Effects of an Extract of Salmon Milt on Symptoms and Serum TNF and Substance P in Patients With Fibromyalgia Syndrome☆

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ABSTRACT

Purpose: The aim of this study was to evaluate the effects of a dietary supplement containing primarily an extract of salmon's milt (semen) on symptoms and blood levels of proinflammatory molecules in patients with fibromyalgia syndrome (FMS), a chronic, painful musculoskeletal disease without a distinct pathogenesis or treatment. We recently reported increased serum levels of the proinflammatory molecules substance P (SP) and tumor necrosis factor (TNF) in patients with FMS as compared to those in normal controls.

Methods: This prospective, open-label study was conducted in patients with FMS (n = 87; 80 women, 7 men; age range, 18−80 years) selected from 2 clinical centers in Spain. Patients were administered the supplement and were evaluated at weeks 1 (before treatment), 4, 8, and 12 (end of treatment) for clinical parameters of functioning, fatigue, and pain, as well as overall impression. Patients were directed to take 1 capsule per day in the morning for the first 4 weeks, followed by 1 capsule in the morning and 1 capsule in the evening for the remaining 8 weeks. Differences in symptom scores in patients with FMS between weeks 1 and weeks 4, 8, and 12 were evaluated using ANOVA. Blood was obtained and serum separated in patients with FMS at 1 and 12 weeks and in a separate population of healthy controls (n = 20; 15 women, 5 men; age range, 25−65 years). Serum levels of SP and TNF were measured in patients with FMS at 1 and 12 weeks and in healthy controls by ELISA. TNF and SP levels in patients with FMS were compared between weeks 1 and 12, as well as between patients with FMS and untreated controls, using the Mann−Whitney *U* test.

Findings: Clinical parameters of functioning, fatigue, and pain, as well as overall impression, were improved significantly at 4 weeks as compared to 1 week and remained unchanged for the duration of the study (all, $P < 0.0001$). Serum TNF and SP levels were significantly elevated at 1 week in patients with FMS compared to controls and were decreased significantly at 12 weeks as compared to 1 week (all, $P < 0.0001$).

Implications: Our findings indicate that this dietary supplement may significantly improve symptoms in patients with FMS. This is the first time to our knowledge that any molecule has been reported to be associated with a reduction in serum SP level. Consequently, the supplement or its hypothesized main active ingredient, spermine, may be developed as a novel treatment approach to FMS or other neuroinflammatory conditions. ClinicalTrials.gov

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INTRODUCTION

Fibromyalgia syndrome (FMS) is characterized worldwide by chronic widespread musculoskeletal pain, sleep disturbances, stiffness, fatigue, and cognitive dysfunction and is estimated to affect 2%–8% of the adult population.[1–4] FMS is considered to be the most common cause of generalized, musculoskeletal pain in women between the ages of 20 and 55 years.[5] The diagnostic criteria for FMS have evolved over the years.[6,7] The pathogenesis of FMS is still poorly understood. Various studies have reported problems with allergens, central sensitization, chemical exposures, infectious agents, inflammation, irritants, and stress.[8–12]

Some neuroimmune mediators have been reported to be abnormal in patients with FMS, but the findings have been inconclusive.[13–15] TNF was most often elevated in the serum of patients with FMS.[16] We have confirmed that TNF is elevated in the serum of patients with FMS,[17] and we further showed significantly increased serum levels of the neuropeptide substance P (SP),[17] which is known to have proinflammatory activity.[18,19] SP may be secreted in response to physiologic or psychological stresses that are known to worsen FMS.[20,21] SP could then stimulate immune cells, especially mast cells,[22,23] to secrete TNF; mast cells are the only immune cells that store TNF and can secrete it rapidly.[24,25] Mast cells are involved not only in allergic conditions[26] but also in inflammatory diseases.[27,28] We have previously suggested that mast cells may be involved in FMS[29,30] and other comorbid conditions.[31] In particular, mast cells were significantly increased in the papillary dermis of patients with FMS,[32] who also often experience chronic urticaria.[33] Activated mast cells secrete numerous neurosensitizing and proinflammatory mediators[26] that could contribute to FMS symptoms.[27] Mast cells are located perivascularly adjacent to neurons both in the skin[34] and in the brain[35,36] and have been associated with pain.[37,38]

