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Regulation of Lipid-dependent Membrane Enzymes by Hot Nature Diet with Co-supplemented Hemp Seed, Evening Primrose Oils Intervention in Multiple Sclerosis Patients

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Multiple sclerosis (MS) is the most chronic and inflammatory disorder that result in physical disability. Because of limited efficacy and adverse side effects, identifying novel therapeutic and protective agents is important. This study was aimed to assess regulation of surface-membrane enzymes by hemp seed and evening primrose oils as well as Hot nature dietary intervention in Relapsing Remitting MS (RRMS) patients. In this double blind, randomized trial, 100 RRMS patients with EDSS<6 were allocated into 3 groups: "Group A" who received co-supplemented hemp seed and evening primrose oils with advising Hot nature diet, "Group B" who received olive oil as placebo, "Group C" who received the co-supplemented oils. Clinically EDSS and functional score as well as biochemical parameters (blood cells PUFA, FADS2, Serum sPLA2) were assessed at baseline and after 6 months. Mean follow-up was 180±2.9SD days (N=65, 23 M and 42 F aged 34.25±8.07 years with disease duration 6.80±4.33 years). There was no significant difference in studies parameters at baseline. After 6 months, significant improvements in EDSS and functional score were found in the group A and C while EDSS and pyramidal score showed significant increase in group B. Biochemical parameters showed improvement in the A and C groups whereas there was worsening condition for the group B after the intervention.The co-supplemented hemp seed and evening primrose oils with Hot nature diet can have beneficial effects in improving clinical symptoms and signs in RRMS patients which were confirmed by regulation of surface-membrane enzymes.

Key words: Relapsing Remitting Multiple sclerosis, Hot nature diet, Evening primrose (*Oenothera biennis* L), Hemp seed (*Cannabis sativa* L), PUFA, Delta-6-desaturase (FADS2), Phospholipase A2.

Multiple sclerosis (MS) is a relatively common disease with unknown etiology and no cure which results in neurological disability in young adults. This condition affects over two million people worldwide[1] and over 60,000 individuals in Iran[2]. Many of the current treatments are costly, limited in efficacy, and possess unpleasant side effects[3]. Although the exact etiology of developing MS is dependent on both genetic and environmental factors[4], pathological events such as impairment of T helpers (Th) are involved[5]. The major types of Th cells are Th1 cells that produce interferon-γ (IFN-γ), Th2 cells that produce interleukin-4 IL-4[6, 7] and Th1/ Th2 balance is considered one of the risk factors in MS etiology[8, 9,10]. IFN-β treatment shifts the immune

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response from the Th1 to Th2 pattern by enhancing the production of anti-inflammatory Th2 cytokines (ex. IL-4) and decreasing the production of pro-inflammatory Th1 cytokines (ex. IFN-γ).

Traditional Iranian Medicine (TIM) practiced in Iran and Cold and Hot natures (Mizadj) have been believed to exist in TIM and in many other traditional medical theories[11]. The study of Shahabi et al, on IL-4 / IFN-γ ratio showed that the tendency of the Hot nature people was to deviate toward Th2-like immune responses to a greater extent than of the Cold-nature people[12]. In this way, consumption of Hot-nature foods in a person suffering from an autoimmune disease with a deviation toward Th1 immune responses (such as MS) may be useful because they can accelerate warmth of nature and deviation toward Th2 immune responses[13]. Evidence was found that omega3-polyunsaturated fatty acids (ω3-PUFAs), can suppress IFN-γ production in MS patients[14], metabolism of PUFA is controlled by Phospholipase-A2 (PLA2)[15] that appears to play a fundamental role in cell injury in the central nervous system (CNS) as well as in the pathogenesis of MS-like and production of pro-inflammatory mediators. The PLA2 hydrolyzes phospholipids to release arachidonic acid (AA) which can mediate inflammation and demyelination, hallmarks of the CNS autoimmune disease MS[16]. A study showed that PLA2 concentration increased 6-fold in the urine of MS patients with active disease and 4-fold in patients in remission, regardless of immune-modulating therapy[17]. We supposed that combination of hemp seed oil (HSO) and evening primrose oil (EPO) [as co-supplemented oils] with Hot nature diet has effect reduction on pro-inflammatory cytokines and targets this key mechanism of disease and works like approved treatments. HSO has been used as a food /medicine in China for at least 3000 years[18]. It contains over 80% in PUFAs, with ω3/ ω6 ratio between 1:2 and 1:3, which is considered to be optimal for human health[19]. HSO contains phytosterols, terpenes and kinds of tocopherol that not only exhibits potent antioxidative properties for scavenging free radicals, but may also acts on specific signaling pathways for regulating inflammatory responses[20-23]. EPO is being used in increasing amounts in nutritional and pharmaceutical preparations, and may alleviate various chronic disease states[24-26]. Therefore, the co-supplemented oils with Hot nature diet may affect membrane phospholipids fatty acid composition, with increase cell membrane PUFAs, also effects of intervention appear to possess anti-inflammatory roles; and inhibit increase in pro-inflammatory cytokines and PLA2, and may represent novel therapeutic strategies against MS. This study is designed to evaluate effects of co-supplemented oils with Hot nature diet on clinical and biochemical parameters of RRMS patients.

