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ARTICLE

The Effect of a Polysaccharide-Based Multinutrient Dietary Supplementation Regimen on Infections and Immune Functioning in Multiple Sclerosis

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ABSTRACT

Multiple sclerosis (MS) is a progressive neurodegenerative disease associated with increased infection rates, chronic inflammation, and premature death. Optimization of nutritional status via dietary supplementation may improve immune function in people suffering from MS and lead to decreased rates of infection. Fifteen individuals with a diagnosis of relapsing-remitting MS for an average of 12.4 years (SD =7.4; R = 2, 25) were enrolled in a one-year open-label clinical trial. Participants consumed a broad-spectrum dietary supplement regimen containing polysaccharides, phytochemicals, antioxidants, vitamins, and minerals three times per day. The occurrence of infections and a panel of cytokines, growth factors, and T- and B-cell subsets were assessed at baseline and 12 months. Seven female and 8 male participants with an average age of 51.3 years (SD =7.2; R = 38, 65) completed the study. At the end of the intervention, participants had fewer total infections (M = 7.9, SD =8.1 at baseline and M = 2.5, SD =4.3 at 12-month follow-up). At 12 months, IL-2, TNF-α, EGF, and CD95 + CD34+ significantly increased, while IL-1β significantly decreased. No major adverse effects were reported; only mild gastrointestinal intolerance was reported in four cases. A decreased occurrence of infection was observed in MS patients treated with 12 months of a polysaccharide-based multinutrient dietary supplement. Significant changes were also noted in several key biomarkers that would be physiologically favorable to the MS population. Thus, the results of this study suggest an immunomodulatory effect of the dietary supplement regimen studied.

KEYWORDS

CD95 + CD34+; dietary supplement; epidermal growth factor; immune function; infections; interleukin-1β; interleukin-2; multiple sclerosis; polysaccharide; tumor necrosis factor-α

Introduction

Multiple sclerosis (MS) is a neurodegenerative disease characterized by inflammation, demyelination, and plaque formation in the white and, less obviously, gray matter of the central nervous system (CNS) (Compston and Coles 2008). MS affects

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approximately 2.5 million people worldwide, with significant direct and indirect costs to both the patient and society in general (Asche et al. 2010; Campbell et al. 2014; Livingston et al. 2016).

The neurodegenerative processes of MS result from a complex interplay of immune dysfunction including T and B lymphocytes, microglia, and macrophages. The two main forms of MS are relapsing-remitting MS (RRMS) and primary-progressive MS (PPMS). Current FDA-approved MS therapies function as immunomodulators, largely targeting inflammation. All of these disease-modifying therapies (DMTs) have been documented to decrease the frequency of clinical exacerbations, and selected agents (e.g., interferon β-1a and fingolimod) have been shown to decrease debility (Vargas and Tyor 2017). MS therapies are noncurative, and with the exception of the interferon-based DMTs, nearly all are associated with a risk of potentially life-threatening complications.

Progressive multifocal leukoencephalopathy (PML) is one of the most-feared DMT-associated complications. This irreversible, potentially fatal, neurodegenerative disease results from immunosuppressive reactivation of the JC virus, an otherwise benign virus detected in 50%–60% of the population (Vargas and Tyor 2017). Hundreds of PML cases have been reported with natalizumab use (Vargas and Tyor 2017), and more recently, smaller numbers of cases have been reported with two of the first-line DMTs, fingolimod and dimethyl fumarate (van Oosten et al. 2013; Nieuwkamp et al. 2015; Rosenkranz et al. 2015; van Kester et al. 2015). Many other DMT-associated side effects have also been reported, including hepatotoxicity and elevated liver transaminases, gastrointestinal discomfort, flushing, decreased lymphocyte count, bradyarrhythmias (fingolimod), and teratogenicity (teriflunomide) (Vargas and Tyor 2017).

Therefore, dietary supplements have been considered as an adjunct to conventional MS pharmacotherapy and an alternate approach to symptom management. For example, 6 months of vitamin D supplementation showed an increase in serum-transforming growth factor (TGF)-β1 levels compared to placebo (Mahon et al. 2003). Six months of hemp seed and evening primrose oil consumption along with dietary modification were shown to improve clinical scores, relapse rates, and immune functioning in RRMS patients (Rezapour-Firouzi et al. 2013). However, the findings are inconsistent, as vitamin D and polyunsaturated fatty acid supplementation have also been shown to have no positive benefit on various clinical measures of MS (Kampman et al. 2012; Torkildsen et al. 2012). Thus, additional investigation is needed to identify therapies of potential benefit to MS patients.

The science of glycomics (i.e., the investigation of the structure and biosynthesis of organic saccharides; Varki et al. 2015) may provide new insights into a potentially beneficial class of therapeutic nutrients for MS patients. Dietary supplements containing concentrated forms of polysaccharides have led to various clinical improvements in multiple myeloma (Cholujova et al. 2013), hepatocellular carcinoma (Bang et al. 2010), hyperlipidemia (Veldman et al. 1999), atherogenesis (McCarty 1997), chronic fatigue syndrome (See et al. 1998), and attention deficit hyperactivity disorder (Dykman and Dykman 1998). Our group has demonstrated novel immunomodulatory changes with the use of polysaccharide dietary supplements in several studies. In response to a hydrolyzed rice bran supplement, healthy adults demonstrated elevated natural killer cell

cytotoxicity and improvements in their inflammatory profile according to various cytokines and growth factors (Ali et al. 2012). Several clinically and statistically significant improvements (e.g., alkaline phosphatase, platelets, neutrophils, neutrophil-lymphocyte ratio, and γ-glutamyl transferase) occurred in response to the hydrolyzed rice bran supplement compared to placebo in adults with nonalcoholic fatty liver disease (Lewis et al. 2018). A reduction in inflammation (decreases in tumor necrosis factor– [TNF-] α and vascular endothelial growth factor [VEGF]) and a 286% increase in CD14+ cells occurred in persons with moderate to severe Alzheimer's disease in response to 12 months of a polysaccharide multinutrient dietary supplement (Lewis et al. 2013).

Given the increasing incidence of MS, the severity of disease-related symptoms, and treatment-associated costs, the investigation of a polysaccharide-based, multinutrient dietary supplement in MS patients is warranted. We conducted an open-label study to investigate the effect of a 12-month course of a polysaccharide-based multinutrient formula on infection incidence and immune function in a sample of MS patients.

