Sónia Augusta Oliveira Ribeiro

Influence of polyunsaturated fatty acids on multiple sclerosis and use for treatment

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(Sónia Augusta Oliveira Ribeiro)

Trabalho apresentado à Universidade Fernando Pessoa como parte dos requisitos para obtenção do grau de licenciada em Ciências da Nutrição.

Orientadora: Profª Doutora Rita Guerra

Coorientadora: Profª Doutora Cláudia Silva

I. Dedicatória

Aos meus pais, que me proporcionaram a oportunidade de seguir o meu sonho de me tornar Nutricionista, apoiando-me sempre.

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II. Index

III. Figures and tables index

IV. Abbreviations list

AA – Arachidonic Acid

ALA – Alfa-Linolenic Acid

ARR – Annual Relapse Rate

CNS – Central Nervous System

DHA – Docosahexaenoic Acid

EAE – Experimental Autoimmune Encephalomyelitis

EDSS – Expanded Disability Status Scale

EPA – Eicosapentaenoic Acid

EPO – Evening-Primrose Oil

GdE - Gadolinium Enhancing

GLA – γ-Linolenic Acid

HR – Hazard Ratio

HSO – Hempseed Oil

LA – Linoleic Acid

MMP-9 – Matrix Metalloproteinase-9

MRI – Magnetic Resonance Imaging

MS – Multiple Sclerosis

MUFAs – Monounsaturated Fatty Acids

PL – Phospholipid

PUFAs – Polyunsaturated Fatty Acids

RAPA – Rapamycin

RRMS – Relapsing-Remitting Multiple Sclerosis

SD – Standard Deviation

SFAs – Saturated Fatty Acids

TAG – Triacylglycerol

V. Title/Authors/Academic affiliation

Influence of polyunsaturated fatty acids on multiple sclerosis and use for treatment

A influência dos ácidos gordos polinsaturados na esclerose múltipla e o uso para tratamento

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VI. Resumo

Muitos dos pacientes com esclerose múltipla usam terapias complementares alternativas como parte do tratamento e dos seus sintomas, entre estas a suplementação com ácidos gordos polinsaturados que é a mais comummente utilizada. O interesse no uso de ácidos gordos polinsaturados em pacientes com esclerose múltipla tem vindo a aumentar. O objetivo desta revisão foi perceber de que forma estes ácidos gordos podem influenciar a esclerose múltipla e qual a sua utilidade para o tratamento da mesma. Para a elaboração desta revisão foram realizadas duas pesquisas na PubMed usando as expressões de pesquisa "("polyunsaturated fatty acids") AND multiple sclerosis" e "[(("fatty acids" OR polyunsaturated OR unsaturated) AND multiple sclerosis AND (trial OR cohort OR prospective study)) NOT review]". Foram incluídos ensaios clínicos, estudos realizados em animais e estudos de coorte, resultando num total de trinta artigos. Os resultados mais promissores sugerindo um efeito benéfico dos ácidos gordos polinsaturados na esclerose múltipla são os provenientes dos estudos realizados em animais, mas estes não podem ser diretamente extrapolados para os humanos. A maioria dos ensaios clínicos analisados, bem como os estudos de coorte, sugerem potenciais benefícios do uso destes ácidos gordos na esclerose múltipla, mas as intervenções usadas incluíram também outras substâncias, recomendações alimentares ou tratamentos farmacológicos. Esta revisão conclui que os ácidos gordos polinsaturados podem ter um impacto positivo na esclerose múltipla quando combinados com outras substâncias, dietas especiais ou tratamentos farmacológicos. No entanto, são necessários mais estudos para determinar o papel efetivo e exclusivo destes ácidos gordos na esclerose múltipla.

Palavras-Chave: Esclerose Múltipla, Ácidos Gordos Polinsaturados, Ácidos Gordos, Tratamento Nutricional.

VII. Abstract

Many patients with multiple sclerosis use complementary alternative therapies as part of the treatment and its symptoms, being the polyunsaturated fatty acids' supplementation among the most commonly used. In fact, there is a growing interest on the use of polyunsaturated fatty acids by multiple sclerosis patients. This review therefore aims to understand how these fatty acids can influence multiple sclerosis and their utility in the treatment. To conduct this review two researches on PubMed were made using the search expressions “("polyunsaturated fatty acids") AND multiple sclerosis” and “[(("fatty acids" OR polyunsaturated OR unsaturated) AND multiple sclerosis AND (trial OR cohort OR prospective study)) NOT review]”. Clinical trials, studies conducted in animals and cohort studies were included resulting in a total of thirty articles. The most promising results suggesting a beneficial effect of polyunsaturated fatty acids on multiple sclerosis come from studies conducted in animals, but the results cannot be directly extrapolated to humans. Most of the reviewed clinical trials as well as the cohort studies suggest potential benefits from the use of these fatty acids in multiple sclerosis but the interventions used also included other substances, dietary recommendations, or pharmacological treatments. This review concludes that polyunsaturated fatty acids can have a positive impact on multiple sclerosis when combined with other substances, special diets or even pharmacological treatments. Nevertheless, more studies are needed to determine the effective and exclusive role of these fatty acids in multiple sclerosis.

Keywords: Multiple Sclerosis, Polyunsaturated Fatty Acids, Fatty Acids, Nutritional Treatment.

1. Introduction

Multiple sclerosis (MS) is an inflammatory neurological chronic disease characterized by the destruction of the myelin sheath (demyelination) caused by a disruptive immune system (1,2). The myelin sheath is a protective layer on brain, spinal cord and optic nerves' axons, and thus its reduction and progressive loss lead to various clinical manifestations according to the system involved (3). These symptoms, such as cognitive impairment, loss of vision and pain, are presented in detail in Table 1. It is important to highlight that all these symptoms are deleterious and impose a high burden and suffering to the to the patient. MS manifests in two main forms: relapsing-remitting MS (RRMS), presented by 85% of the patients, characterized by stability periods interrupted by relapses, whose recovery may or may not be complete, but patients experience clinical deterioration; and primary-progressive MS, presented by 15% of the patients, characterized by the presence of progressive and gradual neurological symptoms without remissions since the beginning. In addition to these two forms, there are also the secondary-progressive MS that appears 10 to 20 years after RRMS installation and it is characterized by less frequent remissions replaced by a gradual deterioration of the neurological symptoms over time; and the progressive-relapsing MS, a subtype of primary-progressive MS, characterized by a slow progression with few brain and spinal lesions (3). According to Atlas of MS (4), a worldwide tool that provides the most updated information, this disease is more prevalent in Europe and America, 133 and 122 cases per 100,000 people, respectively, in high income countries for which 174 cases per 100,000 people are reported and among women who currently represent 69% of world cases. In Portugal the more recent estimates indicate there are 56 cases of MS per 100,000 people.

Although it is considered an autoimmune disease, MS has a still unknown but complex and multifactorial pathogeneses, where genetic factors, such as having someone in the family with the disease, and environmental factors interact. Some examples of environmental factors that can contribute to the development of MS are brain injuries, viral infections, namely Epstein-Barr virus infection, nutrition factors, specially the low intake of polyunsaturated fatty acids, physical inactivity, childhood and adolescence obesity, low levels of vitamin D and smoking (1,5).

MS is an incurable disease (6). However, there are some pharmacological therapies available, mainly immunomodulatory agents like interferon beta, glatiramer

acetate, natalizumab and fingolimod whose action reduces the disease activity (7,8). Nevertheless, these therapies can only slow the progression of the disease and despite being able to prevent some symptoms, they are just partially effective. Besides that, these treatment options are only available for RRMS. Therefore, the establishment of more effective treatments, for all MS patients urges.

In accordance with Atlas of MS (4), there are 2.8 million people around the world with MS, an increase of 500,000 cases since 2013. In fact, it is estimated that, at least, 300 people are diagnosed with MS each day. This increase of cases together with the partially effectiveness, adverse effects, and the high cost of the available therapeutic options, are pushing the development of new, more effective, safe, preventive and treatment approaches (6,9). Many patients with MS use complementary alternative therapies as part of the treatment for the disease and its symptoms (10). The intake of polyunsaturated fatty acids (PUFAs), also pointed out as a factor that can affect both disease risk and progression (5,11), is the most common complementary alternative therapy used by MS patients (12). The first evidence that diet lipidic composition was related to MS was made by Swank R. and his coworkers, in 1952 (13), when investigating the geographical distribution of MS and its relationship with nutrition in Norway. In their research, the investigators concluded that people living in coastal regions consumed high levels of fish whereas diet of people living in inland farming communities was characterized by an elevated content of animal fat. Outstanding differences concerning the prevalence of MS in these two communities were also documented: 180 cases in coastal regions and 532 cases in inland farming communities per 100000 inhabitants per year. With these findings, the authors suggested that a high intake of saturated fat increased the risk of developing MS and that a diet based on fish, and thus rich in PUFAs, was associated with a low risk of MS (5,13).