MATERIALS AND METHODS

This double-blind, randomized clinical trial was carried out on 100 RRMS patients to determine the therapeutic and protective effects of Hot nature dietary and the co-supplemented oils. The study was approved by the Neurosciences Research Center (NSRC) and local Ethics Committee of Tabriz University of Medical Sciences. MS patients were contacted and recruited through the MS Society of Tabriz. Patients with a definite diagnosis of MS using the Kurtzke Extended disability status score (EDSS) <6 criteria[27]; with relapsing-remitting type of MS (RRMS); ages 14-55 years were enrolled. Patients with secondary or primary progressive MS, pregnancy, corticosteroid treatment, patients suffered concomitantly from another chronic disease such as rheumatic diseases, serious heart diseases, malignant tumors, and other neurological and inflammatory illnesses were excluded. Patients were allowed to continue their routine medications. A written informed consent was completed prior to the study for all patients. The patients completed a 3-d food record in the first week, a non-quantitative Food Frequency Questionnaires (nqFFQ) to assess food and drinks consumed and dietary habits. They were asked to maintain their usual level of physical activity and not to consume any supplements during the study. The patients were then randomly assigned to receive three dietary interventions:

Group A Those receiving the co-supplemented oils, 18-21g/day (6-7g, three times daily) with advising Hot nature diet,

Group B Those consuming olive oil 18-21g/

day (6-7g, three times daily),

Group C Those receiving the co-supplemented oils, 18-21g/day (6-7g, three times daily) for 6 months.

To achieve this objective, group A was asked to consume "Hot nature diet "with a wide choice of foods and drinks items permitted during each dietary period and delivered at home for 6 months (Appendix A). Groups B and C were asked to consume their usual diet during the intervention. "Hot nature diet"[13] includes foods with Hot nature, low intake of cholesterol, hydrogenated or trans fatty acids and saturated fats (fried foods), consumption of olive or grape seed oils as main oils in diet, eating plenty of fresh fruit and vegetables with Hot nature, nuts and seeds without additives, fish and seafood ,unrefined carbohydrates, drinking plenty of water (avoiding too much drink containing artificial additives, sweeteners or other stimulants), cutting down sugar and refined starch (i.e. non-whole meal bread, cakes, pastries, biscuits, sweets and soft drinks), consumption of dairy products with honey or date and removing foods with Cold nature (Appendix B)[13], avoid of alcohol and smoking. The patients were contacted monthly by telephone to assess compliance. After baseline assessments, 100 patients randomized to three groups according to following diagram (Fig. 1).

Fig 1. Flowchart of the study; 100 patients randomized to three groups; group A: Co-supplemented hemp seed and evening primrose oils and advising Hot nature dietary; group B: Olive oil; group C:Co-supplemented hemp seed and evening primrose oils.

All measures were repeated similarly with same approach and assessors at the end of intervention period.

Measurement of the disability status of patients

A medical history to check clinical status (inc. EDSS and functional score) and medications used were assessed. The functional disability status (disease severity) of each patient was measured by a trained clinician using the Kurtzke EDSS[27]. The EDSS quantifies disability in eight functional systems (FS) and allows neurologists to assign a functional system score (FSS) in each of them. The functional systems are pyramidal, cerebella, brainstem, sensory, bowel and bladder, visual, cerebral and "other". Scales for the total Kurtzke EDSS are from 0 to 10, in which the 0 score indicates no disability at all and 10 indicates death due to MS. Patients' Natures and Temperaments (Mizadj) was determined according to TIM by using a standard questionnaire[12]. The Warmth/ Coldness ratio was calculated for all patients based on the results obtained by the questionnaire[12].

Blood sample processing and analysis

Venous blood samples (10 ml) were collected from the patients before and 6 months after treatment The red blood cells (RBCs) were washed in a 0.85% saline solution and immediately transferred to small glass vials, layered with nitrogen, and stored up to one year at -80°C. Serum was separated and aliquots were stored at -80°C. Total lipids were extracted from RBCs with chloroform/methanol (1:2v/v), then fatty acids were separated from their alcohols and etherified by methanolysis to form fatty acid metyhyl esters (FAME). FAME were injected in gas chromatography and analysis composition to assess long-chain PUFAs[28,29]. Serum FADS2 (D6D) and PLA2 levels from serum were measured by enzyme-linked immunosorbent assay (ELISA) (Uscn Life Science Inc). The absorbance of D6D, PLA2 levels was read at 450 nm. Percentages of PUFAs were measured against an internal standard. RBC membrane PUFAs as µg FA/ml packed RBC analyzed.

Statistical analysis

The Statistical analysis was performed using SPSS software (ver 14.0; SPSS Inc, Chicago, IL). Data was expressed as mean ± standard deviation (SD). Differences in clinical and biochemical variables between pre- and post within each intervention group were analyzed using paired t-test. Statistical significance was defined as $p < 0.05$.

RESULTS

Clinical and biochemical results in RRMS patients

One hundred (34 M and 66 F) patients were enrolled in this study. Figure. 1 summarizes the patient attrition patterns in the study. The dropout rate was 35 from 100 patients (11 in group A, 11 in group B, and 13 in group C). This study was performed during between October 2010 and October 2011. The patients' characteristics and

demographics are shown in (Table 1). The sample consisted of 23 males and 42 females with a mean age of 34.25±8.07 years and mean disease duration of 6.80±4.33 years. There was statistically no significant difference in the mean age, gender, disease duration, interferon intake, and average age at onset between three groups.

Clinical and biochemical results

The clinical results of the trial are summarized in (Table 2). There were significantly

Table 1. Clinical and demographic characteristics of the study patients n=65 (23 men, 42 women)

Variable	Group A (N=23) Mean ± SD	Group B (N=22) Mean ± SD	Group C (N=20) Mean ± SD
Age(years)*	34.2±7.5	35.9±7.8	33.7±7.8
Average age at onset (years)*	25.0±7.5	30.3±8.1	27.6±6.4
Disease duration (years)*	6.26±3.9	7.55±5.08	6.60±4.0
	N (%)	N (%)	N (%)
Interferon intake	22(95.7)	22)100)	19(95)
Gender (M/F)	16/7	11/11	15/5

Group A: Co-supplemented hemp seed and evening primrose oils and advising Hot nature dietary
Group B: Olive oil
Group C: Co-supplemented hemp seed and evening primrose oils.