Methods

Study participants

Patients ($n = 15$) with RRMS were recruited from referrals to the Joanne P. LaGanke MS Center in the North Central Neurology Associates outpatient facility (Cullman, AL) from 2007 to 2008. The study was conducted with the approval of the DFW Micronutrient Council Institutional Review Board for human subject research, which operates within the standards set forth by the Helsinki Declaration of 1975. Each participant provided informed consent before participating in the study. Participants continued their current medications at trial entry and throughout the course of the study as ordered by the treating physician. In addition, participants had to have a diagnosis of MS for at least one year prior to entering the study. Each participant was clinically evaluated by the staff neurologist prior to enrollment in the study to verify the diagnosis of MS.

Inclusion and exclusion criteria

Inclusion criteria were (a) a diagnosis of MS for at least one year as established by the International Panel on MS Diagnosis McDonald Criteria (McDonald et al. 2001), a score of at least 2.0 on the Kurtzke Expanded Disability Status Scale (EDSS; (Kurtzke 1983)), and at least one relapse within the previous one year; (b) signed informed consent; (c) willingness and ability to consume oral dietary supplements; and (d) ability to drink sufficient water ($\geq$ 1 quart/100 pounds of body weight/day). Exclusion criteria were (a) concurrent enrollment in another study; (b) current hospitalization; (c) current pregnancy; or (d) inability or unwillingness to come to the MS Center once per month to receive the dietary supplements, turn in the compliance measure, and complete follow-up testing.

Intervention

Participants were enrolled in a 12-month, open-label study. The polysaccharide-based multinutrient formula used in this study consisted of dietary supplements that have

been sold by several commercial entities for over 20 years. The following Mannatech products were used in this study: Classic Ambrotose, Lecithin, Empact, and Phytaloe (1 teaspoon three times per day of each) and Sport, Plus, Ambrotose AO, and Catalyst (one capsule or tablet three times per day of each). Classic Ambrotose is a glyconutrient product designed to optimize cellular communication by providing necessary saccharides. Lecithin supports the synthesis of brain myelin and white matter and assists with absorption of glyconutrients. Empact contains nutrients designed to support stamina and combat fatigue. Phytaloe is composed of the nutrients of 13 fruits and vegetables. Sport contains nutrients intended to minimize fatigue and enhance stamina and muscular functioning. Plus contains plant sterols to support the endocrine system's natural production and balance of hormones. Ambrotose AO is an antioxidant product that supports the body's ability to protect cells from free radical damage. Catalyst is a vitamin and mineral product that supports the body's energy levels and provides a balanced food supplement of key nutrients for many necessary metabolic functions. The dietary supplements were provided to participants on a monthly basis at the MS Center.

Outcomes and assessments

Each participant completed a basic demographics and medical history questionnaire at baseline. Participants were then questioned every 3 months to assess (a) adherence to the intervention, (b) adverse reactions, (c) health status, and (d) current medications.

Prevalence of infections

At baseline and 12-month follow-up, participants were asked to report infections during the previous year. Participants were asked to report the number of times they had (a) any respiratory infection; (b) viral upper respiratory infection; (c) sinusitis; (d) gastritis; (e) yeast, including *Candida albicans* and candidiasis; (f) dental/oral infection; and (g) other infections not previously listed, such as those in the toenail or gum at each timepoint. The treating neurologist and staff confirmed the occurrence of the infections based on the clinical diagnosis and on the implementation of the medical treatment. An overall number of infections was summed using these categories.

Blood draw and laboratory procedure

Venous blood was obtained at baseline and at 12 months from all participants. Blood samples were collected in EDTA tubes and delivered to the laboratory within 2 hours of collection. Complete blood cell counts and auto five-part differential count determinations were performed on all specimens by a fully automated Coulter AcT5 hematology analyzer (Beckman Coulter, Fullerton, CA). Flow cytometric enumeration of T, B, and NK cell subsets was performed on a four-color flow cytometer, FACS Calibur (BD Biosciences, San Jose, CA), and the different cell populations were analyzed using Cell Quest Pro software (version 5.2, BD Biosciences, San Jose, CA).

Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Hypaque gradient centrifugation. PBMC were recovered from the gradient interface and washed in phosphate buffered saline. Blood was diluted with 1:1 RPMI 1640 (Gibco, Grand Island, NY), layered over Ficoll-Hypaque solution (Pharmacia, Piscataway, NJ), and centrifuged for 30 minutes at 1,500 rpm at ambient temperature. The PBMC were collected, washed with RPMI 1640, and counted and assessed for viability in trypan blue dye. Plasma for cytokine and growth factor detection was separated and stored at -80 °C until used.

Labeled lymphocytes were acquired on a FACS Calibur flow cytometer (BD Biosciences, San Jose, CA) after proper instrument setting, calibration, and compensation (Perfetto et al. 2006; Valiathan et al. 2014). Absolute counts for each cell subset were calculated by multiplying the specific subset percentages to absolute lymphocyte counts/100. The lymphocytes were gated as T cells [CD45 + CD3+] and B cells [CD19 + CD3−]. The T cells [CD45 + CD3+] were further gated as CD4 T helper cells [CD3 + CD4+] and CD8 T cytotoxic cells [CD3 + CD8+]. The CD3− cells other than B cells were further gated as natural killer cells [CD16 + CD56 + CD3−]. The other T cell subsets were analyzed as follows: CD14 (CD14+, CD14 + CD34+, and CD14 + CD90+), CD95 (CD95 + CD3+, CD95 + CD34+, CD95 + CD90+), CD90+, and CD34+.

Multiplex cytokine and growth factor testing

Cytokine and growth factor levels in plasma specimens were measured using a biochip array system, Evidence Investigator (Randox Laboratories Ltd., Crumlin, UK) as reported previously (Sachdeva et al. 2007). The testing platform consists of biochips secured in the base of a well placed in a carrier holding nine biochips in a 3 × 3 format. Each biochip is coated with the capture antibodies specific for each of the 12 cytokines and growth factors (interleukin- [IL-] 2, IL-4, IL-6, IL-8, IL-10, IL-1α, IL-1β, TNF-α, VEGF, interferon- [IFN-] γ, monocyte chemotactic protein– [MCP-] 1, and epidermal growth factor [EGF]) on a particular test region. A sandwich chemiluminescent assay was performed with 100 μL plasma using reagents (including the calibrators and controls) and protocols supplied by the same manufacturer. The light signal generated from each of the test regions on the biochip was detected using a charge-coupled detector camera and imaging system and compared with a calibration curve generated with known standards during the same run. All specimens were run in duplicate, and the concentration of each cytokine present in each plasma specimen was calculated from the standard curve and reported in pg/mL.