This study was undertaken to determine the effects of a dietary supplement containing a proprietary mixture of ingredients from salmon, especially milt (male genitalia when they contain sperm), on the symptoms of FMS as well as on the serum levels of the proinflammatory molecules TNF and SP. This supplement had previously been reported to have antiinflammatory activity in primary skin fibroblasts.[39]

Here we report the effects of a dietary supplement containing primarily salmon milt on the symptoms of FMS and on the serum levels of TNF and SP.

MATERIALS AND METHODS

Study Formulation

The dietary supplement* in this study contains a proprietary mixture of ingredients from salmon milt (male genitalia of salmon when they contain sperm; 46 mg), hydrolyzed collagen (35 mg), salmon protein hydrolysate (6 mg), CoQ10, lutein, and selenium, as previously reported.[40]

Evaluation Forms

The tools used to evaluate this product included: (1) the Fibromyalgia Impact Questionnaire–Revised (FIQ-R), the scale most commonly used to assess the severity and impact of FMS[41]; (2) the Brief Pain Inventory (BPI) (validated Spanish version[42]), which evaluates both pain intensity and interference with daily activities; (3) the Health Short Form (SF)-12, which is the shortened form of the SF-36 and has shown strong correlation with the latter in relation to both physical and mental activity[43]; and (4) the Clinical Global Impression–Improvement scale as assessed by the patient (PGI-I), a Likert scale of 7 points ranging from 1 (much better) to 7 (very much worse), used in clinical trials and other studies to assess patients' perceptions of the evolution of the disease in relation to the prescribed treatment.[44]

Study Design

This prospective, open-label, uncontrolled study was conducted in patients with a ≥13-week history of FMS (n = 87; 80 women, 7 men; age range, 18–80 years). Adult white patients were selected from 2 rheumatology centers in Spain (Clinica DKF,

* Trademark: Celergen (Celergen Ltd, Luxemburg, Luxemburg).

Madrid; and Clinica Medica Clinalgia, Murcia) if they met the 1990 American College of Rheumatology Research Classification Criteria for a diagnosis of FMS and were willing to sign the informed-consent form. Patients were excluded if they: (1) had a comorbid rheumatic disease; (2) had severe osteoarthritis in weight-bearing joints; (3) had an unstable or untreated endocrinopathy; (4) had congestive heart, renal, or hepatic failure; (5) had cancer in the prior 6 months; (6) had uncontrolled systemic hypertension; (7) had dementia; (8) had a history of drug or alcohol dependence; (9) had used of long-acting opioids in the prior 3 months; (10) received an investigational drug or device within 30 days prior to starting the study; (11) had experienced any psychiatric disorder as specified in Axis J of DSM-IV-R other than major depression; (12) had any clinically significant abnormality on clinical laboratory testing; (13) had an allergy to fish; and/or (14) or were breastfeeding or pregnant (women).

At visit 1 (week 1), a general medical history, including previous or ongoing interventions (pharmacologic or not) for the treatment of FMS, was obtained. The inclusion and exclusion criteria were reviewed, the informed consent was explained and signed, and blood was obtained, serum separated, aliquoted and stored at −80 °C.

At visit 2 (week 1), the FIQ-R and the SF-12 and BPI questionnaires were completed. The study formulation was provided with directions to be administered as 1 capsule after breakfast for 1 month, followed by an increase to 1 capsule in the morning and 1 in the evening after meals as an adjunct to any medication the patient was taking before the start of the study.

At visits 3 and 4 (weeks 4 and 8), the FIQ-R, BPI, SF-12, and PGI-I questionnaires were completed. Supplement tolerability was assessed by the recording of the occurrence of any adverse events.

At visit 5 (week 12), the FIQ, BPI, SF-12, and PGI-I questionnaires were completed. The end of the treatment was recorded. Any adverse reactions communicated by the patients were recorded. Another set of blood samples was drawn and stored as before.