Table 2. Effect of intervention on mean (±SD) clinical variables: Mizadj (Warmth/Coldness ratio), Expanded disability status score (EDSS) and relapse rate in trial groups of RRMS patients comparison to baseline

Variables	Group A(N=23)			Group B (N=22)			Group B (N=20)		
	Baseline	6 months	P	Baseline	6 months	P	Baseline	6 months	P
Mizadj	1.05±.63	1.74±.88	0.001	.90±.47	.78±.43	0.116	1.01±.63	1.22±.8	0.017
EDSS	2.76±1.39	1.77±1.7	0.001	3.45±1.41	1.41±3.86	0.005	3.25±1.94	1.83±2.95	0.002

* P for paired-t test
Group A: Co-supplemented hemp seed and evening primrose oils and advising Hot nature dietary
Group B: Olive oil Group C: Co-supplemented hemp seed and evening primrose oils.

Table 3. Effect of intervention on mean (±SD) functional score in trial groups of RRMS pationts comparison to baseline

Variables	Group A(N=23)			Group B(N=22)			Group C(N=20)		
	Baseline	6 months	P	Baseline	6 months	P	Baseline	6 months	P
Pyramidal	.69±1.22	.61±.98	.328	1.32±1.25	1.68±1.21	.029	1.15±1.53	1.25±1.41	.494
Cerebella	.74±1.0	.52±.95	.233	1.04±1.13	1.14±1.08	.680	.95±.94	.70±1.03	.171
brainstem	.57±.89	.39±.72	.103	.68±,99	.81±1.03	.162	.55±1.09	.40±.88	.186
Sensory	.57±.89	.30±.70	.186	.91±1.34	.91±1.11	1.00	.80±1.01	.70±1.22	.606
Bowel/Bladder	1±1.31	.35±.57	.008	1.68±1.55	1.27±1.49	.071	1.21±1.36	1.25±1.37	.666
Visual	.96±1.29	.69±1.14	.208	1.32±1.70	1.45±1.50	.633	1.20±1.58	1.15±1.66	.748
Cerebral	.87±1.01	.43±.66	.009	1.05±.99	.82±1.01	.381	1.30±1.17	1.15±.93	.330
other Functional	.65±.49	.35±.49	.005	.86±.35	.73±.46	.186	.80±.41	.40±.50	.002

* P for paired-t test
Group A: Co-supplemented hemp seed and evening primrose oils and advising Hot nature dietary
Group B: Olive oil Group C: Co-supplemented hemp seed and evening primrose oils.

Table 4. Effect of intervention on mean (±SD) biochemical parameters: Delta-6-Desaturase (D6D), serum phospholipase A2 (PLA2) and polyunsaturated fatty acids (PUFAs) of total cell lipids extracted from blood cell phospholipids in trial groups of RRMS patients; comparison to baseline

Variables	Group A(N=23)			Group B(N=22)			Group C(N=20)		
	Baseline	6 months	P	Baseline	6 months	P	Baseline	6 months	P
PUFA	34.86±4.01	38.17±3	.013	36.1±4.31	36.29±3.78	.877	33.22±3.71	35.92±2.38	.028
D6D	.21±.008	.010±.005	.001	.012±.10	.039±.62	.166	.027±.017	.015±.010	.004
PLA2	.95±.94	.32±.36	.017	.69±.61	1.13±1.50	.302	.66±.84	.27±.34	.042

* P for paired-t test
**µg FA/ml
*** The absorbance of D6D, PLA2 levels was read at 450 nm.
Group A: Co-supplemented hemp seed and evening primrose oils and advising Hot nature dietary
Group B: Olive oil
Group C: Co-supplemented hemp seed and evening primrose oils.

better changes in Mizadj and EDSS in group A and C at the end of the intervention, while olive oil consumption resulted in a significant increase in EDSS. Based on results of (Table 3) globally all functions in groups A and C shows relating improving trend but only, there are statistically significant reduction in bowel/bladder, cerebral and "other functions" in group A and "other functional" scores in group C after 6 month intervention, whereas in group B there is relating trend to worsening in all functions, but only pyramidal function shows statistically significant increase. This results (Tables 2, 3) means that the co-supplemented oils with or without Hot nature diet used in our study might have a therapeutic effect towards MS. (Table 4) indicates that patients (baseline) have elevated serum level of PLA2 activities which might be due to increase

