The Journal of Nutrition
Ingestive Behavior and Neurosciences

High-dose ω-3 Fatty Acid Plus Vitamin D_3 Supplementation Affects Clinical Symptoms and Metabolic Status of Patients with Multiple Sclerosis: A Randomized Controlled Clinical Trial

Ebrahim Kouchaki,[1,2] Maryam Afarini,[1] Javad Abolhassani,[1] Naghmeh Mirhosseini,[4] Fereshteh Bahmani,[3] Seyed Ali Masoud,[1] and Zatollah Asemi[3]

[1] Department of Neurology, School of Medicine; [2] Physiology Research Center; and [3] Research Center for Biochemistry and Nutrition in Metabolic Diseases, Kashan University of Medical Sciences, Kashan, I.R. Iran; and [4] Pure North S'Energy Foundation, Calgary, Alberta, Canada

Abstract

Background: Combined omega-3 fatty acid and vitamin D supplementation may improve multiple sclerosis (MS) by correcting metabolic abnormalities and attenuating oxidative stress and inflammation.

Objective: This study aimed to determine the effects of ω-3 fatty acid and vitamin D cosupplementation on the disability score and metabolic status of patients with MS.

Methods: This was a randomized, placebo-controlled clinical trial with Expanded Disability Status Scale (EDSS) score and inflammation as primary outcomes and oxidative stress biomarkers and metabolic profile as secondary outcomes. Patients, aged 18–55 y, were matched for disease EDSS scores, gender, medications, BMI, and age (n = 53) and randomly received a combined 2 × 1000 mg/d ω-3 fatty acid and 50,000 IU/biweekly cholecalciferol supplement or placebo for 12 wk. The placebos were matched in colour, shape, size, packaging, smell, and taste with supplements. Fasting blood samples were collected at baseline and end of intervention to measure different outcomes. Multiple linear regression models were used to assess treatment effects on outcomes adjusting for confounding variables.

Results: Patients taking ω-3 fatty acid plus vitamin D supplements showed a significant improvement in EDSS (β −0.18; 95% CI: −0.33, −0.04; P = 0.01), compared with placebo. Serum high-sensitivity C-reactive protein (β −1.70 mg/L; 95% CI: −2.49, −0.90 mg/L; P < 0.001), plasma total antioxidant capacity (β +55.4 mmol/L; 95% CI: 9.2, 101.6 mmol/L; P = 0.02), total glutathione (β +51.14 μmol/L; 95% CI: 14.42, 87.87 μmol/L; P = 0.007), and malondialdehyde concentrations (β −0.86 μmol/L; 95% CI: −1.10, −0.63 μmol/L; P < 0.001) were significantly improved in the supplemented group compared with the placebo group. In addition, ω-3 fatty acid and vitamin D cosupplementation resulted in a significant reduction in serum insulin, insulin resistance, and total/HDL-cholesterol, and a significant increase in insulin sensitivity and serum HDL-cholesterol concentrations.

Conclusion: Overall, taking ω-3 fatty acid and vitamin D supplements for 12 wk by patients with MS had beneficial effects on EDSS and metabolic status. This trial was registered at the Iranian website (www.irct.ir) for registration of clinical trials as IRCT2017090133941N20. *J Nutr* 2018;148:1380–1386.

Keywords: ω-3 fatty acid, vitamin D, multiple sclerosis, disability, inflammation, oxidative stress

Introduction

Multiple sclerosis (MS) is defined as a long-lasting inflammatory neurodegenerative disease involving the central nervous system, which affects young and middle-aged adults in the ages ranging from 20 to 55 y (1). MS is evidently more common among women with ~60% of MS cases being female (1). Mental illnesses such as depression might be detected in 50–60% of patients with MS (2). Increased inflammatory markers and oxidative damage have been suggested as a pathogenic mechanism leading to progressive MS (3, 4). In addition, chronic inflammation in these patients might lead to increased insulin resistance and postprandial hyperinsulinemia (5).