Statistical analysis

Data were analyzed using SPSS 24 (IBM Inc., Chicago, IL) for Windows. Frequency and descriptive statistics were calculated on all variables. Repeated-measures analysis of variance (ANOVA) examined the effect of time for infections (baseline and 12 months). For the biomarkers, linear mixed modeling (LMM) assessed the fixed effect of time on changes from baseline to follow-up. LMM with heterogeneous compound symmetry

covariance accounted for any missing data, intercorrelated responses between time-points, and nonconstant variability. The criterion for statistical significance for all tests was $\alpha = .05$.

Results

Sociodemographics

Seven females and eight males enrolled in the study with an average age of 51.3 years (SD =7.2; R = 38, 65). Participants were diagnosed with MS for an average of 12.4 years (SD =7.4; R = 2, 25). The mean baseline EDSS score was 4.1 (SD =1.9, R = 2.0, 9.0). Participants were taking the following MS medications: Tysabri (natalizumab) ($n = 7$), Betaseron (Interferon β-1b) ($n = 5$), intravenous immunoglobulin ($n = 2$), and no DMTs by choice ($n = 1$).

Tolerability

Two participants reported having an increase in soft stools upon starting the intervention. Four participants reported having nausea and vomiting in response to taking the dietary supplements, although all of them had previously reported the same symptoms. In addition, several participants noted difficulty in taking the products on top of the excessive number of medications (e.g., > 10 per day).

Infections

Table 1 displays the descriptive information for all infections at baseline and 12 months. Yeast infections decreased by an average of more than 1 ($F[1, 14] = 5.0$, $p = .04$). Other types of infections decreased by an average of more than 1 ($F[1, 14] = 5.2$, $p = .04$). Total infections decreased by an average of more than 5 ($F[1, 14] = 13.9$, $p = .002$). No other type of infections changed.

Cytokines, growth factors, T-cell subsets, and adult stem cell subsets

Table 2 shows the descriptive values for all 12 cytokines and growth factors. For IL-2, a significant fixed effect was found for time ($F[1, 7.9] = 12.4$, $p < .01$), as IL-2 increased

Table 1. The occurrence of infections at baseline and after 12 months of a polysaccharide-based multinutrient dietary supplement.

Variable	Baseline	12 months
Any respiratory	1.0 ± 1.9 (0, 6)	0.5 ± 1.1 (0, 4)
Viral upper respiratory	1.2 ± 1.7 (0, 6)	0.6 ± 1.1 (0, 4)
Sinusitis	1.2 ± 2.1 (0, 6)	0.3 ± 0.8 (0, 3)
Gastritis	0.4 ± 1.5 (0, 6)	0.1 ± 0.3 (0, 1)
Yeast*	1.5 ± 2.6 (0, 6)	0.1 ± 0.3 (0, 1)
Dental/oral	0.4 ± 1.1 (0, 4)	0.2 ± 0.8 (0, 3)
Other*	2.1 ± 2.7 (0, 6)	0.8 ± 2.1 (0, 6)
Total*	7.9 ± 8.1 (0, 32)	2.5 ± 4.3 (0, 16)

Values are expressed as mean ± standard deviation (minimum, maximum).
*Values are significantly different ($p < .05$) from baseline to 12 months.

Table 2. Cytokines and growth factors at baseline and after 12 months of a polysaccharide-based multinutrient dietary supplement.

Category	Variable	Baseline	12 Months
Proinflammatory cytokines	IL-1α (pg/mL)	0.5 ± 0.8 (0, 2.5)	1.0 ± 1.4 (0.4, 4.2)
	IL-1β (pg/mL)*	4.0 ± 4.4 (0.95, 15.4)	1.7 ± 2.5 (0, 7.0)
	IL-6 (pg/mL)	1.5 ± 1.5 (0, 5.5)	1.3 ± 1.6 (0, 4.8)
	TNF-α (pg/mL)*	2.9 ± 1.4 (0, 4.4)	9.3 ± 5.7 (0, 16.7)
Th-1 cytokines	IL-2 (pg/mL)*	3.5 ± 4.4 (0, 13.3)	13.7 ± 7.6 (0, 22.6)
	IFN-γ (pg/mL)	1.0 ± 1.1 (0, 3.0)	2.8 ± 3.6 (0, 10.2)
Th-2 cytokines	IL-4 (pg/mL)	4.3 ± 3.9 (0, 13.9)	6.9 ± 10.3 (0, 25.7)
	IL-10 (pg/mL)	0.2 ± 0.4 (0, 1.0)	0.4 ± 1.1 (0, 2.8)
Chemokines	MCP-1 (pg/mL)	101.7 ± 49.1 (44.4, 188.9)	100.1 ± 26.4 (75.7, 136.3)
	IL-8 (pg/mL)	12.4 ± 19.1 (0, 48.8)	3.7 ± 2.6 (0, 7.2)
Growth factors	VEGF (pg/mL)	84.0 ± 73.2 (9.5, 240.2)	83.6 ± 37.9 (35.8, 142.2)
	EGF (pg/mL)*	39.2 ± 40.8 (2.16, 113)	75.8 ± 44.2 (9.4, 138.4)

Values are expressed as mean ± standard deviation (minimum, maximum).
*Significantly different ($p < .05$) from baseline to 12 months.

Table 3. T-cell subsets at baseline and after 12 months of a polysaccharide-based multinutrient dietary supplement.

Variable	Baseline	12 Months
White blood cells (cells/μL)	5,670 ± 1,708.8 (3,800, 8,200)	6,000 ± 2,174.9 (3,600, 9,200)
Lymphocytes (%)	32.0 ± 12.3 (15.7, 51.9)	35.4 ± 7.0 (25.7, 48.6)
CD3 + (%)	72.2 ± 8.7 (59, 86.6)	70.8 ± 10.1 (57, 87)
CD3 + (cells/μL)	1,249.7 ± 507.3 (591, 2,112)	1,465.4 ± 541.1 (879, 2,346)
CD3 + CD4 + (%)	50.9 ± 9.2 (40.5, 71)	51.2 ± 8.2 (42, 68)
CD3 + CD4 + (cells/μL)	894.4 ± 429.8 (381, 1,732)	1063.3 ± 417.4 (645, 1,656)
CD3 + CD8 + (%)	20.1 ± 8.1 (10.1, 39.1)	18.6 ± 6.1 (9, 26)
CD3 + CD8 + (cells/μL)	343.3 ± 170.3 (136, 623)	379.7 ± 158.4 (139, 656)
B Cells CD19 + (%)	16.7 ± 7.9 (4.9, 32.5)	17.9 ± 9.3 (8, 34)
B Cells CD19 + (cells/μL)	287.3 ± 157.2 (76, 590)	390 ± 237.1 (113, 678)
NK Cells CD16 + 56 (%)	10.4 ± 4.2 (1.5, 15.8)	11.1 ± 4.1 (4, 15)
NK Cells CD16 + 56 (cells/μL)	181.2 ± 120.0 (37, 453)	251 ± 169.3 (50, 484)
CD3 + CD4+/CD3 + CD8+ ratio	2.9 ± 1.2 (1.1, 4.8)	3.1 ± 1.2 (1.8, 5.3)