Blood was obtained from all patients during visits 2 and 5 (weeks 1 and 12). Serum was separated using standard methodology, distributed in 2 separate plastic tubes, and frozen at −70 °C until analyzed. Samples were number-coded with no personal identifiers. Serum was also obtained from healthy subjects (15 women, 5 men; age range, 25−65 years) who had no history of musculoskeletal pain or inflammatory diseases and were not related to any of the patients with FMS. The serum samples from healthy controls were purchased from BBI Solutions (Cardiff, UK). All serum samples from patients and controls were shipped on dry ice and stored at −80 °C until 1 of 2 samples frozen from each subject from each group was thawed for biomarker analysis.

The clinical protocol was approved on March 4, 2016, by the Comite de Etica de la Universidad Catolica de San Antonio, Murcia, Spain (approval number 6007). All patients signed an appropriate consent form. This trial was registered retrospectively as ClinicalTrials.gov identifier NCT03911882.

Assay of Biomarkers

Serum TNF levels were determined using commercially available ELISA kits from R&D Systems (Minneapolis, Minnesota). Serum SP levels were measured using ELISA kits from Phoenix Pharmaceuticals Inc (Burlingame, California) according to the manufacturers' directions.

Statistical Analysis

All data were validated and inspected for outliers. The results are presented as scattergrams. Normality of distribution was checked with the Shapiro−Wilk test.

Scores at week 1 (no-treatment control) were compared with scores at weeks 4 (1 capsule per day), 8, and 12 (2 capsules per day) using 1-way ANOVA. The multiple uses of the no-treatment control sample for comparison with each one of the treatment timepoints were adjusted using the Bonferroni correction. Multiple comparisons among the scores at weeks 4, 8, and 12 were performed using the Tukey multiple-comparisons test. Results are reported as means (SD) and were considered significant at $P < 0.05$.

Serum levels of TNF and SP were measured in patients with FMS at weeks 1 and 12 and in healthy controls by ELISA, run in duplicate. TNF and SP levels in patients with FMS were compared between weeks 1 and 12, as well as between patients with FMS and controls, using the Mann−Whitney *U* test. Results are reported as means (SD), with differences

considered significant at $P < 0.05$. The analysis was performed using Prism software version 7.0 (GraphPad Software, San Diego, California).

RESULTS

The study started with 100 participants previously diagnosed with FMS, of whom 90 completed and 10 left the study for personal reasons (see Supplemental Figure 1 in the online article at https://doi.org/10.1016/j.clinthera.2019.05.019). Another 3 patients were excluded because of other underlying medical problems. The final FMS group consisted of 87 white patients (80 women, 7 men) with a mean (SD) age of 56.81 (13.49) years. In the subgroup of men, the mean weight was 82.13 (8.46) kg, while in women it was 67.32 (12.78) kg. The educational history indicated that >70% had post–high school education (see Supplemental Figure 2 in the online article at https://doi.org/10.1016/j.clinthera.2019.05.019). Thirty percent were gainfully employed (see Supplemental Figure 3 in the online article at https://doi.org/10.1016/j.clinthera.2019.05.019).

Clinical Assessment

All patients were rated at >50% disability on all symptom indexes (function, impact, symptoms) at the beginning of the study (week 1). The scores of all indexes were significantly reduced following the first week of treatment (week 4; $P < 0.0001$) and then remained unchanged for the duration of treatment (week 12) (Figure 1). There was no statistically significant difference between the scores of at weeks 4, 8, and 12.

Patients also showed significant improvements on pain interference (Figure 2A) and pain severity (Figure 2B) (all, $P < 0.0001$ at week 1 vs weeks 4, 8, and 12). The SF-12 mental component was also improved at week 4 ($P < 0.0001$) (Figure 3A), and the SF-12 physical activity component was improved by week 12 ($P = 0.0004$) (Figure 3B).

