



# Alteration of delta-6-desaturase (FADS2), secretory phospholipase-A2 (sPLA2) enzymes by Hot-nature diet with co-supplemented hemp seed, evening primrose oils intervention in multiple sclerosis patients



Soheila Rezapour-Firouzi<sup>a,b,g,\*</sup>, Seyed Rafie Arefhosseini<sup>b,c</sup>,  
Mehrangiz Ebrahimi-Mamaghani<sup>b</sup>, Behzad Baradaran<sup>d</sup>, Elyar Sadeghihokmabad<sup>a</sup>,  
Somaiyeh Mostafaei<sup>a</sup>, Mohammadali Torbati<sup>e</sup>, Mahtaj Chehreh<sup>f</sup>

<sup>a</sup> Neurosciences Research Center, University of Medical Sciences at Tabriz, Iran

<sup>b</sup> School of Nutrition and Health, University of Medical Sciences at Tabriz, Iran

<sup>c</sup> Nutrition Research Center, University of Medical Sciences at Tabriz, Iran

<sup>d</sup> Immunology Research Center, University of Medical Sciences at Tabriz, Iran

<sup>e</sup> Department of Food Science and Technology Faculty of Nutrition, Food & Drug Organization, University of Medical Sciences at Tabriz, Iran

<sup>f</sup> Islamic Azad University, Tabriz, Iran

<sup>g</sup> Department of Immunology, Microbiology and Genetics, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran

## ARTICLE INFO

### Article history:

Received 6 September 2014

Received in revised form 26 April 2015

Accepted 5 July 2015

Available online 17 July 2015

### Keywords:

Relapsing remitting multiple sclerosis (RRMS)

*Oenothera biennis* L.

*Cannabis sativa* L.

Polyunsaturated fatty acid (PUFA)

Delta-6-desaturase (FADS2)

Secretory phospholipase A2 (sPLA2)

## ABSTRACT

**Background:** The effect of nutrition and dietary supplements as environmental factors has been suggested as possible factors affecting both disease risk and progression in on the course of multiple sclerosis with complex genetic-risk profiles. This study was aimed to assess regulation of surface-membrane enzymes such as Delta-6-desaturase (FADS2), secretory Phospholipase A2(sPLA2) by hemp seed and evening primrose oils as well as Hot-natured dietary intervention in relapsing remitting multiple sclerosis (RRMS) patients.

**Methods and materials:** In this double blind, randomized trial, 100 RRMS patients with Extended disability status score (EDSS) <6 were allocated into 3 groups: "Group A" who received co-supplemented hemp seed and evening primrose oils along with advised Hot nature diet; "Group B", who received olive oil; "Group C", who received the co-supplemented oils. Clinically EDSS and functional score as well as biochemical parameters [blood cells polyunsaturated fatty acid (PUFA), FADS2, sPLA2] were assessed at baseline and after 6 months.

**Results:** Mean follow-up was  $180 \pm 2.9$ SD days ( $N = 65$ , 23 M and 42 F aged  $34.25 \pm 8.07$  years with disease duration  $6.80 \pm 4.33$  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 groups A and C while EDSS and pyramidal score showed significant increase in group B. Alteration of biochemical parameters showed improvement in groups A and C whereas there was worsening condition for group B after the intervention.

**Conclusion:** 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.

© 2015 Elsevier Ltd. All rights reserved.

**Abbreviation:** AA, arachidonic acid; ALA, alpha- linolenic acid; BBB, blood-brain barrier; CNS, central nervous system; COX-2, cyclooxygenase-2; D6D (FADS2), delta-6-desaturase; D5D (FADS1), delta -5-desaturase; DGLA, dihomo-gammalinolenic acid; DHA, docosa-hexanoic acid (key omega-3); EDSS, extended disability status score; EP, evening primrose; EPO, evening primrose oil; FA, fatty acid; FDA, food and drug administration; FR, food records; FS, functional system; FSS, functional system scores; GLA, gamma linoleic acid; HS, hemp seed; HSO, hemp seed oil; IFN, interferon (B1b-B1a-B); IFN-  $\gamma$ , interferon-  $\gamma$ ; IL, interleukin (IL-2 IL-4 IL-10 IL-17); LA, linoleic acid (omega-6 family); LC- PUFA, long chain- polyunsaturated fatty acid; MS, multiple sclerosis; NSRC, neurosciences research center; nqFFQ, non-quantitative food frequency questionnaires; PGE, prostaglandin (E1, E2, E3); PLA2, phospholipase A2; PPMS, primary progressive multiple sclerosis; PRMS, progressive relapsing Multiple sclerosis; PUFA, polyunsaturated fatty acid;  $\omega$ 3-PUFAs, omega3-polyunsaturated fatty acids; RBCs, red blood cells; RRMS, relapsing remitting multiple sclerosis; SDA (STA), stearidonic acid; sPLA2, secretory PLA2s; SPMS, secondary progressive multiple sclerosis; Th, T helper (1–2–17); USFA, unsaturated fatty acid.

