vast majority of research on multiple sclerosis involves either the analysis of clinical markers for the disease (brain lesions, EDSS scores, relapse rates, etc.) or clinical biomarkers (TNF-α, IFN-γ, MMP-9, etc.). The validity of these biomarkers often comes into question, however, and should be considered when reviewing the results from multiple sclerosis research studies. Vladic et al.,[66] for instance, found that cerebrospinal fluid levels and serum levels of TNF-α and IL-6 were not significantly higher in multiple sclerosis patients ($n = 20$) when compared to control patients ($n = 15$) ($p > 0.05$), for example.

This means that changing the levels of these cytokines may not hold as much importance in treating the disease as originally thought.

As of now, there is no consensus on the best biomarkers to use for multiple sclerosis progression and development. MMP-9, IL-17, IL-1β, TNF-α, IFN-γ, and IL-6 are all commonly studied and recognized biomarkers, but their levels should not be interpreted as independent measures for multiple sclerosis or automatic indications of multiple sclerosis progression.

Mediterranean and Western Diet

Only a minimal amount of research has been conducted on the role of specific diets in the development, progression, and treatment of multiple sclerosis. The Western and Mediterranean diets are often investigated in terms of their dietary components: saturated fats, simple sugars, vegetables, etc. Generally speaking, increased total cholesterol levels, LDL cholesterol, triglyceride levels, and BMI are associated with higher relapse rates[60] and increased lesion formation.[61] In comparison, consumption of foods in the Mediterranean diet are associated with a decrease in the likelihood of multiple sclerosis development.[62]

Lipid profiles in multiple sclerosis have been used in a few studies[60,61] that produce contradictory results. Total cholesterol levels and LDL cholesterol levels have been associated with an increase in lesions,[61] but have not been associated with any change in relapse rates.[60] Risk of relapse and lesions are two different outcome measures in these studies, but both are indicative of the progression of the disease and are therefore comparable in terms of their significance. The contrast in this research sparks questions and prompts future research. These studies were conducted in individuals before an official diagnosis of MS, so later research should study lipid profiles in clinically diagnosed MS patients. Additionally, the researchers that discovered a significant relationship between cholesterol levels and increased lesions only followed the participants for two years,[61] while the researchers who found no

relationship between cholesterol levels and relapse rates followed the patients for five years,[60] more than twice as long. The difference in time scale could affect lesion formation and relapse rates independently of lipid profile, as multiple time points in the progression of the disease were included in the five-year study that weren't measured at all in the two-year study.

Although findings are limited, consumption of foods in the standard "Western" diet are believed to negatively impact the progression of multiple sclerosis, while consumption of foods in the Mediterranean diet are thought to be preventative for multiple sclerosis. More research is necessary to determine how the Mediterranean diet impacts multiple sclerosis patients who have already been diagnosed, and how the Mediterranean diet could work in combination with supplements or vitamins.

Concluding Comments

A considerable amount of research has been conducted on the impact of dietary supplements and general dietary patterns in the development and progression of multiple sclerosis. Preliminary results show promise, as ω-3 fatty acids, vitamin D_3, and the Mediterranean diet have been shown to improve MS symptoms and decrease the likelihood of MS development. More research needs to be conducted, however, as not all studies find the same results. As of now, research suggests that dietary interventions are an excellent addition to an MS treatment plan, but they are not a replacement for traditional pharmacological treatments.

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