Polyunsaturated fatty acids are long chain fatty acids, since they possess more than 18 carbon atoms, and present more than 2 double bonds carbon-carbon (6). There are two main groups of PUFAs: the omega-3 family, which includes alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA); and the omega-6 family which integrates the linoleic acid (LA) and the arachidonic acid (AA) (6). Both omega-3 and omega-6 fatty acids are considered essential since the human body is incapable of synthetizing them and the only way of satisfying the nutritional needs is through the intake of food containing them. In order to understand the possible role of PUFAs on MS it is mandatory to considerer inflammation which is characterized by the

production of inflammatory cytokines (14). The increased cytokines levels are related with disease progression in MS, making the reduction of the proinflammatory cytokines a way to benefit these patients (15). It is believed that omega-3 PUFAs have anti-inflammatory properties, which may positively influence the course of the disease (5,6,16). Omega-3 PUFAs can be converted in anti-inflammatory mediators such as resolvins and protectins that exert beneficial actions namely by reducing proinflammatory cytokines' production and levels (6).

A Cochrane Review (17) by Parks and coworkers assessed the effects of dietary interventions, namely PUFAs' supplementation, and concluded that the evidence was insufficient to determine if this intervention had or not effects on MS-related outcomes. However, this review only included clinical trials. Although clinical trials are on the top pyramid of scientific evidence, the conduction of this type of study is challenging and expensive, and so the number of papers on the association between PUFAs' supplementation and MS is still not sufficient to conclude about this.

In order to surpass this limitation, the present review includes not only clinical trials but also trials conducted in mice. Trials conducted in mice allow to determine the safety of substances without putting human lives at risk. Cohort studies were also included given that they can establish the temporal relationship between exposure and illness, which is an important advantage. Moreover, they often include a high number of participants, for whom many characteristics were evaluated, providing relevant findings regarding exposure and disease. The aim of this review is to understand how PUFAs can influence MS and their role in the treatment of this disease.

2. Methodology

To carry out this review a research on PubMed was made. Only clinical trials, trials in animal models and cohort studies were selected and the search expression "("polyunsaturated fatty acids") AND multiple sclerosis" was used. This search provided 99 articles. When the filter "last 10 years" was applied, the number of papers decreased to 42. After abstract reading, it was realized that 28 were not clinical trials, studies conducted in animals nor cohort studies and 5 were not related to the theme. Therefore, 33 articles were excluded and 8 articles were included. Since this search resulted in few studies, a new research was made also on PubMed using the search expression "[(("fatty acids" OR polyunsaturated OR unsaturated) AND multiple sclerosis

AND (trial OR cohort OR prospective study)) NOT review]". This search resulted in 63 results. After applying the time custom range "2001-2021", the number of articles decreased to 49. Of those, 18 were not clinical trials, studies conducted in animals nor cohort studies, 3 were already included, 19 were not related to the theme and 2 reported results previously described in other included studies. Seven papers were therefore included.

The consultation of the bibliographic references of the included articles, resulting from both researches, allowed to obtain 15 additional articles (snowball research): 1 clinical trial and 14 articles with relevant information about MS.

This review included 30 articles in total. In Figure 1 it is presented the flow diagram with the search expressions used as well as all the number of articles included in this review.

3. Review

Trials in animal models

Experimental autoimmune encephalomyelitis (EAE) is typically used to study the pathology and treatment options for MS. EAE is an animal model that mimic MS effects in the central nervous system (CNS) since as patients with MS, these animals present demyelination, neurodegeneration and neuroinflammation (18). EAE is normally induced through immunization with myelin antigens but can also be induced by passively transferring specific CD4 T cells to myelin antigens (19).

In 2013, to clarify the anti-inflammatory effect and the therapeutic potential of EPA in MS, Unoda et al. (19) conducted an experiment in 2 groups of female mice. In the treatment group, mice were fed with a fish-oil-free diet plus 5% of EPA supplementation. In the control group, mice were also fed with a fish-oil-free diet but did not received supplementation. Both groups were exposed to the intervention 2 weeks before EAE induction. This intervention in the treatment group led to a significant and substantial reduction in disease severity score and reduced CNS infiltrating cells, as demonstrated by pathologic histology (numerical data not presented). In view of these results, Unoda et al. concluded that treatment with EPA reduced severity and progression of MS.

Adkins et al. (18), in 2019, compared the effects of 2 forms of DHA, the phospholipid (PL) and the triacylglycerol (TAG), at 2 different doses, 0.3% and 1%, on the EAEs' onset and clinical score. Four weeks after intervention started, EAE was induced and data from onset phase (days 9-16), progression phase (days 17-21) and recovery phase (days 22-28) were separately analyzed. At day 10, significantly fewer mice, specifically 8%, in 1% PL-DHA group showed EAE signs ($p = 0.049$) compared with control group, 42%. At day 11, significantly fewer mice, 36%, in 0.3% PL-DHA group showed EAE signs ($p = 0.037$) when compared with control group, 75%. At day 12, while 92% of mice in the control group showed EAE signs, significantly fewer mice in 0,3% TAG-DHA, 36%, ($p = 0.049$) and in 1% PL-DHA, 50%, ($p = 0.022$) exhibit EAE. Comparing with control group, significantly fewer mice in 0.3% TAG-DHA, 55% vs 91%, showed EAE signs at day 13 ($p = 0.005$). During the onset phase, it was seen that mice treated with DHA exhibited a significantly lower EAE score (values not shown) when compared to control group ($p = 0.028$). Nevertheless, during recovery phase and comparing with control group, EAE score tended to be lower in 0.3% and 1% PL-DHA

groups, although no significant differences were found ($p \geq 0.05$). Mean EAE scores were not described in the original publication and thus not presented here. Given these results, the authors concluded that administering dietary DHA, in both forms and in the concentrations 0.3% TAG-DHA, 0.3% PL-DHA and 1% PL-DHA, delayed the onset of EAE.

In 2020, Rezapour-Firouzi et al. (20) conducted a trial whose purpose was to evaluate the fatty acids composition in the blood and spleen cells of mice with EAE and under treatment with rapamycin (RAPA), used as an inhibitor of the mammalian target of RAPA, and the combination of both. To this end, 30 mice were randomly assigned to 5 different groups of 6 mice each, 15 days after EAE induction: group A received 50 λ (1 λ = 1 μL) of EPO/HSO and 1 μg/kg/50 λ of RAPA; group B received 1 μg/kg/50λ of RAPA; group C received 50λ of EPO/HSO; group D: 1% ethyl alcohol diluted with distilled water and group E which did not suffer EAE induction and did not receive treatment. This intervention allowed the authors to also study its influence on EAE. Regarding this, the authors only described qualitative results: after 2 weeks of intervention, all EAE mice exhibited chronic progressive disease and EAE severity was significantly reduced in groups receiving EPO/HSO treatment with the highest scores in disease severity of mice found in groups A and D and the lowest score found in group C. These results suggested that EPO/HSO applied orally improves EAE in mice.

More recently, in the present year, Feng et al. (21) studied the effects of omega-3 PUFAs on dendritic cells which have an important role in immune response as well as in the pathogenesis of autoimmune disorders. For this, EAE-induced mice were assigned to 2 groups: treatment group, receiving 10 mg/kg of DHA; and control group, receiving 10 mg/kg of placebo. This intervention was performed daily for 2 weeks. It was observed a significant reduction in EAE severity ($p < 0.01$) and in disease incidence (p values not shown) in treatment group compared to control group. Moreover, a decreased production of pro-inflammatory cytokines IL-6 ($p < 0.01$), IFN-γ ($p < 0.01$), IL-17 (p values not shown) and IL-12p40 ($p < 0.01$) and an increased production of IL-10 ($p < 0.01$) in treatment group were observed. These data led authors to conclude that the administration of omega-3 PUFAs has a beneficial effect on clinical EAE scores and disease progression in mice.