\* Corresponding author at: Department of Immunology, Microbiology and Genetics, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran. Tel: +98 9144066938/4133827931.

E-mail addresses: [s.firozi@gmail.com](mailto:s.firozi@gmail.com) (S. Rezapour-Firouzi), [arefhosseini@tbzmed.ac.ir](mailto:arefhosseini@tbzmed.ac.ir) (S.R. Arefhosseini), [ebrahimimamaghani@tbzmed.ac.ir](mailto:ebrahimimamaghani@tbzmed.ac.ir) (M. Ebrahimi-Mamaghani), [Behzad\\_im@yahoo.com](mailto:Behzad_im@yahoo.com) (B. Baradaran), [aeass@yahoo.com](mailto:aeass@yahoo.com) (E. Sadeghihokmabad), [somaiyehmostafaei@yahoo.com](mailto:somaiyehmostafaei@yahoo.com) (S. Mostafaei), [drtorbati@yahoo.com](mailto:drtorbati@yahoo.com) (M. Torbati), [mchehreh@yahoo.com](mailto:mchehreh@yahoo.com) (M. Chehreh).

<http://dx.doi.org/10.1016/j.ctim.2015.07.003>

0965-2299/© 2015 Elsevier Ltd. All rights reserved.

## 1. Introduction

Multiple sclerosis (MS) is a chronic neurological disease that is a major cause of disability in young adults in Iran.<sup>1</sup> Although the exact etiology of developing MS is dependent on both genetic and environmental factors,<sup>2</sup> pathological events such as impairment of T helpers (Th) are involved.<sup>3</sup> Rezapour-Firouzi et al. showed that Th2/ Th1 imbalance is accompanied with alteration of *Mizadj* (*Mizadj*: degree of Warmth/Coldness or degree of Th2/Th1 or degree of Interleukin-4(IL-4)/Interferon-  $\gamma$  (IFN-  $\gamma$ ) as risk factors in MS etiology are controlled by co-supplemented hemp seed and evening primrose oils with Hot-natured diet.<sup>4</sup> Epidemiological studies have demonstrated a relation between MS mortality and dietary fat. Lipids serve important functions as membrane phospholipids constituents'. Furthermore; there is evidence that polyunsaturated fatty acids PUFAs composition in co-supplemented hemp seed and evening primrose oils can suppress IFN- $\gamma$  production in relapsing remitting multiple sclerosis (RRMS) patients.<sup>4</sup> Metabolism of PUFA is controlled by secretory phospholipase-A2 (sPLA2)<sup>5</sup> 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 sPLA2 hydrolyzes phospholipids to release arachidonic acid (AA), which can mediate inflammation and demyelination, hallmarks of the CNS autoimmune disease MS.<sup>6</sup> 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.<sup>7</sup> Hemp seed oil (HSO) contains gamma Linoleic acid (GLA) as well as evening primrose oil (EPO) contains stearidonic acid (SDA), the combination of these oils as a dietary supplement has a potential to reduce prostaglandin E2 (PGE2) and pro-inflammatory cytokines TH1 which targets this key mechanism of the disease and works like approved treatments.<sup>4</sup> GLA and SDA are produced in the body from desaturation of linolenic acid (LA) and alpha- linolenic acid (ALA) 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, thereby affecting permeability and functional properties of cells. The D6D deletion may prevent the conversion of LA and ALA into very long chain-PUFAs.<sup>8,9</sup>

Therefore, we designed a study to investigate the effects of a 9:1 combination HSO with EPO as a supplement to a Hot-nature diet in comparison to the 9:1 combination of HSO with EPO without a special diet and olive oil in the third group, that may affect the composition of membrane phospholipids fatty acids by alteration of D6D activity; also, the effects of intervention appear to possess anti-inflammatory roles by inhibiting the elevation of sPLA2, and may represent novel therapeutic strategies against MS.

## 2. 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 diet 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,<sup>10</sup> with relapsing- remitting type of MS (RRMS), aged 14–55 years were enrolled. These patients had enough ability to present by themselves in EMAM REZA hospital, patients had interviewed by learned interviewers, nutritionists and neurologists. All process for each patient take more or less one hour and a written informed consent was completed prior to the study for each patient in the

**Table 1**

Permissible foods (Hot nature diet) for Group A.