To date, the majority of clinical trials in patients with MS have been focused on either dietary supplements like fish oil or vitamin D (6) or specific diets such as low saturated fat, with/without any supplement (7–10), and data on combined

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Manuscript received March 12, 2018. Initial review completed April 4, 2018. Revision accepted May 8, 2018.
First published online July 3, 2018; doi: https://doi.org/10.1093/jn/nxy116.

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supplementation are scarce. Early studies have reported that fish oil supplementation significantly decreased inflammatory cytokines and nitric oxide (NO) catabolites in patients with MS (10, 11). Previous published trials have documented that vitamin D supplementation decreased parameters of oxidative stress and positively influenced other metabolic profiles in these patients (12, 13). However, in another trial of high-dose vitamin D_3 (cholecalciferol) supplementation (20,000 IU/wk) for 2 y, no effects were examined on parameters of systemic inflammation in patients with MS (14). In addition, fish oil supplementation at a high dosage of 4 g/d for 12 mo did not improve oxidative stress in patients with MS (15).

We hypothesized that combined omega-3 fatty acid and vitamin D_3 supplementation may have synergistic benefits on the disability score, mental health, biomarkers of inflammation and oxidative stress, and metabolic status in patients with MS. The current study was therefore conducted to evaluate the effects of ω-3 fatty acid and vitamin D_3 cosupplementation on disability score, biomarkers of inflammation and oxidative stress, and metabolic profile in patients with MS.

Methods

Trial design

This study was a 12-wk randomized, double-blinded, placebo-controlled clinical trial.

Patients

Patients in the age range of 18–55 y with relapsing-remitting MS (RRMS) according to McDonald criteria, and an expanded disability status scale (EDSS) score of <4.5 (16), who were referred to the Shahid Beheshti Clinic in Kashan, Isfahan State, Iran, between November 2017 and January 2018, were included in this study. Eligible patients should have all of the following information recorded in their documents collected in the MS clinic: date of birth, gender, age at MS onset, confirmed RRMS, number of relapses since the onset and delay between the first 2 relapses, date of the measurement and EDSS scoring at that time (or <3 mo before or after), familial antecedents of MS (defined by the presence of 1 case in first- or second-degree relatives), and the absence of vitamin D_3 and ω-3 fatty acid supplementation before measurement. Exclusion criteria were as follows: pregnancy or lactating during the past 6 mo, a history of nephrolithiasis during the previous 5 y, menopause, defined as no regular menstruation, and unwillingness to use appropriate contraception.

Ethics statements

This study followed the Declaration of Helsinki and all patients signed the informed consent form. The research was approved by the ethics committee of Kashan University of Medical Sciences (KAUMS) and was registered at the Iranian website for registration of clinical trials (http://www.irct.ir) as IRCT2017090133941N20.

Study design

All patients were matched for disease severity based on EDSS, gender, type of medications, BMI, and age. They were then randomly allocated into 2 groups to receive either 2 ω-3 fatty acid capsules daily (containing 500 mg DHA and 106 mg EPA) plus vitamin D_3 as cholecalciferol supplements (50,000 IU/biweekly) (n = 26) or sunflower oil capsules (placebo, n = 27) for 12 wk. High-DHA fish oil capsules (7.6% EPA + 27% DHA) and sunflower oil placebo capsules were donated by Nu-Mega Ingredients Pty Ltd (Melbourne, Australia) and vitamin D_3 capsules were manufactured by the Pharmaceutical Company (Tabriz, Iran). The placebos were matched in colour, shape, size, packaging, smell, and taste with the vitamin D_3 and ω-3 fatty acid capsules. The compliance rate was assessed by measuring serum 25(OH)D (25-hydroxyvitamin D) concentrations. Intake of the ω-3 fatty acid, vitamin D_3, and placebo capsules was monitored through asking participants to return the medication containers. To increase the compliance rate, all patients received brief daily cellphone reminders to take the supplements. Patients were requested to undertake their regular physical activity and not to take any extra nutritional supplements during the 12-wk trial. All patients completed a 3-d food record and 3 physical activity records at the baseline of the study, wk 3, 6, and 9, and at the end of the intervention. Daily macro- and micronutrient intakes were calculated by analyzing food records via nutritionist IV software (First Databank, San Bruno, CA). In the current study, physical activity was described as metabolic equivalents (METs) in h/d. To determine the METs for each patient, we multiplied the duration of reported physical activity (in h/d) by its related METs coefficient, derived from established standard tables (16).