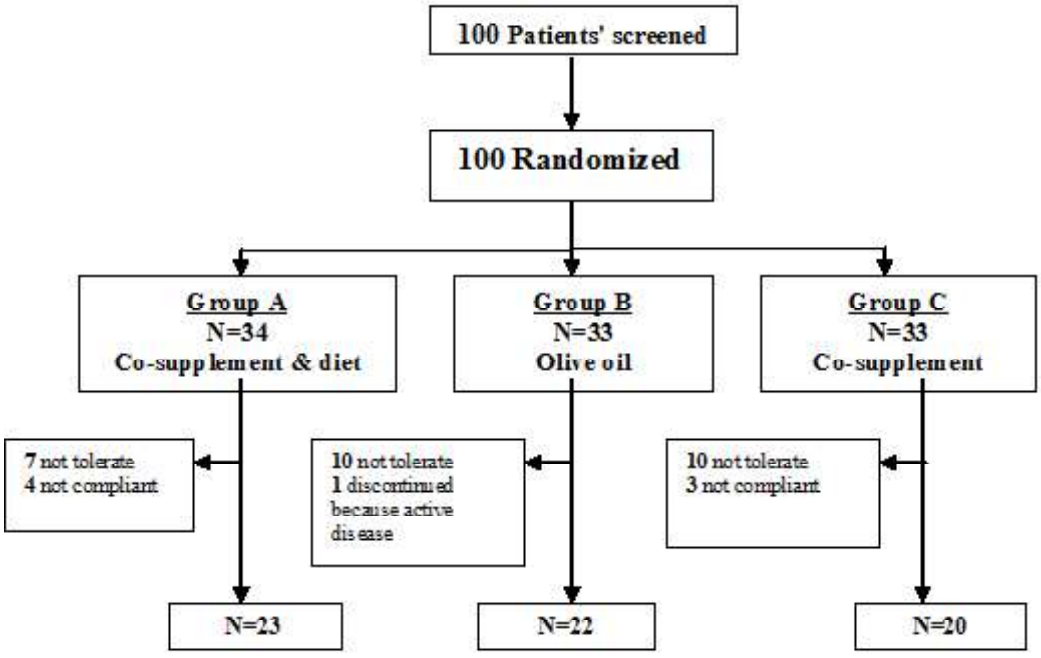

Fig. 1. Flowchart of the study; 100 patients randomized to three groups; group A: Co-supplemented hemp seed and evening primrose oils and advising Hot nature dietary; group B: Olive oil; group C:Co-supplemented hemp seed and evening primrose oils

hydrolysis of membrane phospholipids by PLA2 is a well-known early response to tissue damage in all organ systems including CNS. Mean of D6D, PLA2 and PUFAs concentrations were significantly different before and after consumption of the co-supplemented oils with or without Hot nature diet in groups A and C, respectively. Red blood cells PUFAs rate showed significant increase, while D6D and PLA2 concentrations decreased significantly in groups A and C. No significant changes were found in PUFAs, D6D and PLA2 in group B (Table 4). This result suggests that the observed reduction of D6D was a consequence of the well-described effects of this type of intervention and increase of PUFAs and reduction in expression of PLA2 key enzymes, caused a decrease in mean EDSS. Surprisingly, altering PUFA rate caused a decrease in PLA2 expression, in particular, in the co-supplemented oils and Hot nature diet group.

DISCUSSION

Possible mechanisms

The theory of "Hot and Cold natures" founds its origin from ancient Greece medicine by Hippocrates (Greek physician, 460-375 BC) and Galen (199-129 BC)[30, 31, 32]. Hippocrates says let's our diet be our medicine, and Avicenna said that for each person there is specific foods for himself. The most important rule of all the ancient theories was the maintenance of the balance between the fundamental body elements, among which Warmth and Coldness played a completely essential role[11,32]. To determine a persons' Warmth/Coldness (W/C) or Mizadj, it is observed that intermediate forms or combinations of two or more temperaments, so most people are under influence of both the Hot and the Cold elements[33] and we could evaluate the severity of each nature in a person by W/C ratio (Table 2). It means that in a person with a very Hot nature, W/C ratio is high (such as allergic patients with tendency to Th2-like responses) and in a person with a very Cold nature, W/C ratio is low (such as MS patients with tendency to Th1-like responses). Therefore, an allergen can induce allergic reaction in Hot nature persons with a higher than in cold nature persons, because the former have a greater tendency to Th2-like responses[34]. Based on studies, immune responses during infancy and early childhood are dominated by Th2 cytokines, but the shifting toward Th2 pattern decreases with age[35, 36], and an allergen can induce allergic reaction in child with a higher strength than adults. This is in agreement with TIM's belief that the nature is dominated by Warmth at birth but its Warmth decreases with age[11]. This critical point that we should indicate why MS attacks is observed in the start of adulthood age. Shahabi *et al*, showed the persons of a Hot nature had more deviation of the immune system toward Th2 responses than the persons of a Cold nature, and in agreement with TIM practitioners' view that MS (Th1-mediated autoimmune disease), is more prevalent in Cold nature persons than in Hot nature. This intervention is in agreement with the complications relating to Hot or Cold nature dominance and targets this key mechanism of disease and works like approved treatments. It may explain why therapies that promote a Th1 to Th2 cytokine-shift are beneficial in MS patients.

All approved therapies, besides many of those under investigation appear to possess immunomodulatory and anti-inflammatory roles as the main mechanism of action that Beta-interferons and Glatiramer acetate are on top of this list[37, 38,39]. Based on TIM practitioners' view that Hot nature foods is useful for MS patients, while Cold nature foods aggravates their disease, also women is dominated twice more than men by Cold nature and this confirms autoimmune diseases such as MS is mostly common in women more than men, parameters like weather coldness, lack of sun light exposure and stressful life enhance Coldness in subjects [13]. Increase of W/C ratio (Mizadj) agreeing with increase of EDSS and functional score parameters were significantly better in groups A and C compared to group B, and case groups felt physically and emotionally healthier (Tables 2, 3). A trend favoring in group A was maintained on EDSS and functional score until the end of the study for all measurements while, no therapy exists that can confer prolonged remission in MS and therapeutic agents are only partly effective. Their long-term beneficial effects are uncertain with side effects[40, 41]. Agreeing with above changes , dietary PUFA (co-supplemented oils) and their metabolites affect inflammatory functions and cytokines production during the 6 months, because ω3-

PUFAs, can suppress IFN-γ production in MS patients[14], prior studies have demonstrated a relation between MS mortality and dietary fat[42], and lipids can be found in two structural components; the neuronal membrane (about 50%) and the myelin sheath (about 70%) and a high proportion of lipid 70-85% [43] and the Blood-Brain Barrier (BBB) is a key to the bioavailability of brain essential fatty acid (EFA)[44]. Using chromatographic lipid profiling; we confirmed a significant increased in red blood cells PUFAs rate in groups A and C while in group B is no significant (Table 4).