|                                                                                                                                                                                                                                                                                                                            |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Cereals and grains:</b> wheat bread –beans-peas-cotyledon –soya products– wheat germ in soup- the low rate of catena & rice – macaroni with Soya or mutton-dry wheat germ- macaroni-variety of wheat bread without adding- potatoes puree with milk - forage of meat or HALIM-the low rate of rice- wheat germ porridge |
| <b>Meat and eggs:</b> goose – turkey- mutton-veal-quail –domestic poultry and rooster-shrimp-liver-heart-tongue-brain-variety of south and north fishes (salt water fishes)-caviar-white of the egg                                                                                                                        |
| <b>Dairy products:</b> fresh milk honey –cream honey –kinds of cheese with walnut or dates- concentrate yogurt–shallot mixed concentrate yogurt- Kefir yoghurt                                                                                                                                                             |
| <b>Fats:</b> olive oil-grape seed oil-sesame oil                                                                                                                                                                                                                                                                           |
| <b>Sweets:</b> grape juice–brown sugar–sugar candy with rose water-honey-sesame pudding, SAMANO(it is a kind dish with juice of germinating wheat or malt mixed with flour)-rose jam- orange flower jam-walnut jam                                                                                                         |
| <b>Vegetables:</b> kinds of cabbage-shallot-celery-tarragon-mint-cress- radish- garlic-onion–carrot-kinds of pepper- sweet basil –corrugated variety of cucumber –clover-fenugreek-coriander-savory -grape leaves–turnip–dill–mushroom–pumpkin–spring onion–pennyroyal–parsley–beetroot–eggplant–tomato(low)               |
| <b>Fruits:</b> cantaloupe-olive-black olive-grape-fig-sweet pomegranate- cherry–dates–coconut–banana–mango–pine apple–quince–apple–berry–pear-melon-sweet citreous grapes-                                                                                                                                                 |
| <b>Spice:</b> turmeric–mustard–cinnamon–caraway–ginger–saffron–green cardamom–pepper–vanilla–cocoa powder- nigella seeds- tomato paste (low)-pomegranate paste (low) –lemon juice (low)                                                                                                                                    |
| <b>Nuts:</b> various nuts without additives –pea nut-Indian almond – walnut- sweet almond–pistachio–hazel nut- Soya nut without additives - sun dried grapes–sun flower seeds–sun dried apricot –sesame-melon seed–linseed –pumpkin seed–water melon seed                                                                  |
| <b>Drinks:</b> tea–green tea- the tail of some vegetable- cool drinking with sweet basil –orange flower –mint- fennel- sweet basil- alfalfa bee balm and borage water                                                                                                                                                      |
| <b>Different kind of foods prepared by traditional methods of the above materials</b>                                                                                                                                                                                                                                      |

three groups. Patients with secondary or primary progressive MS, pregnancy, corticosteroid treatment as well as patients suffering 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 [only Interferon: Avonex one time/week] (Table 3). The patients completed a 3-day 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:

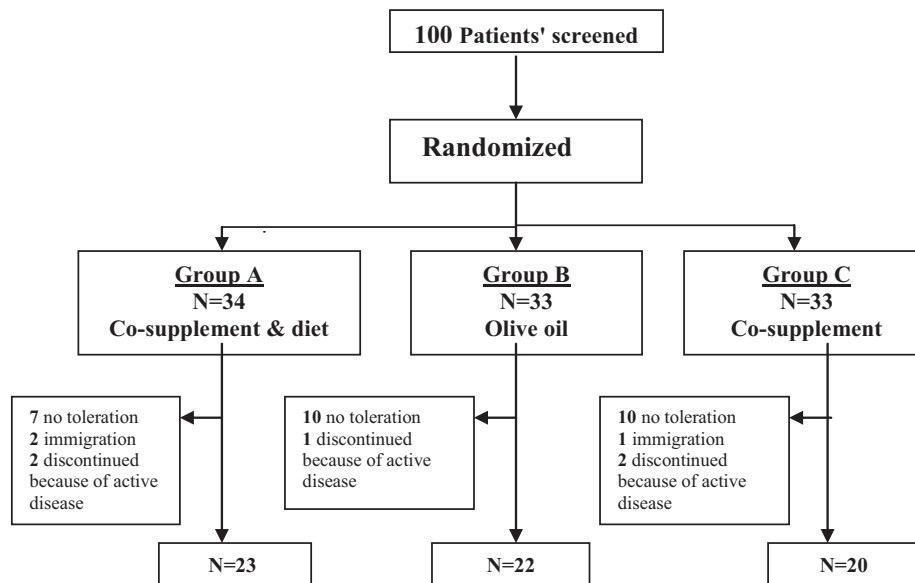
“Group A”: Those receiving the co-supplemented oils, 18–21 g/day (6–7 g, three times daily) with advised *Hot- natured diet* (Table 1)

“Group B”: Those consuming olive oil 18–21 g/day (6–7 g, three times daily),

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

To achieve this objective, group A was asked to consume “*Hot-natured diet*” with a wide choice of foods and drinks items permitted during each dietary period and delivered at home for 6 months.