Sample size

Sample size was calculated using the standard formula for clinical trials, considering type 1 error (α) of 0.05 and type 2 error (β) of 0.20 (power = 80%). According to a previous published study (17), we used 2.65 mg/L as the difference in mean (d) and 3.30 mg/L as SD for high-sensitivity C-reactive protein (hs-CRP) as the key variable. Based on this information, 25 individuals were required to be included in each treatment group. Considering 5 probable dropouts in each group, the final sample size was determined as 30 patients in each group.

Randomization

Randomization was conducted via computer-generated random numbers. Randomization and allocation were concealed from the researchers and patients until the final analyses were completed. The randomized allocation sequence, enrolling patients, and allocating them into intervention groups were performed by a trained staff at the MS clinic.

Assessment of outcomes

The primary outcomes of this study included EDSS score and inflammatory markers. Biomarkers of oxidative stress and metabolic profiles were the secondary outcomes of interest in this study.

Disability score

EDSS scoring was recorded at baseline and 3 mo later, at the end of the intervention. Patients who reported new MS symptoms in the phone interview were invited to the clinic for further evaluation. Relapses, which were defined as new neurologic deficits, lasting longer than 24 h, with no evidence of an infection (18), were recorded throughout the study. All relapses were confirmed by objective neurological examination.

Anthropometric measures

Patients' weight and height were measured after an overnight fast, with the use of a standard scale (Seca, Hamburg, Germany), at both the onset of the study and after 12 wk of the trial. BMI was calculated as kg/m^2.

Biomarkers

Blood samples were collected, after 12 h fasting, at the beginning and end of the trial, at the Kashan reference laboratory. Serum 25(OH)D

Supported by a grant no.1396.64 from the Kashan University of Medical Sciences. The financial support for conception, design, data analysis, and manuscript drafting comes from the Research Center for Biochemistry and Nutrition in Metabolic Diseases, Kashan University of Medical Sciences, Kashan, Iran.

Author disclosures: EK, MA, JA, NM, FB, SAM, and ZA, no conflicts of interest. Address correspondence to ZA (e-mail: asemi_r@yahoo.com).

Abbreviations used: EDSS, expanded disability status scale; FPG, fasting plasma glucose; GSH, total glutathione; hs-CRP, high-sensitivity C-reactive protein; IL-1β, interleukin-1β; IL-6, interleukin-6; MDA, malondialdehyde; METs, metabolic equivalents; MS, multiple sclerosis; NF-κB, nuclear factor kappa B; QUICKI, quantitative insulin sensitivity check index; RRMS, relapsing-remitting MS; TAC, total antioxidant capacity; TNF-α, tumor necrosis factor-α.

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concentrations were measured with the use of an ELISA kit (IDS, Boldon, United Kingdom) and enzyme-linked immunosorbent assay with inter- and intra-assay CVs of <7%. Serum hs-CRP concentrations were measured with the use of an ELISA kit (LDN, Nordhorn, Germany) with the intra- and interassay CVs <7%. Other biomarkers were assessed as follows: plasma NO through the use of the Giess method (19), total antioxidant capacity (TAC) via the ferric reduction antioxidant power method developed by Benzie and Strain (20), glutathione (GSH) applying the Beutler et al. method (21), and malondialdehyde (MDA) concentrations by means of the thiobarbituric acid reactive substance method (22) with the inter- and intra-assay CVs <5%. To measure fasting plasma glucose (FPG) and serum lipid profiles (total cholesterol, HDL-cholesterol, LDL-cholesterol, VLDL-cholesterol, and TGs), the study utilized the most commonly used kits (Pars Azmun, Tehran, Iran). CVs for FPG, total cholesterol, HDL-cholesterol, LDL-cholesterol, VLDL-cholesterol, and TGs were 1.7%, 1.6%, 1.8%, 1.9%, 2.1%, and 1.8%, respectively. Circulating concentrations of serum insulin were assessed through the use of an ELISA kit (Monobind, Lake Forest, CA) with the intra- and interassay CVs <5%. The HOMA-IR and the quantitative insulin sensitivity check index (QUICKI) were calculated according to previously established formulas (23).