All of these studies are summarized in Table 2.

Clinical trials

In 2005, Weinstock-Guttman et al. (22) conducted a clinical trial with 27 subjects to determine if a low-fat diet supplemented with omega-3 fatty acids would positively affect the quality of life in RRMS patients. To this end, the participants were randomly assigned to 2 different groups: the treatment group, which received 6 capsules of 1g of fish oil (65% of omega-3, 1.98 of EPA and 1.32g of DHA) plus the recommendation to a low-fat diet (total consumed fat, including the supplements, should not exceed 15% of total energy intake); and the control group, which received 6 capsules of 1g of placebo (olive oil supplements) plus a controlled low cholesterol diet (total consumed fat not exceeding 30% of total energy intake and saturated fat not exceeding 10%). Both groups also received 400 units of vitamin E, 1 multivitamin tablet (not containing any PUFAs) and at least 500 mg of calcium daily. After 1 year of follow-up, it was observed that EDSS score in the treatment group decreased 0.07 points and increased 0.35 points in the control group (p values were not presented) and the relapse rate also decreased both in treatment (- 0.79, $p = 0.021$) and control (- 0.69, $p = 0.044$) groups, when comparing with the previous year. Therefore, the authors concluded that a low-fat diet supplemented with omega-3 PUFAs can complement the beneficial effects of disease modifying therapies. However, this trial was carried out in a small sample size and participants were submitted to 2 simultaneous interventions.

Shinto et al. (23) conducted, in 2009, a trial composed of 10 patients with RRMS for 6 months. The objective of this trial was to evaluate the omega-3 fatty acids supplementation capacity on the decrease of matrix metalloproteinase-9 (MMP-9) protein levels, on the serum and on the peripheral blood mononuclear cells, in RRMS. To achieve this, the 10 participants took 9.6g/day of fish oil concentrate (containing 2.9g of EPA and 1.9g of DHA) for 3 months. After this period, there was a significant decreased of 58% in peripheral blood mononuclear cells' secreted MMP-9 levels ($p = 0.002$). No significant differences in MMP-9 serum levels after 1 and 3 months of intervention were seen ($p = 0.61$ and $p = 0.68$, respectively). The researchers concluded that omega-3 fatty acids can significantly decrease the levels of MMP-9 secreted by immune cells meaning they can have a potential therapeutic role in RRMS patients. Yet, the sample size was small and there was no control group.

Torkildsen et al. (12) carried out, in 2012, a 24-month clinical trial with 91 participants who had RRMS, a EDSS score of 5 or less and, at least, one clinical relapse. The purpose of this study was to investigate if omega-3 fatty acids, as monotherapy or combined with beta-1a interferon, reduced clinical activity of disease in MS' patients. To

conduct this trial, the treatment group received 1350 mg of EPA and 850 mg of DHA daily and control group only received placebo (corn oil). After 6 months, both groups received 44 μg of beta-1a interferon 3 times a week for 18 months. Although the results are presented graphically and, therefore, it is not possible to enumerate the number of relapses, it is described that no significant differences in relapses' number between the 2 groups were observed neither at 6 months ($p = 0.54$) nor at 24 months ($p = 0.72$). At 24 months, 57% of patients in treatment group and 58% of patients in control group were relapse-free ($p = 0.88$) and the mean EDSS score increased from 1.94 to 2.22 in the treatment group and from 1.86 to 2.19 in the control group. Also at 24 months, 30% of patients in both groups experienced disease progression ($p > 0.99$). These results suggest that omega-3 PUFAs did not exert any beneficial effects on disease activity, neither as monotherapy nor as combined with beta-1a interferon. However, this study had some limitations such as the small sample size and the possible confounding effect due to the use of corn oil as placebo since it was composed of linoleic acid and oleic acid which can also have anti-inflammatory properties.

In 2013, Pantzaris et al. (9) evaluated, through a 30-month clinical study, if 3 new interventions reduced the disease activity in patients with RRMS who were either treated or not with disease-modifying treatment. To this end, 80 patients were randomly assigned to 4 different groups, each one composed of 20 patients. Treatment group A received 1650 mg of EPA + 4650 mg of DHA + 2000 mg de γ-linolenic acid (GLA) + 3850 mg of LA + 600 mg of other omega-3 + 1714 mg of monounsaturated fatty acids (MUFAs) + 650 mg of saturated fatty acids (SFAs) + 0.6 mg of vitamin A + 22 mg of vitamin E + citrus aroma; treatment group B received the same treatment as treatment group A but with the addition of 760 mg of pure γ-tocopherol; treatment group C received 760 mg of pure γ-tocopherol dispersed in 16137 mg of pure virgin olive oil + citrus aroma and the control group received pure virgin olive oil + citrus aroma. A significantly reduction of 70% in annual relapse rate (ARR) ($p = 0.003$) was observed in treatment group B. Such reduction was persistent 12 months after the study in this treatment group. At 2 years, the cumulative probability of disability progression was 10% in treatment group B and 70% in control group ($p = 0.047$). Observing these results, Pantzaris et al. brought to the conclusion that a mixture of omega-3 and omega-6 PUFAs, MUFAs, SFAs, vitamin A, vitamin E and γ-tocopherol helped reducing the annual relapse rate and the disability progression. Yet, the small sample size and the high dropout rate (49%) are presented as limitations of this trial.

Also in 2013, Ramirez-Ramirez et al. (11) conducted a 12-month clinical trial to evaluate the efficacy of fish oil on several cytokines' serum levels, on EDSS and on ARR in RRMS. For this purpose, treatment group received 4g/day of 0.8g of EPA + 1.6g of DHA + excipient, composed of glycerin, purified water, tocopherol, sunflower oil, and titanium dioxide, and the control group received 4g/day of placebo which was treatment group' excipient. This intervention led to the following results: at 12 months, serum TNFα levels decreased 42.9% in treatment group and 0.7% in control group ($p < 0.001$), serum IL-1β levels decreased 50.3% in treatment group and 15.2% in control group ($p < 0.001$), serum IL-6 levels decreased 48.3% in treatment group and 3.8% in control group ($p < 0.001$). Regarding the EDSS score, no differences were found between groups: mean ± standard deviation (SD) in treatment group was 2.2 ± 1 points compared to 2.2 ± 0.8 points in control group ($p = 0.66$). For ARR, again no differences were found between groups: 0.84 ± 0.94 and 1±1 ($p = 0.79$), respectively for treatment group and control group. Investigators concluded that supplementation with 4g/day of fish oil showed efficacy on the reduction of inflammatory cytokines' levels but had no clinical efficacy. This study presented as limitations the small sample size ($n = 50$) and the fact that an evaluation of the severity of each relapse was not conducted nor the time between each one was documented.

Still in 2013, Rezapour-Fizouri et al. (24,25) carried out a 6-month clinical trial with 65 subjects. The participants were divided in 3 groups: treatment group A, receiving 18-21g/day of a co-supplementation of hempseed and evening-primrose oils together with a "hot nature" diet; treatment group B, receiving 18-21g of a co-supplementation of hempseed and evening-primrose oils but no special diet; and control group, receiving 18-21g/day of olive oil. The "hot nature" diet is characterized by the inclusion of foods with hot nature, such as wheat bread, turkey, kefir yoghurt, olive oil, grape juice, cabbage, banana, cinnamon, walnut and tea, the low intake of cholesterol, hydrogenated, trans and saturated fats, the preferential use of olive oil as main oil, the intake of hot nature fresh fruits and vegetables, fish and seafood, dairy products, nuts and seeds without additives and the intake of large amounts of water. It is also characterized by the exclusion of refined carbohydrates, sweet and stimulating drinks and cold nature foods such as starch, processed meat, natural butter, candy, spinach, strawberry, salty foods, spicy nuts and fried foods. This trial allowed to conduct 2 studies with different purposes. In the first study, the authors aimed to evaluate the effects of a co-supplementation of oils with a "hot nature" diet in the fatty acids' composition of erythrocyte membranes of RRMS

patients. After 6 months, IFN-γ, a pro-inflammatory cytokine, mean levels decreased in treatment group A: 0.26 ± 0.04 at baseline vs. 0.24 ± 0.04 at 6 months, p = 0.001, and increased in control group: 0.22 ± 0.06 at baseline vs. 0.24 ± 0.06 at 6 months, p = 0.005; the concentration of IL-4, an anti-inflammatory cytokine, increased in treatment groups A: 0.58 ± 0.50 at baseline vs. 0.69 ± 0.69 at 6 months, p = 0.027, and B: 0.81 ± 0.87 at baseline vs. 0.95 ± 0.91, p = 0.046. The EDSS score significantly decreased in treatments groups A: 2.76 ± 1.39 at baseline vs. 1.7±1.77 at 6 months, p = 0.001, and B: 3.25 ± 1.94 at baseline vs. 2.95 ± 1.83 at 6 months, p = 0.002, whereas EDSS score increased in control group: 3.45 ± 1.41 at baseline vs. 3.86 ± 1.41 at 6 months, p = 0.005. This data suggested that a "hot nature" diet co-supplemented with oils can promote and accelerate anti-inflammatory responses as well as prevent pro-inflammatory cytokine production in patients with MS.