The study showed that daily administration of the co-supplemented oils has beneficially affected the PUFAs of total cell lipids extracted from blood cell phospholipids. In addition, the PUFAs rate was correlated with the EDSS and functional score benefits at the last visit. This results likely due to remyelination that occurs during the early phases of disease; whereas this is rare at more progressed stages[45]. Current estimates of the ω3/ω6 PUFAs ratio in developed countries are as low as 1:25 with recommendations to the public that it should be much higher (ideally 1:4)[46]. The ω6/ω3 ratio in HSO is normally between 2:1 and 3:1, which is considered optimal for human health[18]. Horrobin showed that preliminary results the use of EPO and colchicine combined therapy in MS patients suggest that may be of considerable value[47]. EPO is being used in increasing amounts in nutritional and pharmaceutical preparations, and there are claims that it may alleviate various chronic disease states [24, 25, 26]. The EPO content of 9% GLA is the single most important parameter that is metabolized into DGLA, the natural precursor of PGE. β-Carotene is a pro-vitamin A and gives a characteristic color to EPO [48,49,50]. The HSO / EPO ratio in this study is 9:1, so ω3/ω6 PUFAs ratio reach to 1:4 or higher, that is competitive inhibition of the conversion of dihomo-gamma-linolenic acid (DGLA) to arachidonic acid (AA) resulting in more anti-inflammatory prostaglandin E1(PGE1)[51]. AA is a precursor of pro-inflammatory and pro-aggregator (PGE2), while gamma-linolenic acid (GLA) and DGLA are precursors of anti-inflammatory PGE1 (Appendix E). GLA is produced in the body from desaturation of linolenic acid (LA) by the reaction catalyzed by enzyme delta-6-desaturase (D6D or FADS2). D6D is the rate-limiting step in the PUFAs biosynthetic pathways that are incorporated into cell membranes, which thereby affect permeability, and functional properties of cells. The D6D deletion may prevent the conversion of alpha-linolenic acid (ALA) into very long chain-PUFAs[52, 53]. Based on studies, the activity of D6D, which had become impaired by: aging, diabetes, viral infection, high alcohol intake, high level cholesterol ,high blood pressure, radiation ,stress-related hormones, deficiencies of zinc ,magnesium, biotin, vitamins :C, B6,B3 and excessive level of trans fatty acid[54,55,56], genetic deficient (inactive D5D and D6D enzymes)[57]. Moreover, excessive consumption of GLA occurs in: high rates of cell division, inflammatory, antiviral reaction and Trauma[24].

The noticeable presence of both GLA and stearidonic acid (SDA) in HS and EP oils, typically at a favorable ω6/ω3 ratio of 2:1 allows this enzymatic step with D6D to be efficiently bypassed[58]. Because the produced result of this enzyme (GLA and SDA) is delivered to the patient organism by the co-supplemented oils in this trial, in this way, D6D concentration decreased significantly in groups A and C, while group B showed a non significant (Table 4). These mentioned basic parameters for cellular metabolic pathways could easily replace to this intervention in groups A and C. It is a strong reason for importance of the effects of diet and various nutrients for modulation in developed wrong disordered metabolic interaction in the metabolism of MS patients. In considering the inflammatory role of secretory PLA2 (sPLAs), phospholipids constitute approximately 40%, 60% and 90% of the total lipids in myelin, erythrocyte and mitochondrial, respectively, that play a role in double bio-membrane structure[59,60]. Metabolism of PUFA in membrane phospholipids is controlled by PLA2 and acyltransferases known as the "deacylation-reacylation cycle"[61].

Evidence showed that sPLA2 involvement in diverse inflammatory conditions, implicating almost all of membranes in any organ of the body (such as myelin, erythrocyte and mitochondrial)[62]. The PLA2 hydrolyzes phospholipids to release AA which can mediate inflammation [63]. The AA liberated is converted to PGE2, possibly by cyclooxygenase-2 (COX-2), which is induced by inflammatory stimuli[64], toxicity of AA was associated with increased lipid

peroxidation and mitochondrial damage[65], and may contribute to both acute forms of apoptosis and delayed inflammation induced tissue degeneration, finally demyelination that hallmarks of the CNS autoimmune disease MS[66]. Mean levels of sPLA2 were increased 6-fold in the urine of MS patients with active disease and 4-fold for patients in remission, regardless of immune-modulating therapy[17]. For this reason, PLA2 appears to play a fundamental role in cell injury in the CNS, and plays a key role in the pathogenesis of MS-like with production of pro-inflammatory mediators[67]. However, up till now there are no effective sPLA2 or cPLA2 inhibitors available for clinical use[68], but extracellular PLA2 inhibitors suppress CNS inflammation[69]. In this way, inhibition of specific PLA2 and elevated levels of inflammatory cytokines may represent novel therapeutic strategies against MS. We found that elevated serum of PLA2 activity in the patients (baseline) which is a well-known early response to tissue damage in all organ systems including erythrocyte, myelin in CNS and mitochondrial ,etc. After study, PLA2 concentration decreased significantly in groups A and C and estimated PLA2 and D6D were both inversely correlated with PUFAs and these parameters in B group showed a non significant (Table 4).