Statistical methods

Anthropometric measures and nutrient intake were compared between intervention groups, via independent-samples *t* test. Multiple linear regression models were used to assess treatment effects on the study outcomes after adjusting for confounding variables including the baseline values, age, and BMI. The effect sizes were presented as the mean differences with 95% CIs. The normality of the model residual was tested through the use of the Kolmogorov-Smirnov one-sample test. Outcome log-transformation was applied if the model residual did not have a normal distribution (QUICKI, TGs, and VLDL-cholesterol). Bootstrapping was also used as a sensitivity analysis for CIs and inverse probability weighting was used to explain loss-to-follow-up, but the results did not change substantially. A P value of <0.05 was considered as statistically significant. All statistical analyses were conducted via the Statistical Package for the Social Sciences version 18 (SPSS Inc., Chicago, IL).

Results

At the end of the intervention, 53 patients [treatment (n = 26) and placebo (n = 27)] completed the trial (**Figure 1**). Four patients in the treatment group and 3 in the placebo group were excluded from final analyses due to moving to another city (n = 4) or loss of interest for participation in the research (n = 3). Overall, the compliance rate in this study was high, such that >90% of capsules were consumed throughout the study in both groups. No side effects were reported after coadministration of ω-3 fatty acid and vitamin D_3 capsules in MS patients throughout the study.

Mean age, height, weight, and BMI at baseline and end-of-trial were not significantly different between the intervention groups (**Table 1**).

Mean dietary macro- and micronutrient intakes were also not significantly different between the 2 groups throughout the trial (**Table 2**).

Our findings showed that the coadministration of ω-3 fatty acid and vitamin D_3, for 12 wk, significantly decreased EDSS score [β (difference in the mean outcome measures between treatment groups) −0.18; 95% CI: −0.33, −0.04; P = 0.01] in patients with MS (**Table 3**). Moreover, serum hs-CRP (β −1.70 mg/L; 95% CI: −2.49, −0.90 mg/L; P < 0.001), plasma TAC (β +55.4 mmol/L; 95% CI: 9.2, 101.6 mmol/L; P = 0.02), GSH (β +51.14 μmol/L; 95% CI: 14.42, 87.87 μmol/L; P = 0.007), and MDA (β −0.86 μmol/L; 95% CI: −1.10, −0.63 μmol/L; P < 0.001) improved significantly in the supplemented group, compared with the placebo group. In addition, ω-3 fatty acid and vitamin D_3 combination resulted in a significant reduction in serum insulin (β −2.33 μIU/mL; 95% CI: −4.03, −0.63 μIU/mL; P = 0.008), HOMA-IR (β −0.46; 95% CI: −0.83, −0.08; P = 0.01), and total/HDL-cholesterol (β −0.43; 95% CI: −0.85, −0.006; P = 0.04), and a significant increase in QUICKI (β +0.01; 95% CI: 0.003, 0.02; P = 0.008) and serum HDL-cholesterol concentrations (β +2.30 mg/dL; 95% CI: 0.59, 4.00 mg/dL; P = 0.009) compared with the placebo. Other biomarkers of oxidative stress, FPG, and other lipids did