The second study conducted with the same trial aimed to evaluate the potential therapeutic effects of hempseed and evening-primrose oils in multiple sclerosis. The following results were presented: relapse rate decreased in all groups but only reached significance in the treatment groups (mean ± SD at baseline vs. mean ± SD at 6 months): treatment group A = 0.31 ± 0.21 vs. 0.04 ± 0.20 (p = 0.001); treatment group B = 0.43 ± 0.40 vs. 0.05 ± 0.22 (p = 0.002); control group = 0.38 ± 0.49 vs. 0.18 ± 0.39 (p = 0.053). These results led the authors to conclude that a combination of hempseed and primrose oils showed immunomodulatory effects resulting in significant improvements on EDSS score and on relapse rate comparing to control group. Both studies had the same limitation: the uncontrolled diet can act like a confounding factor.

In 2016, Sorto-Gomez et al. (26) conducted a 1-year clinical trial to evaluate the effect of fish oil on the glutathione reductase activity and on the content of reduced and oxidized glutathione in MS. For this, 50 subjects, with a baseline EDSS score of 0-5 and treated with subcutaneous 250 μg of interferon beta-1b every other day at least 1 year before the trial, were assigned to 2 groups, 25 individuals in each group. For treatment group the intervention was 4g/day of Omega Rx capsules (0.8g of EPA + 1.6g of DHA + excipient composed of cerin, water purified, tocopherol, canola oil, sunflower oil, natural rosemary flavor and citric acid); control group received 4 capsules/day of placebo (Perfect Source Natural Products). The percentage of EPA significantly changed (mean ± SD): increased in treatment group = 0.88 ± 1.18 at baseline vs 2.68 ± 1.47 at 12 months ($p \leq 0.001$) and decreased in control group = 0.72 ± 0.63 at baseline vs 0.46 ± 0.36 at 12 months (p values not shown). The percentage of DHA also experienced a significant

change (mean ± SD): increased in the treatment group = 2.80 ± 0.86 at baseline vs 5.33 ± 1.08 at 12 months ($p \leq 0.001$) and decreased in control group = 2.95 ± 1.04 at baseline vs 2.93 ± 1.03 (p values not shown). In contrast, it was observed a significant decrease of AA in both groups (mean ± SD): treatment group = 11.01 ± 1.56 at baseline vs 8.86 ± 1.36 at 12 months ($p \leq 0.001$); control group = 10.42 ± 2.71 at baseline vs 10.12 ± 1.87 at 12 months (p values not shown). No differences in glutathione reductase activity nor in the content of reduced and oxidized glutathione were found. These results led authors to conclude that although AA concentration decreased in both groups, fish oil supplementation promoted an increase in EPA and DHA percentage which is indicative of a reduced inflammatory eicosanoids production and an increase of anti-inflammatory mediators such as resolvins and protectins.

Esfahan et al. (27) conducted in 2017, a clinical trial for 12 months to assess the effect of adding fish oil, composed of 180 mg of EPA, 120 mg of DHA and excipient (glycerin, purified water, tocopherol, sunflower oil, and titanium dioxide), to Fingolimod, an oral immunomodulator used in RRMS' treatment, on the cytokines TNFα, IL-1β, IL-6 and IFN-γ. Treatment and control groups were both composed of 25 subjects each. No significant differences on serum TNFα mean levels were observed neither in treatment group: 37.16 Pg/ml at baseline vs. 21.20 Pg/ml at 12 months ($p = 0.308$) nor in the control group: 28.79 Pg/ml at baseline vs. 16.47 Pg/ml at 12 months ($p = 0.165$). However, significant differences in serum IL-1β mean levels were found both in treatment group: 13.76 Pg/ml at baseline vs. 13.17 Pg/ml at 12 months ($p = 0.022$), and in control group: 14.00 Pg/ml at baseline vs. 13.15 Pg/ml at 12 months ($p < 0.001$). Significant differences in serum IL-6 mean levels were detected in control group: 6.33 Pg/ml at baseline vs. 6.05 Pg/ml at 12 months ($p = 0.003$) but not in treatment group: 6.19 Pg/ml at baseline vs. 6.17 Pg/ml at 12 months ($p = 0.94$). Moreover, no significant differences on serum IFN-γ mean levels were observed neither in treatment group: 6.38 Pg/ml at baseline vs. 5.92 Pg/ml at 12 months ($p = 0.39$) nor in control group: 6.98 Pg/ml at baseline vs. 5.99 Pg/ml at 12 months ($p = 0.28$). When comparing mean EDSS score changes in both groups, no significant differences were observed: 3.10 at baseline vs. 2.30 at 12 months in treatment group ($p = 0.026$); 2.55 at baseline vs. 1.68 at 12 months in control group ($p = 0.037$). In contrast with results from Ramirez-Ramirez et al. (11), supplementation with fish oil did not reduce inflammatory cytokines' levels nor improved disability score of patients. However, in this latter study from Esfahan and colleagues, the quantity of fish oil used was 1g/day and lower than the one used in the study from Ramirez-Ramirez et al. This

can be recognized as a limitation, as also the small sample size (n = 52), insufficient case numbers (n = 25), small duration of the study (12 months) and the provision of tocopherol as placebo which may had confounded the results given its antioxidant properties.

Finally, in 2018, Kouchaki et al. (28) conducted a clinical trial to determine the effects of a 12 weeks' co-supplementation with omega-3 fatty acids and vitamin D3 in disability score and in metabolic status of MS patients. This co-administration of omega-3 PUFAs and vitamin D3 significantly reduced EDSS score in MS patients, 2.3 at baseline vs. 2.2 at 12 weeks ($p = 0.01$) which led authors to conclude that taking PUFAs omega-3 and vitamin D3 supplements simultaneously for 12 weeks has beneficial effects on MS patients' disability score. Nonetheless, the circulating fatty acids' profiles were not evaluated before and after supplementation which constitutes a limitation of this study. Characteristics of treatment and control groups, as well as the interventions, main results, conclusions and limitations of all studies are summarily described in Table 2.

Cohort studies

In order to prospectively investigate the association between PUFAs' dietary intake and the risk of MS, evaluated by Cox regression analysis and expressed as Hazard Ratio (HR), Bjørnevik et al. (29) carried out, in 2017, a cohort study involving 80920 women from Nurses' Health Study and 94511 women from Nurses' Health Study II. It was shown that PUFAs total intake was associated with lower MS risk: HR = 0.69, 95% CI 0.51-0.93 ($p = 0.03$) and that ALA intake was also associated with lower MS risk: HR = 0.62, 95% CI 0.46-0.84 ($p = 0.009$). No significant associations between LA, EPA and DHA intake and the risk of MS were found (data not shown). These data suggested that low PUFAs intake can be a modifiable risk factor for MS. However, this study was based on self-reported diets information and there were no biochemical markers of PUFAs, which are limitations.