Values are expressed as mean ± standard deviation (minimum, maximum).

from baseline to 12 months (mean difference =10.2; SE =2.9; 95% CI [3.5, 16.9]; $p < .01$). For IL-1β, a significant fixed effect was found for time (F[1, 9.6] = 6.8, $p = .03$), as IL-1β decreased from baseline to 12 months (mean difference = 2.5; SE =1.0; 95% CI [0.4, 4.7]; $p = .03$). For TNF-α, a significant fixed effect was found for time (F[1, 7.4] = 8.7, $p = .02$), as TNF-α increased from baseline to 12 months (mean difference =6.5; SE =2.2; 95% CI [1.3, 11.6]; $p = .02$). For EGF, a significant fixed effect was found for time (F[1, 6.6] = 7.5, $p = .03$), as EGF increased from baseline to 12 months (mean difference =31.5; SE =11.5; 95% CI [3.9, 59.0]; $p = .03$).

Table 3 shows the descriptive values of the T-cell subsets, including CD3+, CD3 + CD4+, CD3 + CD8+, CD19+, and CD16 + 56+, none of which significantly changed. Table 4 shows the descriptive values of the CD14+, CD34+, CD90+, and CD95+ subsets. For CD95 + CD34 + (absolute number), a significant fixed effect was found for time (F[1, 7.1] = 8.9, $p = .02$), as CD95 + CD34+ increased from baseline to 12 months (mean difference =6.8; SE =2.3; 95% CI [1.4, 12.2]; $p = .02$). For CD14 + CD34 + (%), a significant fixed effect was found for time (F[1, 17.2] = 5.6, $p = .03$), as CD14 + CD34 + (%) increased from baseline to 12 months (mean difference =2.9; SE =1.2; 95% CI [0.3, 5.4]; $p = .03$). For CD14 + CD90 + (%), a significant fixed

Table 4. CD14, CD34, CD90, and CD95 subsets at baseline and after 12 months of a polysaccharide-based multinutrient dietary supplement.

Variable	Baseline	12 Months
CD34 + (%)	0.67 ± 0.45 (0.2, 1.4)	0.54 ± 0.45 (0.2, 1.5)
CD34 + (cells/μL)	10.5 ± 8.2 (0, 28)	11.3 ± 9.8 (3, 32)
CD90 + (%)	3.8 ± 3.3 (0.9, 10.8)	4.5 ± 7.5 (1, 21.3)
CD90 + (cells/μL)	62.3 ± 52.0 (14, 159)	78 ± 111.5 (18, 327)
CD95 + CD3 + (%)	38.3 ± 6.3 (29.8, 50)	40.3 ± 6.2 (35.6, 53.4)
CD95 + CD3 + (cells/μL)	641.8 ± 205.6 (368, 1,018)	838.7 ± 318.5 (505, 1,266)
CD95 + CD34 + (%)	0.32 ± 0.34 (0, 0.9)	0.54 ± 0.22 (0.3, 1)
CD95 + CD34 + (cells/μL)*	4.6 ± 6.0 (0, 18)	11.3 ± 6.1 (6, 21)
CD95 + CD90 + (%)	1.3 ± 1.0 (0.5, 3.2)	2.7 ± 4.1 (0.4, 11.9)
CD95 + CD90 + (cells/μL)	20.6 ± 18.2 (0, 57)	47.3 ± 62.7 (7, 183)
CD14 + (%)	8.2 ± 4.7 (4.9, 20.7)	6.3 ± 1.6 (4.6, 9.4)
CD14 + CD34 + (%)*	0.74 ± 1.08 (0, 3.1)	2.7 ± 5.13 (0, 14.3)
CD14 + CD90 + (%)*	1.9 ± 1.8 (0, 5.9)	1.2 ± 0.9 (0, 2.4)
CD14 + CD95 + (%)	0.35 ± 1.0 (0, 3.2)	0.44 ± 0.67 (0, 1.6)

Values are expressed as mean ± standard deviation (minimum, maximum).
*Significantly different ($p < .05$) from baseline to 12 months.

effect was found for time ($F[1, 6.3] = 6.6$, $p = .04$), as CD14 + CD90 + (%) decreased from baseline to 12 months (mean difference =0.6; SE =0.2; 95% CI [0.1, 1.1]; $p = .04$).

Discussion

Current pharmacologic therapies for MS clearly decrease the clinical relapse rate, and selected agents also decrease debility. Risks of therapy with a majority of FDA-approved MS drugs are numerous and can be potentially life threatening. Because of the wide range of adverse effects associated with MS and DMTs, and the lack of curative outcomes, MS patients have expanded their use of other therapies, including dietary supplements (Leong et al. 2009).

In the current study, we showed significant decreases in yeast, other, and total infections, with a combined annual decrease of more than 5 infections. Of paramount importance to the care of MS patients is decreasing the rate of infections, as they are the leading cause of mortality in this patient population and a common cause of morbidity (Goodin et al. 2012). The treatment costs for common infections in MS patients place a substantial burden on the U.S. health care system (Nelson et al. 2015). Patients with MS are significantly more likely than those without MS to develop serious infections that lead to both hospitalization and infection-related mortality (Allizond et al. 2015; Nelson et al. 2015). A Swedish registry compared incidences of infection in different cohorts in post–hospital admission and found that MS patients were significantly more likely to develop an infection than the general population and those who had another immune-mediated disease (Montgomery et al. 2013). The increased susceptibility to infection appears to be related to both the effect of immunomodulatory or immunosuppressive medications (Winkelmann et al. 2014) and a secondary consequence of the debility of the disease (Nelson et al. 2015). Another study found that MS patients were nearly twice as likely as hospital patients without MS to have serious infections (19.2 vs. 10.3 per 1,000, $p < .01$) and more than twice as likely to suffer fatal infections (1.2 vs. 0.5 per 1,000, $p = .03$), even after controlling for the use of

immunosuppressive medications (Nelson et al. 2015). Thus, our findings have potential implications for morbidity, mortality, and cost associated with infections in MS patients.