The above findings imply that compared to olive oil, the co-supplemented oils with Hot nature diet produced a significant reduction in clinical symptoms and signs, and the patients general health and well-being improved that due to evidences on base higher PUFA in peripheral tissue (red blood cells) and maybe in brain tissue and mitochondrial, etc. These results support the hypothesis of EFA abnormalities in MS patients, and indicate that the problem could well be one of conversion of EFA to LC-PUFA (according to: appendix C), as originally suggested before. It is important to note that supplementation with PUFAs may require additional vitamin E intake to prevent increased peroxidation of membrane lipids[70], while the total amount of tocopherols of (α-, β-, γ-tocopherol, δ-tocopherol) HSO is high between 80 and 110 mg/100 g, with γ-tocopherol as the main tocopherol (85%) that exhibit potent antioxidative properties for scavenging free radicals[20]. We must notice that the co-supplemented oils are foodstuffs and do not act as rapidly as most medications, so any effects will take time to appear. It means, most patients who respond to supplementation usually report noticeable benefits within one or two months. The minimum trial period should be at least six months, as studies have shown that it takes 10-12 weeks for PUFA levels in brain cell membranes to return to normal levels after a long-standing deficiency[71]. In this intervention, minor adverse events was rare and much less than for medication commonly prescribed for RRMS patients and on recommendation, the co-supplemented oils might be given alone or during treatment with synthetic drugs to permit reduction of dose level of the later, and can administer orally.

CONCLUSION

Regarding the beneficial properties of this intervention, effects co-supplemented oils and Hot nature diet appear to possess anti-inflammatory roles; and have prophylactic and therapeutic properties in improving clinical symptoms and signs in RRMS patients which were confirmed by regulation of surface-membrane enzymes. Confirmation of importance of Warmth or Coldness of foods was advocated by many traditional medical theories, maybe one of the other conclusions of the present study.

Limitations

The limited duration of the intervention caused by budget limitation and consumption of the Co-supplemented oils as syrup is the other important one. So we would not able to encapsulate the supplement for patients' consumption by a protective coating or membrane, the high prevalence of dropout in this study is caused by the mentioned parameters. Uncontrolled diet is the other important confounding factor.

Future directions

1. Based on studies the use of co-supplemented hemp seed and evening primrose oils (8:2 ratio is better, which is considered to be optimal for human health) either alone or in conjunction of different immunomodulation therapy synthetic drugs (which may have synergistic effects with each other) for longer periods in MS patients.
2. Provide a rationale for performing additional functional studies on the D-6-desaturase (FADS1) and D-5-desaturase (FADS2) gene transcription in all groups of MS patients and healthy adults, because, a strong correlation

between MS and a rapid fall of delta-6-desaturase activity has been shown after intervention.

3. Hot nature diet and Supplementing with hemp seed and evening primrose oils may prevent several inflammatory diseases and neurodevelopmental and neurodegenerative disorders, because the changes in lipid biology identified in MS may be relevant to other psychiatric conditions.

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REFERENCES

1. Mao P, Reddy PH. Is multiple sclerosis a mitochondrial disease? *Biochimica et Biophysica Acta* 2010; **1802**: 66-79.
2. Iranian MS Forum. Increasing prevalence of MS,a not solved puzzle.2012.available at http://www.ms-links.org/2012/03/blog-post
3. Lublin F. History of modern multiple sclerosis therapy. *J Neurol* 2005; 252.
4. Pryse-phillips w and Sloka JS. Etiopathogenesis and Epidemiolgy:Clues to Etiology.in:Handbook of multiple sclerosis(cook SD ed),Taylor & francis Group, 2006; New york:1-39.
5. Minagar A,Alexander JS. Blood-brain barrier disruption in multiple sclerosis. *Mult Scler* 2003; **9**: 540-549.
6. Fukaura H, Kent SC, Pietrusewicz MJ, Khoury S J, Weiner HL, Hafler, DA. Induction of circulating myelin basic protein and proteolipid protein-specific transforming growth factor-beta1-secreting Th3 T cells by oral administration of myelin in multiple sclerosis patients. *J. Clin. Invest* 1996; **98**: 70-77.
7. Hafler DA, Kent SC, Pietrusewicz MJ, Khoury SJ, Weiner HL, Fukaura H. Oral administration of myelin induces antigen-specific TGF-beta 1 secreting T cells in patients with multiple sclerosis. *Ann. N. Y. Acad, Sci*. 1997; **835**:120-231.
8. McKenzie BS, Kastelein RA, Cua DJ. Understanding the IL-23-IL-17 immune pathway. *Trends Immunol* 2006; **27**:17-23.
9. Harrington LE, Hatton RD, Mangan PR. Interleukin17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat Immunol* 2005; **6**:1123-1132.
10. Park H, Li Z, Yang XO. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin-17. *Nat Immunol* 2005; **6**:1133-1141.
11. Avicenna. The Cannon of Medicine, 6 ed. Tehran: Sorush Publisher, 2004, [In Persian].
12. Shahabi S, Muhammad Hassan Z, Mahdavi M, Dezfoli M, Torabi Rahvar M, Naseri M, Hosseni Jazani N, Khalkhali HR. Hot and Cold Natures and Some Parameters of Neuroendocrine and Immune Systems in Traditional Iranian Medicine: A Preliminary Study. *The Journal of Alternative and Complementary Medicine* 2008; **14**(2): 147-156.
13. Mirzaei H. Multiple sclerosis. [In Persian]. Online document at: www.dr.myblog.ir/Post-1256.ASPX, 2010; Accessed June 25.
14. Gallai V, Sarchielli V, Trequattrini A, Franceschini M, Floridi A, Firenzi C. Cytokine secretion and eicosanoid production in the peripheral blood mononuclear cells of MS patients undergoing dietary supplementation with omega-3 fatty acids. *J Neuroimmunol* 1995; **56**:143-53.
15. Sun GY, Xu J, Jensen MD, Simonyi A. Phospholipase A2 in the central nervous system: implications for neurodegenerative diseases, *Journal of Lipid Research* 2004; **45**: 205-213.
16. Kalyvas A, Baskakis C, Magrioti V, Constantinou-Kokotou V, Stephens D, López-Vales R, Lu Jian-Q, Wee Yong V, Dennis EA, Kokotos G, Samuel D. Differing roles for members of the phospholipase A2 superfamily in experimental autoimmune encephalomyelitis. *Brain* 2009; **132**(5):1221-1235.
17. Cunningham TJ, Yao L, Oetinger M, Cort L, Blankenhorn EP , Greenstein JI. Secreted phospholipase A2 activity in experimental autoimmune encephalomyelitis and multiple sclerosis. *Journal of Neuroinflammation* 2006; **3**: 26.
18. De Padua LS, Bunyaprafatsara N, Lemmens RHMJ. Plant Resources of South-East Asia. *Medicinal and Poisonous Plants* 1999; **1**(12): 167-175.
19. Simopoulos AP, Leaf A, Salem N. Workshop statement on the essentiality of and recommended dietary intakes from omega-6 and omega-3 fatty acids. *Prostaglandins Leukot Essent Fatty Acids,* 2000; **63**:119-21.
20. Matthaus B, Brühl L. Virgin hemp seed oil: An interesting niche product, *Eur. J. Lipid Sci.*