Once more, Bjørnevik et al., but in 2018 (30), aimed to investigate the association between the ALA levels and the disease activity in MS. Authors therefore conducted a cohort study with the same participants of Torkildsen et al. (12) clinical trial (previously described). In order to evaluate T1 gadolinium enhancing (GdE) lesions, new T2 lesions and new combined unique activity, which is the sum of T1 GdE lesions and new or enlarging T2 lesions, magnetic resonance imaging (MRI) scans were conducted. Although higher levels of ALA were significantly associated with lower odds of MRI activity ($p = 0.028$), it was observed that ALA levels were not significantly associated

with new T1-GdE lesions (p = 0.14) neither with Expanded Disability Status Scale (EDSS) progression (p = 0.13). Higher ALA levels were correlated with a lower EDSS score in different moments of follow-up (p = 0.040) but not with new relapses (p = 0.082). These data suggest that higher levels of ALA were associated with a lower MRI activity in MS patients, suggesting that omega-3 PUFAs have beneficial effects on disease activity. However, the limited sample size, the short follow-up period and the fact that ALA levels were not associated with T1 GdE lesions nor with EDSS progression are presented as limitations of this study.

Information about the participants of these studies as well as its main conclusions and limitations are presented in Table 2.

4. Discussion

At the moment, pharmacological therapy, namely immunomodulatory drugs, is the first-line treatment of MS (31). However, due to its side effects, high costs and since they are only partially effective, patients with MS seek for alternative therapies that can help them manage the disease (22).

Nutrition is considered one of the environmental factors implied in MS' pathogenesis, but no special diet or supplement are part of this disease therapy (11). Yet, it is believed that more than one third of MS patients are trying or have tried to control disease progression through PUFAs supplementation (10).

After Swank et al. (13) suggested that a diet rich in PUFAs was associated with a low risk of MS, many studies have been conducted with the purpose of assessing their therapeutic benefits. Nevertheless, as it is described in the Cochrane Review (17) that included only clinical trials, the existing evidence seems insufficient to determine whether there is a positive effect of PUFAs on MS. For this reason, the present review includes not only clinical trials but also trials conducted in animals and cohort studies in order to clarify the evidence on the role of PUFAs on MS treatment.

The most promising results are the ones from studies conducted in animals: Unoda et al. (19) observed that a fish-oil-free diet supplemented with EPA reduced severity and progression of MS in mice, suggesting a benefit from this intervention; Adkins et al. (18), when comparing the effects of 2 different types and dosages of DHA, noted that this fatty acid delayed the onset of EAE; Rezapour-Firouzi et al. (20) conducted a trial whose results suggest that EPO and HSO, oils rich in PUFAs, improve EAE; and finally Feng et al. (21) observed that administering omega-3 PUFAs can improve clinical EAE scores

and disease progression in mice. Although mice are not the living creatures genetically more similar to humans, trials with these animals are widely used to evaluate not only the quality and safety of products and/or substances but also their effects. Besides using *in vivo* models, these studies provide data and useful evidence to create new hypothesis, therapies and associations. However, as promising as the results may be, the results obtained from studies conducted in animals, and namely in mice, cannot be extrapolated to humans. These studies can only suggest that administering EPA, DHA or PUFAs rich oils can be done in humans with a low risk of a negative outcome. Nevertheless, the positive outcomes in MS in humans need still to be further accessed.

Cohort studies are important studies not only because they allow to determine the temporal relationship between the exposure to a certain factor and the development of an illness but also because they include a high number of subjects whose characteristics can provide important information regarding exposure and disease. The cohort studies conducted by Bjørnevik et al. in 2017 (29) and in 2018 (30) also suggest a positive effect of PUFAs in MS. Yet, both of studies present limitations such as self-reported data, lack of biochemical markers, small sample size and short follow-up period. Therefore, new cohort studies should be conducted considering and surpassing these limitations. Such studies would provide information on the use of PUFAs for MS treatment, that could be used to outline nutritional guidelines.

Analyzing the results derived from clinical trials, it can be observed some controversy. In contrast with the other 9 studies, Torkildsen et al. (12) and Esfahan et al. (27) failed to prove any possible benefit of PUFAs in MS. It is important to note that both trials present significant limitations such as the use of corn oil and tocopherol as placebos which could have acted like confounding factors. Also, the dosage of fish oil used in the trial conducted by Esfahan et al. (27) can also be a limitation since that a trial conducted by Ramirez-Ramirez et al. (11), also using fish oil containing EPA, DHA and excipient, but in a higher dosage, showed the efficacy of these fatty acids on decreasing inflammatory cytokines' levels.

In general, most of the reviewed clinical trials shows positive results suggesting potential benefits from the use of PUFAs by MS patients. The inflammatory process present in MS is characterized by an increased secretion of reactive oxygen species and a reduced antioxidant defense mechanism, both caused by an extreme production of pro-inflammatory cytokines. Shinto et al. (23), Ramirez-Ramirez et al. (11), Rezapour-Firouzi et al. (24) and Sorto-Gomez et al. (26) successfully proved that supplementation with fish

oil and with other oils rich in PUFAs can contribute to the reduction of pro-inflammatory cytokines levels, therefore promoting a decrease of the inflammation in MS. Weinstock-Guttman et al. (22) and Rezapour-Firouzi et al. (25) also suggested a positive effect of PUFAs on the EDSS score and on the relapse rate of MS patients. Nevertheless, although these trials have presented beneficial effects, none of them evaluated the exclusive role of PUFAs. All the trials used interventions including PUFAs and recommendations for low fat diets or treatments such as the modifying therapies like interferon beta. Pantzaris et al. (9) and Kouchaki et al. (28) also observed positive results in their studies but, likewise, the interventions included not only PUFAs but also other fatty acids, such as MUFAs and SFAs, and vitamins, such as vitamin A, vitamin E and γ-tocopherol. Therefore, it is possible that one should not consider a nutrient alone, such as PUFAs, but the combined effect of different nutrients, food intake and food pattern.

In conclusion, even though most analyzed studies have shown positive results, they cannot be exclusively attributed to PUFAs but also to additional components of the interventions, as previously described. This way, this review concludes that PUFAs can have a positive impact on MS, especially in by lowering the inflammatory process, when combined with other substances, such as tocopherol, low fat diets or even with the MS treatments, such as interferon beta. The possible beneficial effect of PUFAs on MS is an important and motivating sign that further investigations in this theme should be conducted, particularly using PUFAs supplementation alone as intervention, in order to determine the effective role of these fatty acids in MS.

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6. Figures and tables.

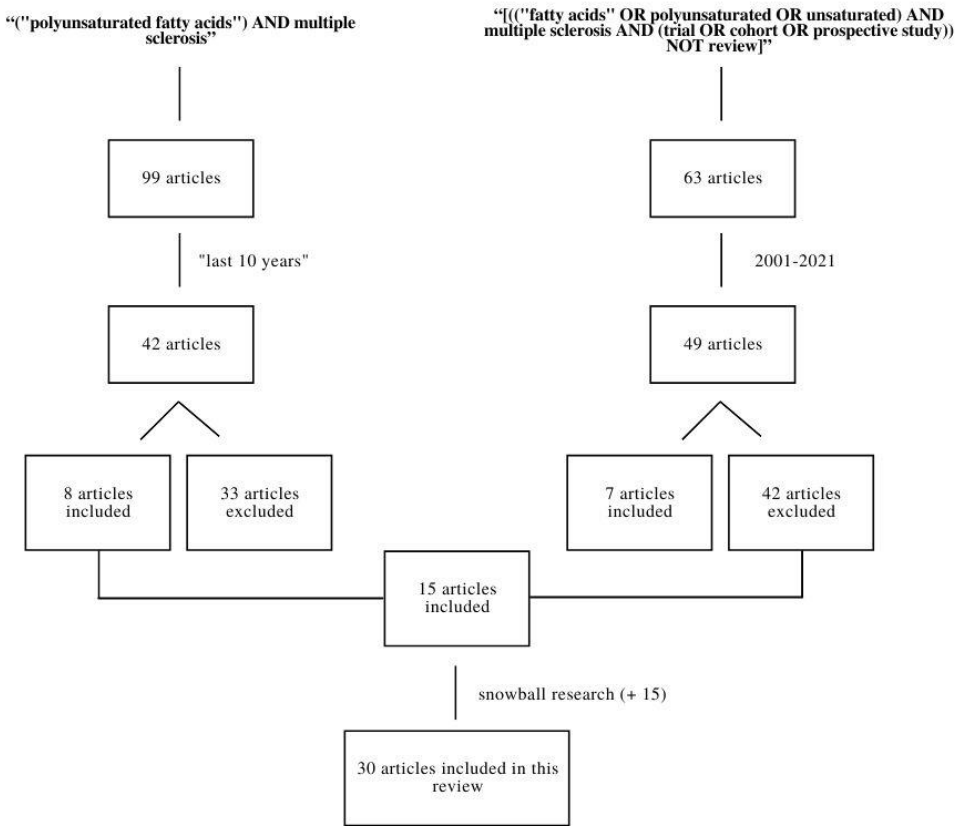

Figure 1. Flowchart regarding included and excluded studies and search expressions used.