Perhaps related to the decrease in infections at follow-up, a sharp increase was noted in IL-2. However, this would not be an expected result due to the stable DMTs used by these patients. IL-2 is a pleiotropic cytokine that has many known functions, including a significant role in balancing the response to pathogens versus a dysregulated response that leads to autoimmunity (Malek and Castro 2010; Liao et al. 2011; Waters et al. 2018). Along with its activities in conjunction with IL-2R, it is highly involved in T helper– (Th-) 1 and Th-2 differentiation, which is crucial for counteracting and preventing infection (Liao et al. 2011). Thus, it is possible in this study that our dietary supplement regimen enabled an increase in IL-2 that corresponded with a simultaneous decrease in infections. A randomized, double-blind trial of 6 months of vitamin D supplementation showed no effect on several cytokines, including IL-2 mRNA, but did significantly increase TGF-β1 (Mahon et al. 2003).

Also potentially related to the reduction in the number of infections was an increase in TNF-α at 12 months. TNF-α is a proinflammatory cytokine that has been shown to be key in how the immune system responds to bacteria, viruses, and fungus, particularly yeast (Petursdottir et al. 2002; Cui et al. 2016), and was shown to be positively related to *Candida* spp in pneumonia patients (Albert et al. 2014). Although others have shown that TNF-α proliferates activated T- and B-cells (Sugarman et al. 1985), the T-cell subsets assessed in this study did not change. In addition, TNF is recognized as part of the underlying pathology of MS, given that (a) autopsy specimens of MS patients reveal high TNF levels in active lesions, (b) TNF is higher in MS compared to healthy controls, and (c) TNF level is significantly related to the severity of lesions (Beck et al. 1988; Hofman et al. 1989; Maimone et al. 1991; Sharief and Hentges 1991).

Pharmacological efforts in systemic inflammatory conditions have moved toward an emphasis on anti-TNF therapy, but this has resulted in paradoxical problems, including adverse neurologic events and demyelination of the CNS in some instances (Andreadou et al. 2013). In addition, TNF-α stimulates the differentiation of oligodendrocyte precursor cells into myelinating oligodendrocytes, thus enhancing remyelination of demyelinated lesions in the CNS (Arnett et al. 2001; Finsen et al. 2002). Similar to our study, another investigation showed that the macrophages of mice fed a diet containing fish oil showed an elevation in TNF-α (Petursdottir et al. 2002), even though fish oil, due to its high content of omega-3 fatty acids, is commonly recognized as anti-inflammatory (Calder 2011). Our findings on TNF-α have to be considered in light of the totality of our results, especially given that the TNF family of receptors and their complex actions in MS have not been fully elucidated.

IL-1β levels significantly decreased in this study. IL-1β is theorized to be proinflammatory in the CNS in MS, and its expression in the PBMCs of MS patients has been shown to be 300% more than in healthy controls (Heidary et al. 2014). Similarly, one group of MS patients who received antibodies to IFN-γ showed a significant decrease in IL-1β after 12 months of treatment (Skurkovich et al. 2001). Conversely, IL-1β increased in patients receiving natalizumab compared to those taking glatiramer acetate after one year (Oreja-Guevara et al. 2012). To our knowledge, the only other dietary supplement

studies showing any effect on IL-1β involve fish oil, again focusing primarily on their omega-3 fatty acid content. Significant reductions were seen in both TNF-α and IL-1β in their synthesis and overall levels in separate studies (Caughey et al. 1996; Bertolotto et al. 1999; Ramirez-Ramirez et al. 2013). However, even though reductions in these markers were demonstrated, clinical responses stayed the same (Ramirez-Ramirez et al. 2013). Given that "cytokine dysregulation" has previously been noted among TNF-α and IL-1β during RRMS and that physiological counter-regulatory mechanisms during the relapse phase are defective (Hollifield et al. 2003), our finding that TNF-α and IL-1β inversely changed may be more understandable in this context.

EGF significantly increased in response to our dietary supplement regimen, which could be important given its role in the system of growth factors and receptors that function together to stimulate cellular growth. EGF levels have been found to discriminate between RRMS and PPMS, with plasma levels being lower in PPMS (Tejera-Alhambra et al. 2015). EGF has been found to improve the proliferation and differentiation of neurons, astrocytes, and oligodendrocytes (Compston et al. 1997; Gonzalez-Perez et al. 2009) and provided along with growth hormone in mild and severe experimental autoimmune encephalomyelitis showed a higher clinical score and survival rate (del Barco et al. 2011). In a sample of MS patients, EGF in the cerebrospinal fluid was significantly lower than in other noninflammatory neurological diseases (Scalabrino et al. 2010). To our knowledge, the present study is the first to show an increase in EGF in response to dietary supplements. Low levels of EGF and other myelin-related molecules, such as cobalamin, in the spinal cord of MS patients have been documented and may retard the process of remyelination of lesions (Scalabrino et al. 2015). Therefore, our finding of a higher level of EGF in response to dietary supplementation could be an important new discovery showing that enhanced nutrition may improve remyelination.

The number of CD95 + CD34+ cells increased at 12 months in our study. CD95 (Fas antigen) is a cell surface receptor that is a member of the TNF super family and is capable of inducing apoptosis (Nagata and Golstein 1995). Fas expression is usually low on CD34+ bone marrow progenitor cells (Nagafuji et al. 1995) but can be elevated *in vitro* with several cytokines (Anthony et al. 1998). However, mobilization, including the use of growth factors, in the peripheral blood progenitor cells from patients with leukemia and lymphoma had a very minimal effect on Fas expression in CD34+ progenitor cells (Anthony et al. 1998). In bone marrow transplantation, purified CD34+ cells are susceptible to a Fas-mediated colony hematopoietic cell suppression (Saheki et al. 2000), but we cannot hypothesize the significance of this finding related to any previous work in MS patients. Nonetheless, in general, Fas plays a key role in regulating immune responses by elimination of self-reactive T cells and by T-cell-mediated cytotoxicity (Saheki et al. 2000), and it is expressed on various normal tissues (Leithauser et al. 1993) and T lymphocytes, neutrophils, monocytes, and eosinophils (Miyawaki et al. 1992; Owen-Schaub et al. 1992). Thus, proliferation of Fas would appear to be of benefit for MS patients who are faced with severe autoimmune dysfunction. Our study shows an increase in Fas after dietary supplementation, compared to the use of IFN-γ and/or TNF-α to induce the expression of Fas on the surface of CD34+ cells after culture (Nagafuji et al. 1995).