Groups B and C were asked to consume their usual diet during the intervention. “*Hot nature diet*” (Table 1) 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),



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

**Table 2**  
Impermissible foods (Cold nature diet) for group A.

|                                                                                                                                                                            |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Cereals and grains:</b> Rice-lentils-vetch-potato-starch-barley bread- corn-bean broad                                                                                  |
| <b>Meats:</b> Beef-machine chicken- fishes live in river- egg yolk- SIRABI (sheep's leg & intestine) -chicken liver - canned fishes -processed meat-sausage type-hamburger |
| <b>Dairy products:</b> Dairy without walnut or dates- sour Dughe (sour yogurt diluted with water) - milk powder-whey-different kind of ice cream                           |
| <b>Fats:</b> solid suet- natural butter and liquid vegetable fats-fats link to meat and poultry-palm oil                                                                   |
| <b>Sweets:</b> Zoolbia Confectionary type Bamie -junk foods including types of toffee, candy,chocolate,chips,snack                                                         |
| <b>Vegetables:</b> Rhubarb-lettuce-cucumber- spinach-green beans-green peas-green bean broad-okra-beetroot leaves                                                          |
| <b>Fruits:</b> Peach-strawberry- nectarines- meddler-water melon-kiwi-greenengage-sour pomegranate-blue berry-sour citrus-sour cherry-rhubarb-sour fruits-plum             |
| <b>Spice:</b> unripe grapes-unripe grapes juice-different sauce-tamarind- salty foods- Sun dried fruit (LAVASHAK) -sumac-sorrel                                            |
| <b>Nuts:</b> dried with sulfur-sulfur raisin-salty and spicy nuts                                                                                                          |
| <b>Drinks:</b> Nonalcoholic Beer -soft drink and alcoholic- sour Dughe                                                                                                     |
| <b>Fried foods- canned and semi canned foods-different kind of sandwich and pizza and other fast foods-fermentation foods</b>                                              |

consumption of dairy products with honey or date and removing foods with *Cold nature* (Table 2), avoiding of alcohol and smoking. The patients were contacted monthly by telephone to assess compliance. After baseline assessments, 100 patients were randomized to three groups according to the following diagram (Fig. 1).

**Table 3**  
Clinical and demographic characteristics of the study patients n=65 (23 men, 42 women).

| Variable                                                        | Group A(N=23)<br>Mean $\pm$ SD | Group B(N=22)<br>Mean $\pm$ SD | Group C(N=20)<br>Mean $\pm$ SD |
|-----------------------------------------------------------------|--------------------------------|--------------------------------|--------------------------------|
| Age(years)                                                      | 34.2 $\pm$ 7.5                 | 35.9 $\pm$ 7.8                 | 33.7 $\pm$ 7.8                 |
| Average age at onset (years)                                    | 25.0 $\pm$ 7.5                 | 30.3 $\pm$ 8.1                 | 27.6 $\pm$ 6.4                 |
| Disease duration (years)                                        | 6.26 $\pm$ 3.9                 | 7.55 $\pm$ 5.08                | 6.60 $\pm$ 4.0                 |
| Interferon intake(avonex: interferon $\beta$ 1a, one time/week) | N (%) 22(95.7)                 | N (%) 22(100)                  | N (%) 19(95)                   |
| Gender (M/F)                                                    | 7/16                           | 11/11                          | 5/15                           |

Group A: Co-supplemented hemp seed and evening primrose oils and advised Hot-nature diet.

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 the intervention period. Researchers, patients and those involved in the data collection and assessment (neurologists and nutritionists) as well as data analysis were blind regarding the type of interventions.

### 2.1. Measurement of the disability status of patients

A medical history to check clinical status (inc. EDSS and functional score) and medications used was taken.<sup>4</sup> The functional disability status (disease severity) of each patient was measured by a trained clinician using the Kurtzke EDSS.<sup>10</sup> The EDSS quantifies disability in eight functional systems (FS) and allows neurologists to assign a functional system score (FSS) to each of them. The functional systems are pyramidal, cerebellar, brainstem, sensory, bowel and bladder, visual, cerebral and "other".

### 2.2. 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  $-80^{\circ}\text{C}$ . Serum was separated and aliquots were stored at  $-80^{\circ}\text{C}$ . Total lipids were extracted from RBCs with chloroform/methanol (1:2 v/v), then fatty acids were separated from their alcohols and etherified by methanolysis to form fatty acid methyl esters (FAME). FAME was injected in gas chromatography

**Table 4**Effect of intervention on mean ( $\pm$ SD) Extended disability status score (EDSS) and functional score in trial groups of RRMS patients comparison to baseline.