Table 1. Symptoms of multiple sclerosis according to the affected body site or structure.

Affected site	**Symptoms**
Brain	Cognitive impairment; hemisensory and motor; affective; epilepsy (rare); focal cortical deficits (rare); tremor; clumsiness and poor balance; vertigo; impaired swallowing and speech
Optical Nerve	Unilateral painful loss of vision
Spinal Cord	Weakness; painful spasms; bladder dysfunction; erectile impotence; constipation
Other	Pain; fatigue; temperature sensitivity; exercise intolerance

Table 2. Characteristics of the included studies.

Publication (year)	Participants	Intervention	Main Results	Conclusions	Limitations
Trials in animal models					
Unoda et al. (2013)	Female mice Treatment group: n = 5 Control group: n = 5	Treatment Group: fish-oil-free diet + 5% of EPA[i] supplementation Control Group: fish-oil-free diet without supplementation Induction of EAE[ii] 14 days after intervention started	- Significant and substantial reduction in EAE severity score in the treatment group - Reduced CNS[iii] infiltrating cells in the treatment group	Treatment with EPA lead to reduced severity and progression of EAE	Trial conducted in animals
Adkins et al. (2019)	12 female mice in each group	0.3% PL-DHA[iv] group: 18.7 µmol/g of PA[v] + 2.51 µmol/g of SA[vi] + 68.2 µmol/g of OA[vii] + 3 µmol/g of VA[viii] + 0.38 µmol/g of EA[ix] + 118 µmol/g of LA[x] + 0.027 µmol/g of AA[xi] + 2.21 µmol/g of ALA[xii] + 0.108 µmol/g of EPA+ 5.46 µmol/g of DHA[xiii]	- At day 10, 8% of mice in 1% PL-DHA group and 42% in control group showed EAE signs (p = 0.049) - At day 11, 36% of mice in 0.3% PL-DHA group and 75% in control group showed EAE signs (p = 0.037) - At day 12, 92% of mice in the control	Administration of dietary DHA delayed the onset of EAE	- Lack of mice terminated at the end of each phase to determine temporal differences in tissue fatty acid profiles and their association

		0.3% TAG-DHA[xiv] group: 18.3 µmol/g of PA + 2.37 µmol/g of SA + 65.6 µmol/g of OA + 2.52 µmol/g of VA + 0.35 µmol/g of EA + 114 µmol/g of LA + 0.027 µmol/g of AA + 2.08 µmol/g of ALA + 0.132 µmol/g of EPA + 5.48 µmol/g of DHA 1% PL-DHA group: 16.2 µmol/g of PA + 2.26 µmol/g of SA + 60.5 µmol/g of OA + 2.18 µmol/g of VA + 0.37 µmol/g of EA+ 105 µmol/g of LA + 0.036 µmol/g of AA + 1.84 µmol/g of AA + 0.308 µmol/g of EPA + 22.6 µmol/g of DHA 1% TAG-DHA group: 16.9 µmol/g of PA + 2.23 µmol/g of SA + 58.5 µmol/g of OA + 2.62 µmol/g of VA + 0.34 µmol/g of EA +	group showed EAE signs, 36% in 0.3% TAG-DHA (p = 0.049) and 50% in 1% PL-DHA (p = 0.022) exhibit EAE - Comparing with control group, significantly fewer mice (55%) in 0.3% TAG-DHA showed EAE signs at day 13 (p = 0.005) - At the end of onset phase, no difference was observed in the number of mice with EAE in DHA groups and in control group (p values not shown) - During recovery phase, EAE score tended to be lower in 0.3% and 1% PL-DHA groups (no significant difference was found)		with clinical scores; - Absence of feeding experimental diets to mice without disease; - Small sample size due to a high mortality (50% in control group and in TAG-DHA group, 42% in 0.3% PL-DHA and 33% in 1% PL-DHA and 1% TAG-DHA)

		107 µmol/g of LA + 0.026 µmol/g of AA + 1.98 µmol/g of ALA + 0.400 µmol/g of EPA + 21.4 µmol/g of DHA <u>Control group:</u> 18.8 µmol/g of PA+ 2.41 µmol/g of SA + 64.8 µmol/g of OA + 1.91 µmol/g of VA + 0.37 µmol/g of EA + 113 µmol/g of LA + 0.024 µmol/g of AA + 2.02 µmol/g of ALA + 0.030 µmol/g of EPA + 0.065 µmol/g of DHA Induction of EAE 4 weeks after the intervention started			
Rezapour-Firouzi et al. (2020)	6 female mice in each group	<u>Group A:</u> 50 λ[xv] of EPO/HSO and 1 µg/kg/50 λ of RAPA[xvi] <u>Group B:</u> 1 µg/kg/50 λ of RAPA <u>Group C:</u> 50 λ of EPO/HSO	- All EAE mice exhibited chronic progressive disease - EAE severity was significantly reduced by treatment of EPO/HSO - The highest scores in disease severity of	The results suggest that EPO/HSO applied orally improve EAE in mice	Trial conducted in animals

		Group D: 1% ethyl alcohol diluted with distilled water Group E: mice did not suffer EAE induction and did not receive treatment	mice were found in groups A and D - The lowest score was found in group C		
Feng et al. (2021)	Mice (n not described)	Treatment group: 10mg/kg of DHA daily for 2 weeks Control group: 10mg/kg of placebo daily for 2 weeks	- Significant reduction in EAE severity ($p < 0.01$) and disease incidence in treatment group compared to control group - Decreased production of pro-inflammatory cytokines IL-6 ($p < 0.01$), IFN-γ ($p < 0.01$), IL-17 (p values not shown) and IL-12p40 ($p < 0.01$) in treatment group; - Increased production of IL-10 ($p < 0.01$) in treatment group	There is a beneficial effect on clinical EAE scores and on disease progression of EAE mice that received dietary n-3 fatty acids	Trial conducted in animals
Cohort Studies					

Bjørnevik et al. (2017)	80920 women from Nurses' Health Study aged 30-50 years (in 1976) and 94511 women from Nurses' Health Study II aged 25-42 years (in 1989)					No intervention	- PUFAs[xvii] total intake was associated with lower MS[xviii] risk (HR[xix] = 0.69, 95% CI[xx]: 0.51-0.93; p = 0.03) - ALA intake was associated with lower MS risk (HR = 0.62, 95% CI: 0.46–0.84; p = 0.009) - No significant association between LA, EPA and DHA intake and the risk of MS was found (data not shown)	Low PUFAs intake can be a modifiable risk factor for MS	- Self-reported information about diet; - Lack of biochemical markers of PUFAs
Bjørnevik et al. (2018)		Q[xxiii]1	Q2	Q3	Q4	No intervention	- ALA levels were not significantly associated with new T1-GdE[xxiv] lesions (p = 0.14); - ALA levels were correlated with a lower EDSS score in different moments of follow-up (p = 0.040) but not with EDSS progression (p = 0.13);	Higher levels of ALA were associated with a lower MRI[xxv] activity in MS patients, suggesting that omega-3 PUFAs have beneficial effects on disease	- Limited sample size; - Relatively short follow-up time (24 months)
	Participants, n	22	22	22	21				
	Women, n	14	16	11	16				
	Men, n	8	6	11	5				
	Mean age (SD[xxi]), years	39.0 (7.3)	39.5 (9.4)	40.2 (6.9)	36.5 (9.8)				