Limitations

This study has several noteworthy limitations. The dietary supplement regimen has a wide variety of polysaccharides, phytonutrients, vitamins, and minerals, which makes it impossible to delineate those nutrients that may be most responsible for the effects shown in the study. In addition, we did not assess dietary intake, physical activity level, or caregiver support, so we are unsure how these variables could have affected the results of the study. Study coordinators were not blinded to the study participants, but they assessed participants for all studies occurring simultaneously, so their influence on this study should have been no different than on any other. We did not restrict or change the use of medications by our participants at baseline, given the ethical considerations associated with such decision. Finally, this study had a small sample size; a larger number of participants might have produced different results.

Conclusions

Current pharmacologic treatments for MS have significantly changed the trajectory of disease burden, but are balanced against a significant risk of intolerability and/or safety, creating a need for additional treatment options (Damal et al. 2013). The polysaccharide-based multinutrient dietary supplement used in this study resulted in a decreased total number of infections at 12 months. Several biomarkers significantly changed as well (i.e., increases in IL-2, TNF-α, EGF, and CD95 + CD34+ and a decrease in IL-1β). The current results compel further study to validate the findings and explore relationships between mechanism (e.g., the expression of Fas mRNA) and effect. If significant, dietary supplements have the potential to significantly improve the MS population and reduce some of the global financial burden due to MS.

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Declaration of interest

Reginald McDaniel has received income as a seller of the dietary supplements used in this study. Christopher LaGanke, Laura Bloom, Sharon Goldberg, Lucas C. Lages, Laura A. Lantigua, Steven E. Atlas, Judi M. Woolger, and John E. Lewis have no conflicts of interest to report.

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Data availability statement

The data that support the findings of this study are available from the corresponding author (JEL) upon reasonable request.

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References

Albert M, Williamson D, Muscedere J, Lauzier F, Rotstein C, Kanji S, Jiang X, Hall M, Heyland D. 2014. Candida in the respiratory tract secretions of critically ill patients and the impact of antifungal treatment: A randomized placebo controlled pilot trial (Cantreat study). Intensive Care Med. 40(9):1313–1322.

Ali KH, Melillo A, Leonard S, Asthana D, Woolger J, Wolfson A, McDaniel HR, Lewis JE. 2012. An open-label, randomized clinical trial to assess the immunomodulatory activity of a novel oligosaccharide compound in healthy adults. Funct Foods Health Dis. 2(7):265–279.

Allizond V, Scutera S, Rossi S, Musso T, Crocilla C, Cavalla P, Trebini C, Marra ES, Cuffini AM, Banche G. 2015. Polymorphonuclear cell functional impairment in relapsing remitting multiple sclerosis patients: preliminary data. PLoS One. 10(6):e0131557.

Andreadou E, Kemanetzoglou E, Brokalaki C, Evangelopoulos ME, Kilidireas C, Rombos A, Stamboulis E. 2013. Demyelinating disease following anti-TNFA treatment: a causal or coincidental association? Report of four cases and review of the literature. Case Rep Neurol Med. 2013:671935.

Anthony RS, McKelvie ND, Craig JI, Parker AC. 1998. Fas antigen (cd95) expression in peripheral blood progenitor cells from patients with leukaemia and lymphoma. Leuk Lymphoma. 30(5-6):449–458.

Arnett HA, Mason J, Marino M, Suzuki K, Matsushima GK, Ting JP. 2001. TNF alpha promotes proliferation of oligodendrocyte progenitors and remyelination. Nat Neurosci. 4(11):1116–1122.

Asche CV, Singer ME, Jhaveri M, Chung H, Miller A. 2010. All-cause health care utilization and costs associated with newly diagnosed multiple sclerosis in the United States. J Manag Care Pharm. 16(9):703–712.

Bang MH, Van Riep T, Thinh NT, Song L. H, Dung TT, Van Truong L, Van Don L, Ky TD, Pan D, Shaheen M. 2010. Arabinoxylan rice bran (MGN-3) enhances the effects of interventional therapies for the treatment of hepatocellular carcinoma: a three-year randomized clinical trial. Anticancer Res. 30(12):5145–5151.

Beck J, Rondot P, Catinot L, Falcoff E, Kirchner H, Wietzerbin J. 1988. Increased production of interferon gamma and tumor necrosis factor precedes clinical manifestation in multiple sclerosis: Do cytokines trigger off exacerbations? Acta Neurol Scand. 78(4):318–323.

Bertolotto A, Malucchi S, Capobianco M, Manzardo E, Guastamacchia G, Milano E, Audano L, Mutani R. 1999. Quantitative PCR reveals increased levels of tumor necrosis factor-alpha mRNA in peripheral blood mononuclear cells of multiple sclerosis patients during relapses. J Interferon Cytokine Res. 19(6):575–581.

Calder PC. 2011. Fatty acids and inflammation: the cutting edge between food and pharma. Eur J Pharmacol. 668(Suppl 1):S50–S58.

Campbell JD, Ghushchyan V, Brett McQueen R, Cahoon-Metzger S, Livingston T, Vollmer T, Corboy J, Miravalle A, Schreiner T, Porter V, et al. 2014. Burden of multiple sclerosis on direct, indirect costs and quality of life: National US estimates. Mult Scler Relat Disord. 3(2):227–236.

Caughey GE, Mantzioris E, Gibson RA, Cleland LG, James MJ. 1996. The effect on human tumor necrosis factor alpha and interleukin 1 beta production of diets enriched in n-3 fatty acids from vegetable oil or fish oil. Am J Clin Nutr. 63(1):116–122.

Cholujova D, Jakubikova J, Czako B, Martisova M, Hunakova L, Duraj J, Mistrik M, Sedlak J. 2013. MGN-3 arabinoxylan rice bran modulates innate immunity in multiple myeloma patients. Cancer Immunol Immunother. 62(3):437–445.

Compston A, Coles A. 2008. Multiple sclerosis. Lancet. 372(9648):1502–1517.

Compston A, Zajicek J, Sussman J, Webb A, Hall G, Muir D, Shaw C, Wood A, Scolding N. 1997. Glial lineages and myelination in the central nervous system. J Anatomy. 190(2):161–200.

Cui S, Hassan RY, Heintz-Buschart A, Bilitewski U. 2016. Regulation of *Candida albicans* interaction with macrophages through the activation of hog pathway by genistein. Molecules. 21(2):162

Damal K, Stoker E, Foley JF. 2013. Optimizing therapeutics in the management of patients with multiple sclerosis: a review of drug efficacy, dosing, and mechanisms of action. Biologics. 7:247–258.

del Barco DG, Montero E, Coro-Antich RM, Brown E, Suarez-Alba J, Lopez L, Subiros N, Berlanga J. 2011. Coadministration of epidermal growth factor and growth hormone releasing peptide-6 improves clinical recovery in experimental autoimmune encephalitis. Restor Neurol Neurosci. 29(4):243–252.