| Variables        | Group A<br>(N=23) |                |                | Group B<br>(N=22) |                 |                | Group C (N=20)  |                 |                |
|------------------|-------------------|----------------|----------------|-------------------|-----------------|----------------|-----------------|-----------------|----------------|
|                  | Baseline          | 6 months       | P <sup>*</sup> | Baseline          | 6 months        | P <sup>*</sup> | Baseline        | 6 months        | P <sup>*</sup> |
| Pyramidal        | .69 $\pm$ 1.22    | .61 $\pm$ .98  | .328           | 1.32 $\pm$ 1.25   | 1.68 $\pm$ 1.21 | .029           | 1.15 $\pm$ 1.53 | 1.25 $\pm$ 1.41 | .494           |
| Cerebella        | .74 $\pm$ 1.0     | .52 $\pm$ .95  | .233           | 1.04 $\pm$ 1.13   | 1.14 $\pm$ 1.08 | .680           | .95 $\pm$ .94   | .70 $\pm$ 1.03  | .171           |
| brainstem        | .57 $\pm$ .89     | .39 $\pm$ .72  | .103           | .68 $\pm$ .99     | .81 $\pm$ 1.03  | .162           | .55 $\pm$ 1.09  | .40 $\pm$ .88   | .186           |
| Sensory          | .57 $\pm$ .89     | .30 $\pm$ .70  | .186           | .91 $\pm$ 1.34    | .91 $\pm$ 1.11  | 1.00           | .80 $\pm$ 1.01  | .70 $\pm$ 1.22  | .606           |
| Bowel/Bladder    | 1 $\pm$ 1.31      | .35 $\pm$ .57  | .008           | 1.68 $\pm$ 1.55   | 1.27 $\pm$ 1.49 | .071           | 1.21 $\pm$ 1.36 | 1.25 $\pm$ 1.37 | .666           |
| Visual           | .96 $\pm$ 1.29    | .69 $\pm$ 1.14 | .208           | 1.32 $\pm$ 1.70   | 1.45 $\pm$ 1.50 | .633           | 1.20 $\pm$ 1.58 | 1.15 $\pm$ 1.66 | .748           |
| Cerebral         | .87 $\pm$ 1.01    | .43 $\pm$ .66  | .009           | 1.05 $\pm$ .99    | .82 $\pm$ 1.01  | .381           | 1.30 $\pm$ 1.17 | 1.15 $\pm$ .93  | .330           |
| other Functional | .65 $\pm$ .49     | .35 $\pm$ .49  | .005           | .86 $\pm$ .35     | .73 $\pm$ .46   | .186           | .80 $\pm$ .41   | .40 $\pm$ .50   | .002           |
| EDSS             | 2.76 $\pm$ 1.39   | 1.77 $\pm$ 1.7 | 0.001          | 3.45 $\pm$ 1.41   | 3.86 $\pm$ 1.41 | 0.005          | 3.25 $\pm$ 1.94 | 2.95 $\pm$ 1.83 | 0.002          |

Group A: Co-supplemented hemp seed and evening primrose oils and advised Hot-nature diet.

Group B: Olive oil.

Group C: Co-supplemented hemp seed and evening primrose oils.

<sup>\*</sup> P for paired-t test

and the composition was analyzed to assess long-chain PUFAs.<sup>11,12</sup> Serum FADS2 (D6D) and sPLA2 levels from serum were measured by enzyme-linked immunosorbent assay (ELISA) (Uscn Life Science Inc). The absorbance of D6D, sPLA2 levels was read at 450 nm. Percentages of PUFAs were measured against an internal standard. RBCs membrane PUFAs packed as  $\mu$ g FA/ml were analyzed.

### 2.3. Statistical analysis

The statistical analysis was performed using SPSS software (ver 14.0; SPSS Inc, Chicago, IL). The data were expressed as mean  $\pm$  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.

## 3. Results: clinical and biochemical results in RRMS patients

One hundred (34 M and 66 F) patients were enrolled in this study. Fig. 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 between October 2010 and October 2011. The patients' characteristics and demographics are shown in (Table 3).

The sample consisted of 23 males and 42 females with a mean age of  $34.25 \pm 8.07$  years and mean disease duration of  $6.80 \pm 4.33$  years. There was statistically no significant difference in the mean age, gender, disease duration, interferon intake, and average age at the onset between the three groups.

### 3.1. Clinical and biochemical results

The clinical results of the trial are summarized in (Table 4). There were significantly better changes in functional scores in groups A and C at the end of the intervention. Based on the results of (Table 4) globally all functions in groups A and C show relatively improving trend, but there are statistically significant reductions only 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 a relative trend to worsening in all functions, but only pyramidal function shows statistically significant increase.

These results (Tables 4) means that the co-supplemented oils with or without Hot-natured diet used in our study might have a therapeutic effect on MS. (Table 5) indicates that patients (baseline) have elevated serum level of sPLA2, which activities which might be due to the increasing in hydrolysis of membrane phospholipids by sPLA2 is a well-known early response to tissue damage in

all organ systems including CNS. Mean of D6D and sPLA2 concentrations were significantly different before and after consumption of the co-supplemented oils with or without Hot-natured diet in groups A and C, respectively. Red blood cells PUFAs rate showed significant increase, while D6D and sPLA2 concentrations decreased significantly in groups A and C. No significant changes were found in PUFAs, D6D and sPLA2 in group B (Table 5).

This result suggests that the observed reduction of D6D was a consequence of the well-described effects of this type of intervention, and that an increase in PUFAs and reduction in expression of sPLA2 key enzymes caused a decrease in mean EDSS. Surprisingly, altering PUFAs rate causes a decrease in sPLA2 expression, in particular, in the co-supplemented oils and Hot-natured diet group.