	Mean EDSS[xxii] at baseline (SD)	1.8 (0.7)	1.9 (0.8)	2.0 (0.9)	1.9 (0.9)		- ALA was not correlated with new relapses (p = 0.082)	activity in MS	
Clinical Trials									
Weinstock-Guttman et al. (2005)	Participants, n Women, % Men, % Mean age (SD), years Mean (SD) EDSS	Treatment Group 13 84.6 15.4 39.9 (10.0) 2.0 (1.3)		Control Group 14 85.7 14.3 45.1 (7.7) 1.9 (0.6)		<u>Treatment group:</u> 6 capsules/day of 1g of fish oil (65% omega-3, 1.98g/day of EPA and 1.32g/day of DHA) + very low dietary fat intake recommendation (≤ 15% of total energy intake, including the supplements) <u>Control group:</u> 6g capsules/day of 1g of placebo (olive oil supplements) + low cholesterol controlled diet (total fat ≤ 30% and saturated fat < 10% of total energy intake) <u>All participants</u> received 400 units of vitamin E, 1 multivitamin tablet (not containing any PUFAs)	- EDSS score decreased 0.07 points in the treatment group (p values not shown); - Relapse rate decreased in both groups comparing with the previous year (treatment group: -0.79, p = 0.021; control group: -0.69, p = 0.044)	A low-fat diet supplemented with omega-3 PUFAs can complement the beneficial effects of disease modifying therapies	- Small sample size (n = 27); - Two simultaneous interventions

				and at least 500 mg of calcium per day			
Shinto et al. (2009)	Participants, n Women, % Men, % Mean age (SD), years Mean (SD) EDSS		10 90 10 47.6 (9.0) 3.20 (0.23)	10 patients with RRMS[xxvi] received omega-3 PUFAs supplementation in fish oil concentrate form for 3 months: 9.6g/day containing 2.9g of EPA and 1.9g of DHA	- Significant decreased of 58% in PBMC[xxvii] secreted MMP-9[xxviii] levels after 3 months of supplementation with omega-3 (p = 0.002) - No significant difference in MMP-9 serum levels after 1 and 3 months of intervention (p = 0.61 and p = 0.68, respectively)	Omega-3 fatty acids can significantly decrease the levels of MMP-9 secreted by immune cells meaning they can have a potential therapeutic role in RRMS patients	- Small sample size (n = 10); - Lack of control group
Torkildsen et al. (2012)		Treatment group	Control group	<u>Treatment group:</u> 1350 mg of EPA + 850 mg of DHA daily for 6 months <u>Control group:</u> placebo daily for 6 months After 6 months, both groups received 44 µg of beta-1a interferon (in addition to the	- No significant differences in the number of relapses between the 2 groups neither at 6 months (p = 0.54) nor at 24 months (p = 0.72) - At 24 months, 57% of patients in treatment group and 58% of patients in	Omega-3 PUFAs did not exert any beneficial effects on disease activity neither as monotherapy nor as combined	Small sample size (n = 91)
	Women, n	30	29				
	Men, n	16	16				
	Mean age (SD), years	38.8 (8.4)	38.3 (8.4)				
	Mean (SD) EDSS	1.94 (0.78)	1.86 (0.86)				

	Mean relapses during the previous year (SD), n People with previous omega-3 PUFAs treatment	1.7 (0.7) 18		1.6 (0.7) 20	intervention initially assigned) 3 times a week for 18 months	control group were relapse-free (p = 0.88) - At 24 months, mean EDSS score increased from 1.94 to 2.22 in the treatment group and from 1.86 to 2.19 in the control group (p values were not presented) - At 24 months, 30% of patients in both groups experienced disease progression (p > 0.99)	with beta-1a interferon	
Rezapour-Firouzi et al. (2013)	 Participants, n Women, n Men, n Mean age (SD), years	Treatment group A 23 16 7 34.2 (7.5)	Treatment group B 20 15 5 33.7 (7.8)	Control group 22 11 11 35.9 (7.8)	<u>Treatment group A:</u> 18-21g/day of co-supplementation of hempseed and primrose oils in a 9:1 ratio + advising "Hot nature" diet <u>Treatment group B:</u> 18-21g/day of co-supplementation of hempseed and primrose oils in a 9:1 ratio	- IFN-γ[xxix] (pro-inflammatory cytokine) mean levels decreased in treatment group A (0.26 ± 0.04 at baseline vs. 0.24 ± 0.04 at 6 months, p = 0.001) and increased in control group (0.22 ± 0.06 at baseline vs. 0.24 ± 0.06 at 6 months, p = 0.005); - Increase of IL-4[xxx] (anti-inflammatory cytokine)	A "Hot nature" diet co-supplemented with oils can promote and accelerate anti-inflammatory responses as well as to prevent pro-inflammatory cytokine production in	The uncontrolled diet can act as a confounding factor

					Duration of treatment was 6 months <u>Control group:</u> 18-21g/day of olive oil	concentration in treatment groups A (0.58 ± 0.50 at baseline vs. 0.69 ± 0.69 at 6 months, p = 0.027) and B (0.81 ± 0.87 at baseline vs. 0.95 ± 0.91, p = 0.046); - EDSS score significantly decreased in treatments groups A (2.76 ± 1.39 at baseline vs. 1.7±1.77 at 6 months, p = 0.001) and B (3.25 ± 1.94 at baseline vs. 2.95 ± 1.83 at 6 months, p = 0.002) and increased in control group (3.45 ± 1.41 at baseline vs. 3.86 ± 1.41 at 6 months, p = 0.005)	patients with MS	
Rezapour-Firouzi et al. (2013)						- EDSS score significantly improved in treatment groups A (2.76 ± 1.39 at baseline vs. 1.77 ± 1.7	A daily dose of 18-21g of a combination of hempseed	

							at 6 months, p = 0.001) and B (3.25 ± 1.94 at baseline vs. 1.83 ± 2.95 at 6 months, p = 0.002); - Relapse rate decreased in all groups but only reached significancy in the treatment groups (mean ± SD at baseline vs. mean ± SD at 6 months): treatment group A = 0.31 ± 0.21 vs 0.04 ± 0.20 (p = 0.001); treatment group B = 0.43 ± 0.40 vs. 0.05 ± 0.22 (p = 0.002); control group = 0.38 ± 0.49 vs. 0.18 ± 0.39 (p = 0.053)	and primrose oils for 6 months showed immunomodulatory effects resulting in significant improvements on EDSS score and on relapse rate comparing to control group	
Pantzaris et al. (2013)	Women, n (p = 1.000)	Treatment group A 15	Treatment group B 15	Treatment group C 15	Control group 15	<u>Treatment group A:</u> 1650 mg of EPA + 4650 mg of DHA + 2000 mg of GLA[xxxii] + 3850 mg of LA + 600 mg of other omega-3 + 1714 mg of MUFAs[xxxiii] + 650 mg of SFAs[xxxiv]	- Significant reduction of 70% in ARR (p = 0.003) persistent 12 months after de study in treatment group B - At 2 years, the cumulative probability of disability	A mixture of omega-3 and omega-6 PUFAs, MUFAs, SFAs, vitamin A, vitamin E	- Small sample size (n = 80); - High dropout rate (49%)

	Men, n Mean age (SD), years (p = 0.982) People with ≤ 1 relapses, % Mean EDSS score (SD) (p = 0.775) ARR[xxxi] (p = 0.946)	5 38.0 (11.9) 40 2.52 (1.23) 1.17	5 36.9 (8.4) 45 2.15 (1.05) 1.21	5 37.7 (8.7) 40 2.42 (1.21) 1.16	5 38.1 (10.9) 35 2.39 (0.93) 1.05	+ 0,6 mg of vitamin A + 22 mg of vitamin E + citrus aroma <u>Treatment group B:</u> 1650 mg of EPA + 4650 mg of DHA + 2000 mg of GLA + 3850 mg of LA + 600 mg of other omega-3 + 1714 mg of MUFAs + 650 mg of SFAs + 0,6 mg of vitamin A + 22 mg of vitamin E + 760 mg of pure γ-tocopherol + citrus aroma <u>Treatment group C:</u> 760 mg of pure γ-tocopherol dispersed in 16137 mg of pure virgin oil + citrus aroma <u>Control group:</u> pure virgin oil + citrus aroma	progression was 10% in treatment group B and 70% in control group (p = 0.047)	and γ-tocopherol reduced the annual relapse rate and the disability progression	