Dykman KD, Dykman RA. 1998. Effect of nutritional supplements on attentional-deficit hyperactivity disorder. Integr Physiol Behav Sci. 33(1):49–60.

Finsen B, Antel J, Owens T. 2002. TNF alpha: Kill or cure for demyelinating disease? Mol Psychiatry. 7(8):820–821.

Gonzalez-Perez O, Romero-Rodriguez R, Soriano-Navarro M, Garcia-Verdugo JM, Alvarez-Buylla A. 2009. Epidermal growth factor induces the progeny of subventricular zone type b cells to migrate and differentiate into oligodendrocytes. Stem Cells. 27(8):2032–2043.

Goodin DS, Ebers GC, Cutter G, Cook SD, O'Donnell T, Reder AT, Kremenchutzky M, Oger J, Rametta M, Beckmann K, et al. 2012. Cause of death in MS: Long-term follow-up of a randomised cohort, 21 years after the start of the pivotal IFNbeta-1b study. BMJ Open. 2(6):e001972.

Heidary M, Rakhshi N, Pahlevan Kakhki M, Behmanesh M, Sanati MH, Sanadgol N, Kamaladini H, Nikravesh A. 2014. The analysis of correlation between IL-1B gene expression and genotyping in multiple sclerosis patients. J Neurol Sci. 343(1-2):41–45.

Hofman FM, Hinton DR, Johnson K, Merrill JE. 1989. Tumor necrosis factor identified in multiple sclerosis brain. J Exp Med. 170(2):607–612.

Hollifield RD, Harbige LS, Pham-Dinh D, Sharief MK. 2003. Evidence for cytokine dysregulation in multiple sclerosis: peripheral blood mononuclear cell production of pro-inflammatory and anti-inflammatory cytokines during relapse and remission. Autoimmunity. 36(3):133–141.

Kampman MT, Steffensen LH, Mellgren SI, Jorgensen L. 2012. Effect of vitamin D3 supplementation on relapses, disease progression, and measures of function in persons with multiple sclerosis: exploratory outcomes from a double-blind randomised controlled trial. Mult Scler. 18(8):1144–1151.

Kurtzke JF. 1983. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology. 33(11):1444–1452.

Leithauser F, Dhein J, Mechtersheimer G, Koretz K, Bruderlein S, Henne C, Schmidt A, Debatin KM, Krammer PH, Moller P. 1993. Constitutive and induced expression of Apo-1, a new member of the nerve growth factor/tumor necrosis factor receptor superfamily, in normal and neoplastic cells. Lab Invest. 69(4):415–429.

Leong EM, Semple SJ, Angley M, Siebert W, Petkov J, McKinnon RA. 2009. Complementary and alternative medicines and dietary interventions in multiple sclerosis: what is being used in South Australia and why? Complement Ther Med. 17(4):216–223.

Lewis JE, Atlas SE, Higuera OL, Fiallo A, Rasul A, Farooqi A, Kromo O, Lantigua LA, Tiozzo E, Woolger JM, et al. 2018. The effect of a hydrolyzed polysaccharide dietary supplement on biomarkers in adults with nonalcoholic fatty liver disease. Evid Based Complement Alternat Med. 2018:1751583

Lewis JE, McDaniel HR, Agronin ME, Loewenstein DA, Riveros J, Mestre R, Martinez M, Colina N, Abreu D, Konefal J, et al. 2013. The effect of an aloe polymannose multinutrient complex on cognitive and immune functioning in Alzheimer's disease. J Alzheimers Dis. 33(2):393–406.

Liao W, Lin JX, Leonard WJ. 2011. IL-2 family cytokines: new insights into the complex roles of il-2 as a broad regulator of t helper cell differentiation. Curr Opin Immunol. 23(5):598–604.

Livingston T, Fay M, Iyer R, Wells W, Pill MW. 2016. Quantifying differences in health care consumption for the management of multiple sclerosis within privately and publicly insured health care programs. J Manag Care Spec Pharm. 22(12):1385–1391.

Mahon BD, Gordon SA, Cruz J, Cosman F, Cantorna MT. 2003. Cytokine profile in patients with multiple sclerosis following vitamin d supplementation. J Neuroimmunol. 134(1-2):128–132.

Maimone D, Gregory S, Arnason BG, Reder AT. 1991. Cytokine levels in the cerebrospinal fluid and serum of patients with multiple sclerosis. J Neuroimmunol. 32(1):67–74.

Malek TR, Castro I. 2010. Interleukin-2 receptor signaling at the interface between tolerance and immunity. Immunity. 33(2):153–165.

McCarty MF. 1997. Glucosamine may retard atherogenesis by promoting endothelial production of heparan sulfate proteoglycans. Med Hypotheses. 48(3):245–251.

McDonald WI, Compston A, Edan G, Goodkin D, Hartung HP, Lublin FD, McFarland HF, Paty DW, Polman CH, Reingold SC, et al. 2001. Recommended diagnostic criteria for multiple sclerosis: guidelines from the international panel on the diagnosis of multiple sclerosis. Ann Neurol. 50(1):121–127.

Miyawaki T, Uehara T, Nibu R, Tsuji T, Yachie A, Yonehara S, Taniguchi N. 1992. Differential expression of apoptosis-related Fas antigen on lymphocyte subpopulations in human peripheral blood. J Immunol. 149(11):3753–3758.

Montgomery S, Hillert J, Bahmanyar S. 2013. Hospital admission due to infections in multiple sclerosis patients. Eur J Neurol. 20(8):1153–1160.

Nagafuji K, Shibuya T, Harada M, Mizuno S, Takenaka K, Miyamoto T, Okamura T, Gondo H, Niho Y. 1995. Functional expression of fas antigen (cd95) on hematopoietic progenitor cells. Blood. 86(3):883–889.

Nagata S, Golstein P. 1995. The fas death factor. Science. 267(5203):1449–1456.

Nelson RE, Xie Y, DuVall SL, Butler J, Kamauu AW, Knippenberg K, Schuerch M, Foskett N, LaFleur J. 2015. Multiple sclerosis and risk of infection-related hospitalization and death in us veterans. Int J MS Care. 17(5):221–230.

Nieuwkamp DJ, Murk JL, van Oosten BW, Cremers CH, Killestein J, Viveen MC, Van Hecke W, Frijlink DW, Wattjes MP, Consortium PMLiDMP. 2015. PML in a patient without severe lymphocytopenia receiving dimethyl fumarate. N Engl J Med. 372(15):1474–1476.