Technol 2008; **110**: 655-661.
21. Hendriks H, Malingre TM, Batterman S, Bos R. The essential oil of *Cannabis sativa* L. *Pharmaceutisch Weekblad* 1978; **113**: 413-424.
22. Nissen L, Zatta A, Stefanini I, Grandi S, Sgorbati B , Biavati B , Monti A . Characterization and antimicrobial activity of essential oils of industrial hemp varieties (*Cannabis sativa* L.). Fitoterap , 2009; 7.
23. Oomah BD, Busson M, Godfrey DV, Drover JCG. Characteristic of hemp (*Cannabis sativa* L.) seed oil. *Food Chem* 2002; **76**: 33-43.
24. Horrobin DF. Nutritional and medical importance of GAMA-Linolenic acid ,prog.*lipid Res* 1992; **2**:163-194.
25. Huang, YS, Mills DE. Linolenic Acid: Metabolism and its Roles in Nutrition and Medicine. AOCS Press, Champaign 1995; IL, USA.
26. Fan YY, Chapkin RS. Importance of dietary glinolenic acid in human health and nutrition? *J. Nutr* 1998;**128**: 1411-1414.
27. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an Expanded Disability Status Scale (EDSS). *Neurology* 1983; **33**:1444-1452.
28. Van Jaarsveld PJ, Smuts CM, Tichelaar HY, Kruger M, Benadé AJS. Effect of palm oil on plasma lipoprotein concentrations and plasma low-density lipoprotein composition in non-human primates. *Int J Food Sci Nutr* 2000; **51**: S21-S30.
29. Folch J, Lees M, Sloane-Stanley GH .A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 1957; **226**: 497-509.
30. Chiappelli F, Prolo P, Cajulis OS. Evidence-based research in complementary and alternative medicine History. *Evid Based Complement Alternat Med* 2005; **2**:453-458.
31. Ody P. The Complete Medicinal Herbal. New York: DK Publication 1993.
32. Ott J. Pharmacophilia, or the Natural Paradise. Kennewick, WA: The Natural Products 1997; Co; 47-62.
33. Abduvaliev AA. Modern views on the theory of nature (mizadj) by ibn sina in medicine. *Lik Sprava* 2003; **3-4**:102-105.
34. Abbas AK, Lichtman AH. Cellular and Molecular Immunology, 5 ed. Philadelphia: Saunders 2003.
35. Adkins B, Bu Y, Guevara P. The generation of the memory in neonates versus adults: Prolonged primary Th2 effector function and impaired development of th1 memory effector function in murine neonates. *J Immunol* 2001; **166**(2): 918-925.
36. Holt PG, Jones CA. The development of the immune system during pregnancy and early life. *Allergy* 2000; **55**: 688-697.
37. Yong VW,Chabot S,Stuve O,Williams G. Interferon beta in the treatment of multiple sclerosis:mechanisms of action. *Neurology* 1998; **51**: 682-689.
38. Rieks M,Hoffmann V,Aktas O,Juschka M,Spitzer I,Brune N,Schimrigk S,Przuntek H,Pohlau D. Induction of apoptosis of CD4+ Tcells by immuno-modulatory therapy of multiple sclerosis with glatiamer acetate. *Eur Neurol.* 2003; **50**:200-206.
39. Lindsey JW. EAE:History,Clinical signs,and DISEASE Course,in Experimental Models of Multiple Sclerosis. Springer 2005.
40. Johnson KP, Brooks BR, Cohen JA, Ford CC, Goldstein J, Lisak RP, Myers LW, Panitch HS, Rose JW, Schiffer RB. Copolymer 1 reduces relapse rate and improves disability in relapsing-remitting multiple sclerosis: results of a phase III multicenter, double-blind placebo-controlled trial. The Copolymer 1 Multiple Sclerosis Study Group. *Neurology* 1995; **45**: 1268-1276.
41. Filippini G, Munari L, Incorvaia B, Ebers GC, Polman C, D'Amico R ,Rice GP. Interferons in relapsing remitting multiple sclerosis: a systematic review. *Lancet* 2003; **361**: 545-552.
42. Esparza ML, Sasaki S & Kesteloot H. Nutrition, latitude, and multiple sclerosis mortality: an ecologic study. *Am. J. Epidemiol* 1995; **142**: 733-737.
43. Morrell P, Quarles RH. Myelin formation, structure and biochemistry. *Philadelphia*, 1999; 69-93.
44. Yehuda S, Rabinovitz S, Mostofsky DI. Essential fatty acids and the brain: From infancy to aging, *Neurobiology of Aging* 2005; **26**S: S98-S102.
45. Lassmann H. Mechanisms of demyelination and tissue damage in multiple sclerosis. *Acta Neurol. Belg* 1999; **99**: 6-10.
46. Delaleu N, Immervoll H, Cornelius J, Jonsson R. Biomarker profiles in serum and saliva of experimental Sjogren's Syndrome: associations with specific autoimmune manifestations. *Arthritis Res Ther* 2008; **10**: R22.
47. Christie WW., The analysis of evening primrose oil, *Industrial Crops and Products* 1999; **10**: 73-83.
48. Horrobin DF., Multiple sclerosis: The rational basis for treatment with colchicine and evening primrose oil. *Medical Hypotheses* 1979; **5**: 365-378
49. Pruthi S, Wahner-Roedler DL, Torkelson CJ. Vitamin E and evening primrose oil for