						Each group received 19.5 ml per day of the mixture assigned for 30 months			
Ramirez-Ramirez et al. (2013)	Participants, n Women, % (p = 0.89) Men, % Mean age (SD), years (p = 0.85) Mean EDSS score (SD) (p = 0.87) Total relapses before the study (SD) (p = 0.82)		Treatment group 25 16.6 83.4 35.1 (7.6) 2.10 (0.98) 5.44 (4.30)		Control group 25 17.7 82.3 34.7 (7.8) 2.06 (0.84) 5.80 (6.93)	<u>Treatment group:</u> 4g/day of EPA (0.8 g) + DHA (1.6 g) + excipient (glycerin, purified water, tocopherol, sunflower oil, and titanium dioxide) for 12 months <u>Control group:</u> 4g/day of placebo (glycerin, purified water, tocopherol, sunflower oil, and titanium dioxide) for 12 months	- At 12 months, serum TNFα[xxxv] levels decreased 42.9% in treatment group and 0.7% in control group ($p < 0.001$); serum IL-1β[xxxvi] levels decreased 50.3% in treatment group and 15.2% in control group ($p < 0.001$); serum IL-6[xxxvii] levels decreased 48.3% in treatment group and 3.8% in control group ($p < 0.001$) - No differences in the EDSS score (mean ± SD): treatment group = 2.2 ± 1 points; control group = 2.2 ± 0.8 points ($p = 0.66$) - No differences in the ARR (mean ± SD): treatment group = 0.84 ± 0.94; control	Supplementation with 4g/day of fish oil showed efficacy on the reduction of inflammatory cytokines' levels but had no clinical efficacy	- Small sample size (n = 50); - Lack of information about relapses' severity and time between each one

					group = 1±1 (p = 0.79)		
Sorto-Gomez et al. (2016)	50 people aged 18-55 years, baseline EDSS score of 0-5 and treated with subcutaneous 250 μg of interferon beta-1b every other day at least 1 year before the trial			<u>Treatment group (n = 25):</u> 4g/day of Omega Rx capsules (0.8g of EPA + 1.6g of DHA + excipient (cerin, water purified, tocopherol, canola oil, sunflower oil, natural rosemary flavor and citric acid) for 1 year <u>Control group (n = 25):</u> 4 capsules/day of placebo (Perfect Source Natural Products) for 1 year	- Significant change in the percentage of EPA (mean ± SD): increase in the treatment group = 1.82 ± 2.18 and decrease in control group = 0.30 ± 0.53 ($p \leq 0.001$) - Significant change in the percentage of DHA (mean ± SD): increase in treatment group = 2.59 ± 1.16 and decrease in control group = 0.13 ± 0.58 ($p \leq 0.001$) - Significant decrease of AA in both groups (mean ± SD): treatment group = 2.18 ± 1.69; control group = 0.36 ± 1.76 - No differences in glutathione reductase activity nor in the content of reduced and oxidized glutathione were found	Fish oil supplementation promoted an increase in EPA and DHA percentage leading to a decrease of AA concentration (reduced inflammatory eicosanoids production and increased anti-inflammatory mediators)	Not described in the trial

Esfahan et al. (2017)	Treatment group Women, n (p = 0.355) Men, n Mean age (SD), years (p = 0.20) Mean EDSS score (SD) (p = 0.24)	Control group 13 12 35.2 (9.9) 3.10 (1.28)	16 11 31.4 (8.4) 2.55 (1.38)	<u>Treatment group:</u> 0.5 mg/day of Fingolimod capsule + 1 g/day fish oil capsule (180 mg of EPA, 120 mg of DHA, and excipient (glycerin, purified water, tocopherol, sunflower oil, and titanium dioxide)) <u>Control group:</u> 0.5 mg/day of Fingolimod capsule + placebo capsule (glycerin, purified water, tocopherol, sunflower oil, and titanium dioxide)	- No significant differences on serum TNFα levels were observed in both groups (mean (SD) at baseline vs. mean (SD) at 12 months): treatment group = 37.16 (92.74) Pg/ml vs. 21.20 (36,45) Pg/ml (p = 0.308); control group = 28.79 (47.46) Pg/ml vs. 16.47 (16.64) Pg/ml (p = 0.165) - Significant differences in serum IL-1β were found in both groups (mean (SD) at baseline vs. mean (SD) at 12 months): treatment group = 13.76 (0.74) Pg/ml vs. 13.17 (0.52) Pg/ml (p = 0.022); control group = 14.00 (1.03) Pg/ml vs. 13.15 (0.61) Pg/ml (p < 0.001)	Supplementation with 1g/day of fish oil did not reduced inflammatory cytokines' levels nor improved disability score of patients	- Small sample size (n = 52); - Insufficient number of cases (n= 25); - Short duration of the trial (12 months); - Use of tocopherol in placebo (which may contribute to confounding findings given its antioxidant nature); - Small dosage of fish oil

					- Significant differences in serum IL-6 levels were detected in control group (mean (SD) = 6.33 (0.58) Pg/ml at baseline vs. 6.05 (0.47) Pg/ml at 12 months) (p = 0.003) but not in treatment group (mean (SD) = 6.19 (0.35) Pg/ml at baseline vs. 6.17 (0.76) Pg/ml at 12 months) (p = 0.94) - No significant differences on serum IFN-γ levels were observed in any group (mean (SD) at baseline vs. mean (SD) at 12 months): treatment group: 6.38 (1.51) Pg/ml vs. 5.92 (1.31) Pg/ml (p = 0.39); control group = 6.98 (3.87) Pg/ml vs. 5.99 (0.84) Pg/ml (p = 0.28)		

					- When comparing EDSS score changes in both groups, no significant differences were observed (mean (SD) at baseline vs. mean (SD) at 12 months): treatment group = 3.10 (1.28) vs. 2.30 (0.88) (p = 0.026); control group = 2.55 (1.38) vs. 1.68 (1.17) (p = 0.037)		
Kouchaki et al. (2018)	Participants, n Mean age (SD), years (p = 0.37)	Treatment group 26 33.3 (6.5)	Control group 27 35.2 (9.2)	Treatment group: 2 capsules daily (containing 500 mg DHA and 106 mg EPA) + vitamin D3 as cholecalciferol supplements (50000 IU/biweekly) Control group: placebo (sunflower oil capsules)	Significant reduction EDSS score in the treatment group: 2.3 at baseline vs. 2.2 at 12 weeks (p = 0.01)	Taking PUFAs omega-3 and vitamin D3 supplements simultaneously for 12 weeks has beneficial effects on MS patients' disability score	Circulating fatty acids profiles were not evaluated before and after the supplementation

[i] EPA – Eicosapentaenoic Acid
[ii] EAE – Experimental Autoimmune Encephalomyelitis
[iii] CNS – Central Nervous System
[iv] PL-DHA – Phospholipid DHA
[v] PA – Palmitic Acid
[vi] SA - Stearic Acid
[vii] OA – Oleic Acid
[viii] VA - Vaccenic Acid
[ix] EA - Eicosanoid Acid
[x] LA - Linoleic Acid
[xi] AA - Arachidonic Acid
[xii] ALA – Alpha-Linolenic Acid
[xiii] DHA – Docosahexaenoic Acid
[xiv] TAG-DHA - Triglyceride DHA
[xv] λ – Lambda unit (equivalent to 1 microlitre (μL)
[xvi] RAPA - Rapamycin
[xvii] PUFAs – Polyunsaturated Fatty Acids
[xviii] MS – Multiple Sclerosis
[xix] HR – Hazard Ratio
[xx] CI – Confidence interval
[xxi] SD – Standard Deviation
[xxii] EDSS – Expanded Disability Status Scale
[xxiii] Q – Quartile of baseline alpha-linolenic acid levels
[xxiv] T1-GdE - T1 gadolinium enhancing
[xxv] MRI – Magnetic Resonance Imaging
[xxvi] RRMS – Relapsing-remitting Multiple Sclerosis
[xxvii] PBMC - Peripheral Blood Mononuclear Cells
[xxviii] MMP-9 - Matrix Metalloproteinase-9
[xxix] IFN-γ - Interferon-γ
[xxx] IL4 – Interleukin-4
[xxxi] ARR – Annual Relapse Rate
[xxxii] GLA - γ-linolenic Acid
[xxxiii] MUFAs – Monounsaturated Fatty Acids
[xxxiv] SFAs – Saturated Fatty Acids
[xxxv] TNAα - Tumor Necrosis Factor Alpha
[xxxvi] IL-1β - Interleukin 1 beta
[xxxvii] IL-6 - Interleukin-6