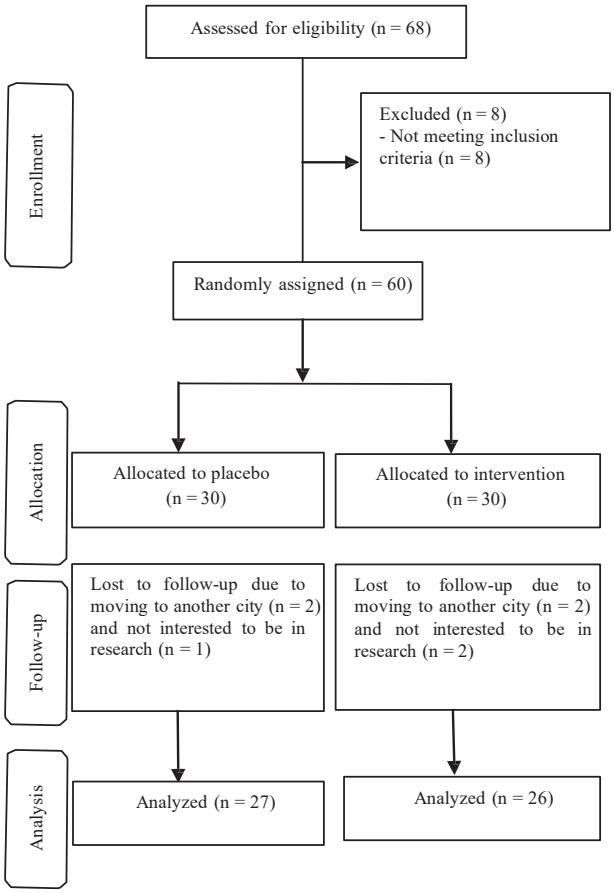

FIGURE 1 Summary of patient flow.

TABLE 1 General characteristics of study patients [1]

	Placebo group (n = 27)	ω-3 fatty acid plus vitamin D_3 group (n = 26)	P [2]
Age, y	35.2 ± 9.2	33.3 ± 6.5	0.37
Height, cm	161.6 ± 6.4	163.2 ± 8.5	0.41
Body weight, kg			
Baseline	65.1 ± 9.9	66.8 ± 11.1	0.53
Wk 12	65.0 ± 10.0	66.8 ± 11.1	0.50
Change	−0.1 ± 0.7	0.1 ± 0.4	0.32
BMI, kg/m^2			
Baseline	24.9 ± 3.3	25.1 ± 3.9	0.83
Wk 12	24.8 ± 3.4	25.1 ± 3.9	0.78
Change	−0.1 ± 0.3	0.03 ± 0.1	0.26

[1] Data are means ± SDs.
[2] Obtained from independent *t* test.

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TABLE 2 Dietary intakes of patients with multiple sclerosis who were or were not supplemented with ω-3 fatty acid plus vitamin D_3 for 12 wk[1]

	Placebo group ($n = 27$)	ω-3 plus vitamin D_3 group ($n = 26$)	P[2]
Energy, kcal/d	2100 ± 196	2186 ± 227	0.14
Carbohydrates, g/d	286 ± 36	297 ± 43	0.34
Protein, g/d	79 ± 20	81 ± 16	0.65
Fat, g/d	75 ± 16	79 ± 11	0.35
SFAs, g/d	24 ± 6	26 ± 4	0.37
PUFAs, g/d	23 ± 6	24 ± 6	0.49
MUFAs, g/d	21 ± 7	22 ± 5	0.55
Cholesterol, mg/d	197 ± 110	219 ± 107	0.46
ω-3 fatty acid, g/d	0.9 ± 0.4	1.0 ± 0.4	0.55
TDF, g/d	18 ± 5	19 ± 4	0.53
Vitamin D, μg/d	2.7 ± 0.7	2.9 ± 0.8	0.52
Vegetables, serving/d	3.6 ± 1.1	4.0 ± 1.0	0.26
Fruits, serving/d	2.9 ± 0.9	3.0 ± 0.8	0.54

[1] Values are means ± SDs. TDF, total dietary fiber.
[2] Obtained from independent *t* test.

not significantly change with ω-3 fatty acid and vitamin D_3 cosupplementation.