## 4. Discussion: possible mechanisms

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%<sup>13</sup> and the Blood-Brain Barrier (BBB) is a key to the bioavailability of brain essential fatty acid (EFA).<sup>14</sup> It is a strong reason for the importance of the effects of diet and various nutrients for modulation in developed wrong disordered metabolic interaction in the metabolism of MS patients. Phospholipids constitute approximately 40%, 60% and 90% of the total lipids in myelin, erythrocyte and mitochondrial, respectively, which play a role in double bio-membrane structure.<sup>15,16</sup> Metabolism of PUFAs in membrane phospholipids is controlled by sPLA2 and acyltransferases known as the "deacylation-reacylation cycle".<sup>17</sup> 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).<sup>18</sup> The sPLA2 hydrolyzes phospholipids to release AA, which can mediate inflammation.<sup>19</sup> The AA liberated is converted to PGE2, possibly by cyclooxygenase-2 (COX-2), which is induced by inflammatory stimuli<sup>20</sup>; therefore, toxicity of AA was associated with increased lipid peroxidation and mitochondrial damage<sup>21</sup>; For this reason, sPLA2 appears to play a fundamental role in cell injury in the CNS, and it plays a key role in the pathogenesis of MS-like by production of pro-inflammatory mediators.<sup>22</sup> However, up to now there have been no effective sPLA2 inhibitors available for clinical use,<sup>23</sup> but extracellular sPLA2 inhibitors suppress CNS inflammation.<sup>24</sup> In this way, inhibition of specific sPLA2 and elevated levels of inflammatory cytokines may represent novel therapeutic strategies against MS. We found that elevated serum of sPLA2 activity in the patients (baseline) is a well-known early response to tissue damage in all organ systems including erythrocyte and probably, in myelin of CNS and mitochondrial, etc. In our study, sPLA2 concentration decreased significantly in groups A and C and estimated sPLA2

**Table 5**  
Effect of intervention on mean ( $\pm$  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)   |                  |      |
|-----------|------------------|-----------------|------|-----------------|------------------|------|------------------|------------------|------|
|           | Base line        | 6 months        | P*   | Base line       | 6 months         | P*   | Base line        | 6 months         | P*   |
| PUFA      | 34.86 $\pm$ 4.01 | 38.17 $\pm$ 3   | .013 | 36.1 $\pm$ 4.31 | 36.29 $\pm$ 3.78 | .877 | 33.22 $\pm$ 3.71 | 35.92 $\pm$ 2.38 | .028 |
| D6D**     | .21 $\pm$ .008   | .010 $\pm$ .005 | .001 | .012 $\pm$ .10  | .039 $\pm$ .62   | .166 | .027 $\pm$ .017  | .015 $\pm$ .010  | .004 |
| sPLA2**   | .95 $\pm$ .94    | .32 $\pm$ .36   | .017 | .69 $\pm$ .61   | 1.13 $\pm$ 1.50  | .302 | .66 $\pm$ .84    | .27 $\pm$ .34    | .042 |

Group A: Co-supplemented hemp seed and evening primrose oils and advised Hot-nature diet.

Group B: Olive oil.

Group C: Co-supplemented hemp seed and evening primrose oils.

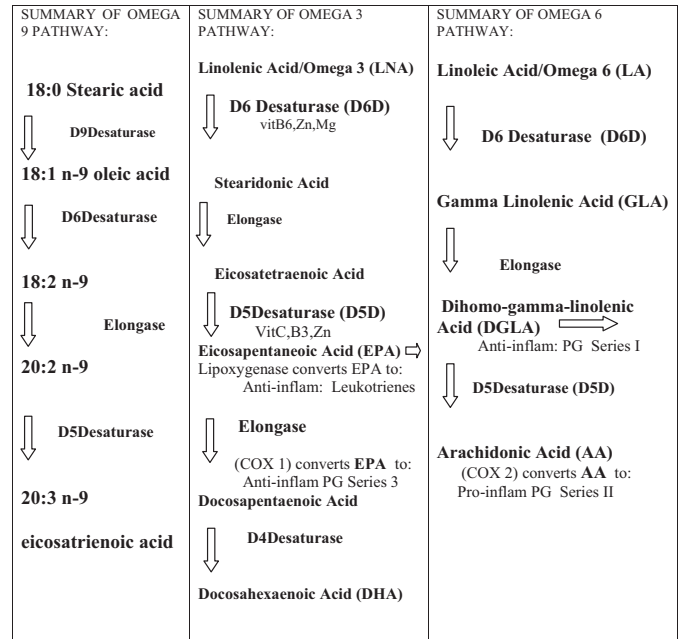
\* P for paired-t test

\*\*  $\mu$ g FA/ml and the absorbance of D6D, PLA2 levels were read at 450 nm.