Oreja-Guevara C, Ramos-Cejudo J, Aroeira LS, Chamorro B, Diez-Tejedor E. 2012. TH1/TH2 cytokine profile in relapsing-remitting multiple sclerosis patients treated with glatiramer acetate or natalizumab. BMC Neurol. 12(1):95.

Owen-Schaub LB, Yonehara S, Crump WL, Grimm EA. 1992. DNA fragmentation and cell death is selectively triggered in activated human lymphocytes by FAS antigen engagement. Cell Immunol. 140(1):197–205.

Perfetto SP, Ambrozak D, Nguyen R, Chattopadhyay P, Roederer M. 2006. Quality assurance for polychromatic flow cytometry. Nat Protoc. 1(3):1522–1530.

Petursdottir DH, Olafsdottir I, Hardardottir I. 2002. Dietary fish oil increases tumor necrosis factor secretion but decreases interleukin-10 secretion by murine peritoneal macrophages. J Nutr. 132(12):3740–3743.

Ramirez-Ramirez V, Macias-Islas MA, Ortiz GG, Pacheco-Moises F, Torres-Sanchez ED, Sorto-Gomez TE, Cruz-Ramos JA, Orozco-Avina G, Celis de la Rosa AJ. 2013. Efficacy of fish oil on serum of TNF α , IL-1 β , and IL-6 oxidative stress markers in multiple sclerosis treated with interferon beta-1b. Oxid Med Cell Longev. 2013:709493

Rezapour-Firouzi S, Arefhosseini SR, Mehdi F, Mehrangiz E-M, Baradaran B, Sadeghihokmabad E, Mostafaei S, Fazljou SMB, Torbati M-A, Sanaie S, Zamani F. 2013. Immunomodulatory and therapeutic effects of hot-nature diet and co-supplemented hemp seed, evening primrose oils intervention in multiple sclerosis patients. Complement Ther Med. 21(5):473–480.

Rosenkranz T, Novas M, Terborg C. 2015. PML in a patient with lymphocytopenia treated with dimethyl fumarate. N Engl J Med. 372(15):1476–1478.

Sachdeva N, Yoon HS, Oshima K, Garcia D, Goodkin K, Asthana D. 2007. Biochip array-based analysis of plasma cytokines in HIV patients with immunological and virological discordance. Scand J Immunol. 65(6):549–554.

Saheki K, Fujimori Y, Takemoto Y, Kakishita E. 2000. Increased expression of FAS (Apo-1, CD95) on CD34+ haematopoietic progenitor cells after allogeneic bone marrow transplantation. Br J Haematol. 109(2):447–452.

Scalabrino G, Galimberti D, Mutti E, Scalabrini D, Veber D, De Riz M, Bamonti F, Capello E, Mancardi GL, Scarpini E. 2010. Loss of epidermal growth factor regulation by cobalamin in multiple sclerosis. Brain Res. 1333:64–71.

Scalabrino G, Veber D, De Giuseppe R, Roncaroli F. 2015. Low levels of cobalamin, epidermal growth factor, and normal prions in multiple sclerosis spinal cord. Neuroscience. 298:293–301.

See DM, Cimoch P, Chou S, Chang J, Tilles J. 1998. The in vitro immunomodulatory effects of glyconutrients on peripheral blood mononuclear cells of patients with chronic fatigue syndrome. Integr Physiol Behav Sci. 33(3):280–287.

Sharief MK, Hentges R. 1991. Association between tumor necrosis factor-alpha and disease progression in patients with multiple sclerosis. N Engl J Med. 325(7):467–472.

Skurkovich S, Boiko A, Beliaeva I, Buglak A, Alekseeva T, Smirnova N, Kulakova O, Tchechonin V, Gurova O, Deomina T, et al. 2001. Randomized study of antibodies to IFN-G and TNF-A in secondary progressive multiple sclerosis. Mult Scler. 7(5):277–284.,.

Sugarman BJ, Aggarwal BB, Hass PE, Figari IS, Palladino MA, Jr, Shepard HM. 1985. Recombinant human tumor necrosis factor-alpha: Effects on proliferation of normal and transformed cells in vitro. Science. 230(4728):943–945.

Tejera-Alhambra M, Casrouge A, de Andres C, Seyfferth A, Ramos-Medina R, Alonso B, Vega J, Fernandez-Paredes L, Albert ML, Sanchez RS. 2015. Plasma biomarkers discriminate clinical forms of multiple sclerosis. PLoS One. 10(6):e0128952

Torkildsen O, Wergeland S, Bakke S, Beiske AG, Bjerve KS, Hovdal H, Midgard R, Lilleas F, Pedersen T, Bjornara B, et al. 2012. ω-3 fatty acid treatment in multiple sclerosis (OFAMS Study): a randomized, double-blind, placebo-controlled trial. Arch Neurol. 69(8):1044–1051. Omega

Valiathan R, Deeb K, Diamante M, Ashman M, Sachdeva N, Asthana D. 2014. Reference ranges of lymphocyte subsets in healthy adults and adolescents with special mention of t cell maturation subsets in adults of South Florida. Immunobiology. 219(7):487–496.

van Kester MS, Bouwes Bavinck JN, Quint KD. 2015. PML in patients treated with dimethyl fumarate. N Engl J Med. 373(6):583–584.

van Oosten BW, Killestein J, Barkhof F, Polman CH, Wattjes MP. 2013. PML in a patient treated with dimethyl fumarate from a compounding pharmacy. N Engl J Med. 368(17):1658–1659.

Vargas DL, Tyor WR. 2017. Update on disease-modifying therapies for multiple sclerosis. J Investig Med. 65(5):883–891.

Varki A, Cummings RD, Esko JD, Stanley P, Hart G, Aebi M, Darvill A, Kinoshita T, Packer NH, PJJ, et al. 2015. Essentials of glycobiology. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Veldman FJ, Nair CH, Vorster HH, Vermaak WJ, Jerling JC, Oosthuizen W, Venter CS. 1999. Possible mechanisms through which dietary pectin influences fibrin network architecture in hypercholesterolaemic subjects. Thromb Res. 93(6):253–264.

Waters RS, Perry JS, Han S, Bielekova B, Gedeon T. 2018. The effects of interleukin-2 on immune response regulation. Math Med Biol. 35(1):79–119.

Winkelmann A, Loebermann M, Reisinger EC, Zettl UK. 2014. Multiple sclerosis treatment and infectious issues: Update 2013. Clin Exp Immunol. 175(3):425–438.