Discussion

We evaluated the effect of coadministration of ω-3 fatty acid and vitamin D_3 at high doses, to the best of our knowledge for the first time, on disability and metabolic status in patients with MS. The results showed that taking ω-3 fatty acid and vitamin D_3 supplements together for 12 wk had beneficial effects on EDSS score, serum hs-CRP, plasma TAC, GSH, MDA, insulin metabolism, HDL-, and total/HDL-cholesterol.

Effect on clinical signs

Patients with MS are predisposed to multiple complications, such as increased risk of cardiovascular disease, dyslipidemia (24), insulin resistance (25), other morbidities, and an increased mortality rate (26). Combination of ω-3 fatty acid and vitamin D_3 supplements for 12 wk led to a significant reduction in these patients' disabilities. Our findings were in line with other studies showing that DHA and EPA supplementation for 2 y resulted in a significant reduction in EDSS score in patients with MS (27). Furthermore, it was suggested that fish oil given together with vitamins and dietary advice could improve clinical outcome in patients newly diagnosed with MS (27). A high-dose vitamin D_3 supplement added to routine care of pregnant women with MS was shown to have a significant impact on EDSS and number of relapses during pregnancy and within 6 mo after delivery (28). In another study, vitamin D deficiency was significantly associated with higher risk of disability in patients with MS (29). However, there are discrepancies among different studies looking into the association of different nutrients with MS. For example, Ramirez et al. (10) showed that high-dose fish oil supplementation (4 g/d) for 12 mo did not affect EDSS score in patients with RRMS. In a meta-analysis conducted by James et al. (30), there was no significant relation between high-dose vitamin D supplementation and risk of MS relapses. The inconclusive results of different studies might be related to their methodology including doses, administering combined compared with individual nutrients, duration of intervention, and other possible confounding factors. ω-3 fatty acid might be beneficial in MS patients through immune modulation. Its intake would reduce the synthesis of the proinflammatory leukotriene B4 and prostaglandin E2 (31) and it can increase the synthesis of the less inflammatory leukotriene B5 and prostaglandin E3 (32). ω-3 fatty acid intake also would affect the synthesis of cytokines (33), which in turn might improve EDSS in these patients. The beneficial impacts of vitamin D_3 on mental health in patients with MS can be explained through its role for increasing the expression of the tyrosine hydroxylase gene and promoting the bioavailability of some neurotransmitters such as dopamine, noradrenaline, and adrenaline (34, 35).

Effect on biomarkers of inflammation and oxidative stress

The cosupplementation of ω-3 fatty acid and vitamin D_3 for 12 wk was found to significantly decrease inflammatory markers including serum hs-CRP and plasma MDA and increase plasma total antioxidant capacity and GSH concentrations in patients with MS. Our findings were in agreement with other studies involving ω-3 fatty acid supplementation indicating decreased production of proinflammatory markers such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and IL-6 (36, 37). In a meta-analysis which evaluated the effects of fish oil supplementation in patients with chronic heart failure, circulating inflammatory markers decreased after 3–12 mo of supplementation (38). We have previously shown that the combination of ω-3 fatty acid and vitamin D_3 for 6 wk had beneficial effects on hs-CRP, TAC, GSH, and MDA in women with gestational diabetes (GDM) (17). Moreover, vitamin D_3 administration at a dosage of 100,000 IU monthly for 12 wk decreased oxidative stress mediators of arterial stiffness in overweight and obese individuals (39). On the other hand, supplementation with 1000 mg EPA and 400 mg DHA per d for 18 wk did not show any significant effect on inflammatory markers like hs-CRP and IL-6 concentrations in a healthy population (40). In another study, supplementation with different doses of EPA plus DHA (300, 600, 900, and 1800 mg/d) for 5 mo did not change IL-6, TNF-α, and CRP concentrations in healthy individuals (41). We also have indicated that 50,000 IU/wk vitamin D_3 supplements for 8 wk did not influence hs-CRP concentrations, yet increased TAC and GSH concentrations in patients with major depressive disorder (12). Increased gene expression of peroxisome proliferator-activated receptors by ω-3 fatty acid might inhibit the activation of nuclear factor kappa B (NF-κB) (42), which reduces the production of inflammatory markers. Less production of parathyroid hormone by vitamin D supplementation (43) might be involved in decreasing the production of inflammatory factors including CRP. ω-3 fatty acid and vitamin D_3 both were also found to have remarkable anti-inflammatory and antioxidant properties (44, 45). Vitamin D_3 might decrease production of reactive oxygen species and proinflammatory cytokines (46).