and D6D were both inversely correlated with PUFAs, and these parameters in group B proved a non-significant (Table 5). The above findings imply that compared to olive oil, the co-supplemented oils with Hot-natured diet produced a significant reduction in clinical symptoms and signs, and the patients general health and well-being improved due to evidence on the base of higher PUFAs in peripheral tissue (red blood cells) and probably, in brain tissue and mitochondrial, etc. Decrease in EDSS agreeing with functional score parameters were significantly better in groups A and C compared to group B, and case groups felt physically and emotionally healthier (Tables 4). A favorable trend 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 confirm prolonged remission in MS and therapeutic agents are only partly effective. Their long-term beneficial effects are uncertain with side effects.<sup>25,26</sup> The co-supplemented oils and their metabolites affect inflammatory functions and cytokines production during the 6 months by improvement in red blood cells PUFAs rate in groups A and C, while in group B this is not significant,<sup>4</sup> and PUFAs rate was correlated with the EDSS and functional score benefits at the last visit. The results are likely due to remyelination during the early phases of disease, though this is rare at more progressed stages.<sup>27</sup> Current estimates of the  $\omega$ 3/ $\omega$ 6 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).<sup>28</sup> The  $\omega$ 6/ $\omega$ 3 ratio in HSO is normally between 2:1 and 3:1, which is considered optimal for human health.<sup>29</sup> The FA profile of HSO is remarkably similar to that of black currant seed oil, which also seems to have a beneficial impact on immunologic vigor.<sup>30,31</sup> From a nutritional point of view, up to 7% GLA and 2.5% SDA are very interesting. The noticeable presence of both GLA and SDA in HS and EP oils, typically at a favorable  $\omega$ 3/ $\omega$ 6 ratio of 1:2 allows this enzymatic step with D6D to be efficiently by passed.<sup>32</sup> Because the produced result (GLA and SDA) of this enzyme 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 results (Table 4). These mentioned basic parameters for cellular metabolic pathways could easily replace this intervention in groups A and C. 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 (Fig. 2), as suggested before.

The EPO content of 9% GLA is the single most important parameter that is metabolized into DGLA, the natural precursor of PGE1.<sup>33–35</sup> The HSO/EPO ratio in this study is 9:1, so  $\omega$ 3/ $\omega$ 6 PUFAs ratio reaches to 1:2.5 or higher, which is competitive inhibition of the conversion of dihomo-gamma-linolenic acid (DGLA) to AA resulting in more anti-inflammatory PGE1.<sup>36</sup> AA is a precursor of pro-inflammatory and pro-aggregator PGE2, while GLA and DGLA are precursors of anti-inflammatory PGE1 (Fig. 2).

GLA and SDA are produced in the body from desaturation of LA and ALA by the reaction catalyzed by enzyme delta-6-



**Fig. 2.** The polyunsaturated fatty acids biosynthetic pathway.

desaturase (D6D or FADS2). D6D is the rate-limiting step in the PUFAs biosynthetic pathways that are incorporated into cell membranes, thereby affecting permeability, and functional properties of cells. The D6D deletion may prevent the conversion of LA and ALA into very long chain-PUFAs.<sup>8,9</sup> Based on studies, the activity of D6D 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,<sup>37–39</sup> genetic deficient (inactive D5D and D6D enzymes).<sup>40</sup> Moreover, excessive consumption of GLA occurs in: high rates of cell division, inflammatory, antiviral reaction and Trauma.<sup>41</sup> Therefore, there is a correlation between MS and a rapid fall of D6D activity which has been shown after intervention, providing a rationale for performing additional functional studies on the D-6-desaturase (FADS2) and D-5-desaturase (FADS1) gene transcription in all groups of MS patients and healthy adults is necessary.

## 5. Conclusion

Regarding the beneficial properties of this intervention, the of effects co-supplemented oils and Hot-natured 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 such as FADS2 and sPLA2.

## Conflict of interests

There was not any conflict of interests to declare.