management of cyclical mastalgia: a randomized pilot study. *Altern Med Rev* 2010; **15**(1): 59-67.
50. Jantti J, Evening primrose oil and olive oil in the treatment of rheumatoid arthritis. *Clinical Rheumatology* 1989; **8**: 238-244.
51. Milijanovic B, Trivedi K, Dana M, Gilbard J, Buring J, Schaumberg D. Relation between dietary n-3 and n-6 fatty acids and clinically diagnosed dry eye syndrome in women. *Am J Clin Nutr* 2005; **82**: 887-93.
52. Baylin A, Ruiz-Narvaez E, Kraft P, and Campos H. Linolenic acid, D6-desaturase(FADS2) gene polymorphism, and the risk of nonfatal myocardial infarction. *Am J Clin Nutr*, 2007; **85**: 554-560.
53. Nakamura MT, Nara TY. Structure, function, and dietary regulation of delta6, delta5,and delta9 desaturases. *Annu.Rev Nutr* 2004; **24**: 345-376.
54. Horrobin DF.Gamma-linolenic acid: An intermediate in essential fatty acid metabolism with potential as an ethical pharmaceutical and as a food. *Rev Contemp Pharmacother* 1990; **1**: 1-45.
55. Horrobin DF. Importance of g-linolenic acid. *Prog Lipid Res* 1992; **31**: 163-194.
56. Bates CE. Racially Determined Abnormal Essential Fatty Acid and Prostaglandin Metabolism and Food Allergies Linked to Autoimmune, Inflammatory, and Psychiatric Disorders among Coastal British Columbia Indians. *Medical Hypotheses* 1988; **25**: 103-409.
57. Okuyama H, Kobayashi T, Watanabe S. Dietary fatty acidsthe N-6/N-3 balance and chronic elderly diseases. Excess linoleic acid and relative N-3 deficiency syndrome seen in Japan. *Prog Lipid Res* 1997; **3**: 409-457.
58. Laule C, Vavasour IM, Kolind SH, Li DKB, Traboulsee TL, Moore GRW, MacKay AL. Magnetic Resonance Imaging of Myelin. *The Journal of the American Societ* 2007; **4**: 460-484.
59. DiMauro S, Hirano M. Mitochondrial encephalomyopathies: an update. *Neuromuscul. Disord*. 2005; **15**(4): 276-286.
60. Sun AY, Wang Q, Simonyi A, Sun GY.Botanical phenolics and brain health. *Neuromolecular Med* 2008; **10**(4): 259-274.
61. Yedgar S, Cohen Y, Shoseyov D. Control of PLA2 activities for the treatment of inflammatory conditions. *Biochimica et Biophysica Acta.* 2006; **1761**: 1373-1382.
62. Kalyvas A , Samuel D. Cytosolic Phospholipase A2 Plays a Key Role in the Pathogenesis of Multiple Sclerosis-like Disease. *Neuron* 2004; **41**: 323-335.
63. Balboa MA, Varela-Nieto I, Lucas KK, Dennis EA. Expression and function of phospholipase A2 in brain. *FEBS Letters* 2002; **531**: 12-17.
64. Caro AA, Cederbaum AI . Role of cytochrome P450 in phospholipase A2- and arachidonic acidmediated cytotoxicity. *Free Radical Biology & Medicine* 2006; **40**: 364 - 375.
65. Kalman B, Laitinen K, Komoly S. The involvement of mitochondria in the pathogenesis of multiple sclerosis. *Journal of Neuroimmunology* 2007; **188** :1-12.
66. Kalyvas A , Samuel D. Cytosolic Phospholipase A2 Plays a Key Role in the Pathogenesis of Multiple Sclerosis-like Disease. *Neuron*, 2004; **41**: 323-335.
67. Thwin MM, Satyanarayanajois, SD, Nagarajarao LM, Sato K,Pachiappan A, Satish LR, Kumar PV, Gopalakrishnakone P. Novel peptide inhibitors of human secretory phospholipase A2 with antiinflammatory activity: solution structure and molecular modeling. *J. Med. Chem*. **50**: 5938-5950. *Neuromolecular Med*, 2007; **10**(4): 259-274.
68. Pinto F, Brenner T, Dan P, Krimsky M, Yedgar S. Extracellular Phospholipase A2 Inhibitors Suppress Central Nervous System Inflammation. *GLIA*. 2003; **44**: 275-282.
69. Meydani M. Effect of long-term fish oil supplementation on vitamin E status and lipid peroxidation in women. *J Nutr* 1991; **121**: 484-91.
70. Bourre JM, Durand G, Pascal G, Youyou A. Brain cell and tissue recovery in rats made deficient in n-3 fatty acids by alteration of dietary fat. *J Nutr* 1988; **119**: 15-22.
71. Gultuna S Koklu S,Yuksel l,Basar O,Uskudar O. Interferon- ?1b augments pulse steroid-Associated hepatoxicity -Hepatitis Monthly. *Autumn* 2008; **8**(4): 317-318.