Effect on glycemic control and lipid profiles

The current study demonstrated that ω-3 fatty acid and vitamin D_3 cosupplementation for 12 wk was associated with significant improvements in glycemic control, insulin sensitivity, and lipid profiles. We have previously shown that the coadministration of vitamin D_3 and ω-3 fatty acid to women with GDM for 6 wk had beneficial effects on fasting glucose,

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TABLE 3 Expanded disability status scale, biomarkers of inflammation and oxidative stress, and metabolic profiles at baseline and after the 12-wk intervention in patients with multiple sclerosis that received ω-3 fatty acid plus vitamin D_3 or placebo[1]

	Placebo group ($n = 27$)		ω-3 fatty acid plus vitamin D_3 group ($n = 26$)		Difference in outcome measures between ω-3 fatty acid plus vitamin D_3 and placebo treatment groups[2]	
Variables	Baseline	Wk 12	Baseline	Wk 12	β (95% CI)	P[3]
Serum 25-hydroxyvitamin D, ng/mL	12.7 ± 2.8	13.0 ± 3.0	14.0 ± 3.1	25.2 ± 7.7	12.53 (10.49, 14.56)	<0.001
EDSS	2.4 ± 0.9	2.5 ± 0.9	2.3 ± 0.6	2.2 ± 0.5	−0.18 (−0.33, −0.04)	0.01
Serum hs-CRP, mg/L	3.9 ± 2.4	4.2 ± 2.5	3.7 ± 2.0	2.6 ± 2.3	−1.70 (−2.49, −0.90)	<0.001
Plasma NO, μmol/L	36.0 ± 4.0	35.2 ± 4.8	34.2 ± 3.7	34.2 ± 3.9	0.41 (−1.38, 2.21)	0.64
Plasma TAC, mol/L	1.0 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	55.4 (9.2, 101.6)	0.02
Plasma GSH, μmol/L	702 ± 119	698 ± 91	751 ± 89	782 ± 108	51.14 (14.42, 87.87)	0.007
Plasma MDA, μmol/L	2.8 ± 0.5	2.9 ± 0.6	3.0 ± 0.6	2.3 ± 0.5	−0.86 (−1.10, −0.63)	<0.001
FPG, mg/dL	89.0 ± 8.6	90.4 ± 8.9	90.6 ± 10.3	88.9 ± 9.8	−2.28 (−5.34, 0.78)	0.14
Serum insulin, μIU/mL	12.7 ± 3.9	13.2 ± 3.8	13.4 ± 3.4	11.4 ± 3.9	−2.33 (−4.03, −0.63)	0.008
HOMA-IR	2.8 ± 0.9	2.9 ± 0.9	3.0 ± 1.0	2.5 ± 1.0	−0.46 (−0.83, −0.08)	0.01
QUICKI	0.33 ± 0.01	0.32 ± 0.01	0.32 ± 0.01	0.33 ± 0.02	0.01 (0.003, 0.02)	0.008
Serum TGs, mg/dL	133 ± 61	136 ± 58	126 ± 69	128 ± 62	−1.70 (−13.65, 10.25)	0.78
Serum VLDL-cholesterol, mg/dL	26.7 ± 12.2	27.3 ± 11.5	25.2 ± 13.7	25.6 ± 12.4	−0.34 (−2.73, 2.05)	0.78
Serum total cholesterol, mg/dL	155 ± 32	162 ± 31	159 ± 41	165 ± 40	−1.77 (−10.93, 7.39)	0.94
Serum LDL-cholesterol, mg/dL	85.0 ± 38.2	90.7 ± 37.0	89.5 ± 33.9	92.5 ± 32.3	−3.96 (−11.48, 3.55)	0.29
Serum HDL-cholesterol, mg/dL	43.3 ± 6.8	44.1 ± 6.8	44.0 ± 6.9	46.6 ± 6.6	2.30 (0.59, 4.00)	0.009
Total/HDL-cholesterol	3.7 ± 0.9	3.8 ± 0.9	3.7 ± 1.0	3.6 ± 0.9	−0.43 (−0.85, −0.006)	0.04