## References

- [1]. Iranian MS. Forum. 2012. Increasing prevalence of MS, a not solved puzzle. available at <<http://www.ms-links.org/2012/03/blog-post/>>.
- [2]. Pryse-phillips W, Sloka JS. Etiopathogenesis and epidemiology: clues to etiology. In: Cook SD, ed. *Handbook of Multiple Sclerosis*. New York: Taylor & Francis Group; 2006:1–39.
- [3]. Minagar A, Alexander JS. Blood-brain barrier disruption in multiple sclerosis. *Mult Scler*. 2003;9:540–549.
- [4]. Rezapour-Firouzi S, Arefhosseini SR, Ebrahimi-Mamaghani M, et al. Immunomodulatory and therapeutic effects of Hot-nature diet and co-supplemented hemp seed, evening primrose oils intervention in multiple sclerosis patients. *Complement Ther Med*. 2013;21:473–480.
- [5]. Sun GY, Xu J, Jensen MD, et al. Phospholipase A2 in the central nervous system: implications for neurodegenerative diseases. *J Lipid Res*. 2004;45:205–213.
- [6]. Kalyvas A, Baskakis C, Magrioti V, et al. Differing roles for members of the phospholipase A2 superfamily in experimental autoimmune encephalomyelitis. *Brain*. 2009;132(5):1221–1235.
- [7]. Cunningham TJ, Yao L, Oettinger M, et al. Secreted phospholipase A2 activity in experimental autoimmune encephalomyelitis and multiple sclerosis. *J Neuroinflamm*. 2006;3:26.
- [8]. Baylin A, Ruiz- Narvaez E, Kraft P, et al. Linolenic acid, D6-desaturase(FADS2) gene polymorphism, and the risk of nonfatal myocardial infarction. *Am J Clin Nutr*. 2007;85:554–560.
- [9]. Nakamura MT, Nara TY. Structure, function, and dietary regulation of delta6, delta5, and delta9 desaturases. *Annu Rev Nutr*. 2004;24:345–376.
- [10]. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology*. 1983;33:1444–1452.
- [11]. Van Jaarsveld PJ, Smuts CM, Tichelaar HY, et al. 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.
- [12]. 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.
- [13]. Morrell P, Quarles RH. Myelin formation, structure and biochemistry. *Philadelphia*. 1999:69–93.
- [14]. Yehuda S, Rabinovitz S, Mostofsky DI. Essential fatty acids and the brain: from infancy to aging. *Neurobiol Aging*. 2005;26S:S98–S102.
- [15]. DiMauro S, Hirano M. Mitochondrial encephalomyopathies: an update. *Neuromuscul Disord*. 2005;15(4):276–286.
- [16]. Sun AY, Wang Q, Simonyi A, et al. Botanical phenolics and brain health. *Neuromol Med*. 2008;10(4):259–274.
- [17]. Yedgar S, Cohen Y, Shoseyov D. Control of PLA2 activities for the treatment of inflammatory conditions. *Biochim Biophys Acta*. 2006;1761:1373–1382.
- [18]. Kalyvas A, Samuel D. Cytosolic phospholipase A2 plays a key role in the pathogenesis of multiple sclerosis-like disease. *Neuron*. 2004;41:323–335.
- [19]. Balboa MA, Varela- Nieto I, Lucas KK, et al. Expression and function of phospholipase A2 in brain. *FEBS Lett*. 2002;531:12–17.
- [20]. Caro AA, Cederbaum AI. Role of cytochrome P450 in phospholipase A2- and arachidonic acid-mediated cytotoxicity. *Free Radical Biol Med*. 2006;40:364–375.
- [21]. Kalman B, Laitinen K, Komoly S. The involvement of mitochondria in the pathogenesis of multiple sclerosis. *J Neuroimmunol*. 2007;188:1–12.
- [22]. Thwin MM, Satyanarayanan SD, Nagarajao LM, et al. Novel peptide inhibitors of human secretory phospholipase A2 with antiinflammatory activity: solution structure and molecular modeling. *J Med Chem*. 2007;50:5938–5950. *Neuromolecular Med* 10 (4): 259–274.
- [23]. Pinto F, Brenner T, Dan P, et al. Extracellular phospholipase A2 inhibitors suppress central nervous system inflammation. *Glia*. 2003;44:275–282.
- [24]. Meydani M. Effect of long-term fish oil supplementation on vitamin E status and lipid peroxidation in women. *J Nutr*. 1991;121:484–491.
- [25]. Johnson KP, Brooks BR, Cohen JA, et al. 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.
- [26]. Filippini G, Munari L, Incurvaia B, et al. Interferons in relapsing remitting multiple sclerosis: a systematic review. *Lancet*. 2003;361:545–552.
- [27]. Lassmann H. Mechanisms of demyelination and tissue damage in multiple sclerosis. *Acta Neurol Belg*. 1999;99:6–10.
- [28]. Delaleu N, Immervoll H, Cornelius J, et al. Biomarker profiles in serum and saliva of experimental Sjogren's Syndrome: associations with specific autoimmune manifestations. *Arthritis Res Ther*. 2008;10:R22.
- [29]. De Padua LS, Bunyaprafatsara N, Lemmens RHMJ. Plant resources of South-East Asia. *Med Poisonous Plants*. 1999;1(12):167–175.
- [30]. Wu D, Meydani M, Leka LS, et al. Effect of dietary supplementation with black currant seed oil on the immune response of healthy elderly subjects. *Am J Clin Nutr*. 1999;70:536–543.
- [31]. Barre DE. Potential of evening primrose, borage, black currant, and fungal oils in human health. *Ann Nutr Metab*. 2001;45:47–57.
- [32]. Okuyama H, Kobayashi T, Watanabe S. Dietary fatty acids: the 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.
- [33]. Christie WW. The analysis of evening primrose oil. *Ind Crops Prod*. 1999;10:73–83.
- [34]. 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.
- [35]. Jantti J. Evening primrose oil and olive oil in the treatment of rheumatoid arthritis. *Clin Rheumatol*. 1989;8:238–244.
- [36]. Miljanovic B, Trivedi K, Dana M, et al. Relation between dietary n-3 and n-6 fatty acids and clinically diagnosed dry eye syndrome in women. *Am J Clin Nutr*. 2005;82:887–893.
- [37]. 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.
- [38]. Horrobin DF. Importance of g-linolenic acid. *Prog Lipid Res*. 1992;31:163–194.
- [39]. 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. *Med Hypotheses*. 1988;25:103–409.
- [40]. Okuyama H, Kobayashi T, Watanabe S. Dietary fatty acids: the 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.
- [41]. Horrobin DF. Nutritional and medical importance of GAMA-Linolenic acid. *Prog Lipid Res*. 1992;2:163–194.