[1]Data are means ± SDs. EDSS, expanded disability status scale; FPG, fasting plasma glucose; GSH, total glutathione; hs-CRP, high-sensitivity C-reactive protein; MDA, malondialdehyde; NO, nitric oxide; QUICKI, quantitative insulin sensitivity check index; TAC, total antioxidant capacity.
[2]"Outcome measures" refers to the change in values of measures of interest between baseline and wk 12. β, difference in the mean outcome measures between treatment groups; ω-3 fatty acid plus vitamin D_3 group = 1 and placebo group = 0.
[3]Obtained from multiple regression model (adjusted for baseline values of each biochemical variable, age, and baseline BMI).

insulin concentrations, HOMA-IR, QUICKI, TGs, and VLDL-cholesterol concentrations (47). Supplementation with 2.4 g/d EPA + DHA for 8 wk to hemodialysis patients also decreased insulin concentrations and HOMA-IR (48). Von Hurst et al. (49) determined that vitamin D supplementation at a dosage of 4000 IU/d for 6 mo significantly improved insulin sensitivity in healthy women. However, there was controversy regarding the impact of vitamin D and/or ω-3 fatty acid on glycemic control. For example, no significant difference was seen in fasting glucose, insulin, HOMA-IR, LDL-cholesterol, leptin, or adiponectin concentrations after the supplementation of 1800 mg/d ω-3 fatty acid for 4 mo in hemodialysis patients (50). In another study, vitamin D supplementation with 1000 IU/d for 12 wk did not influence insulin resistance in healthy overweight or obese women (51). Differences in the design of the studies, lack of considering baseline values of dependent biochemical parameters along with characteristics of study patients, different dosages and types of ω-3 fatty acid and vitamin D used as well as the duration of the intervention might provide some reasons for discrepant findings. ω-3 fatty acid might inhibit proinflammatory markers and suppress gene expression of NF-κB, and so it could improve markers of insulin metabolism (52). Vitamin D_3 might as well improve glycemic control through upregulating the insulin receptor genes (53) and increasing the transcription of insulin receptor genes (53).

This study had a few limitations. In the present study, we did not evaluate circulating fatty acid profiles before and after supplementation. Further, this study did not assess gene expression related to inflammatory cytokines and biomarkers of oxidative stress.

In summary, the current study demonstrated that taking ω-3 fatty acid and vitamin D_3 supplements together for 12 wk by patients with MS has beneficial effects on their MS disability score, inflammation and antioxidant capacity, and metabolic status including insulin metabolism.

Acknowledgments

The present study was supported by a grant from the Vice-chancellor for Research, KAUMS, and Iran. The research was supported by the donation of high-DHA tuna fish oil capsules and sunflower oil placebo capsules from Nu-Mega Ingredients Pty Ltd (Melbourne, Australia). We thank Moein Mobini for a scientific review and edit of the paper. The authors' responsibilities were as follows—ZA: conception and design, conducted statistical analysis, and wrote the manuscript, EK, MA, JA, NM, FB, and SAM: conception, collected the data, and wrote the manuscript; and all authors: approved the final paper.

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