Serum Biomarkers

At the beginning of the study, the mean serum level of TNF was significantly increased in patients with FMS as compared to healthy controls (3.34 [3.1] vs 0.55 [0.41] pg/mL; $p = 0.0001$) (Figure 4A). The

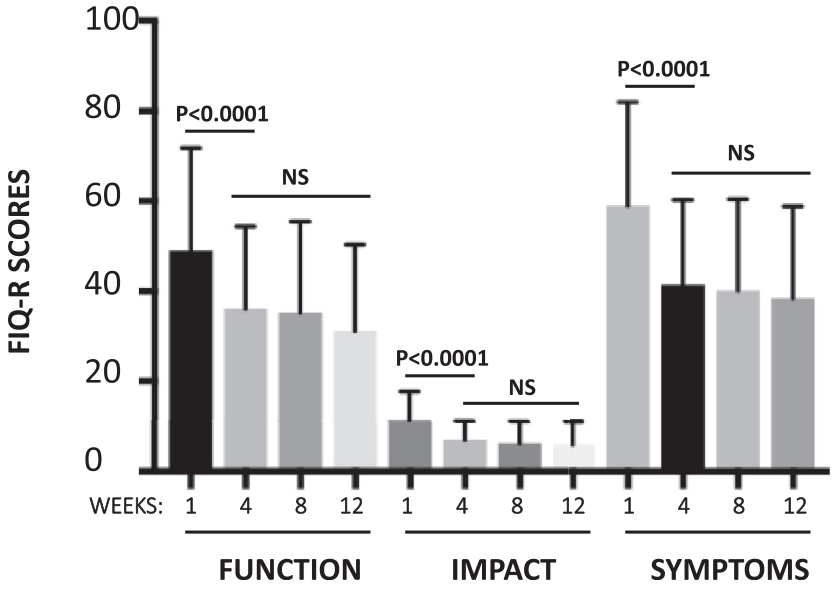

Figure 1. Mean (SD) scores on all 3 domains of the Fibromyalgia Impact Questionnaire—Revised (FIQ-R) at all weeks. Scores at week 1 (no-treatment control) were compared with scores at weeks 4 (1 capsule/d), 8, and 12 (2 capsules/d) using 1-way ANOVA. Multiple scores at weeks 4, 8, and 12 were compared using the Tukey multiple-comparisons test. Differences were considered significant at $P < 0.05$.

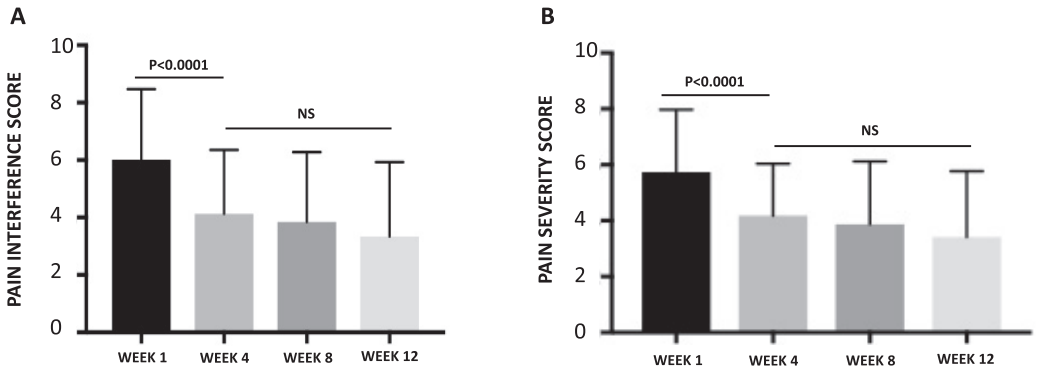

Figure 2. Mean (SD) scores on the interference (A) and severity (B) parts of the pain scale at all weeks. Scores at week 1 (no-treatment control) were compared with scores at weeks 4 (1 capsule/d), 8, and 12 (2 capsules/d) using 1-way ANOVA. Multiple scores at weeks 4, 8, and 12 were compared using the Tukey multiple-comparisons test. Differences were considered significant at $P < 0.05$.

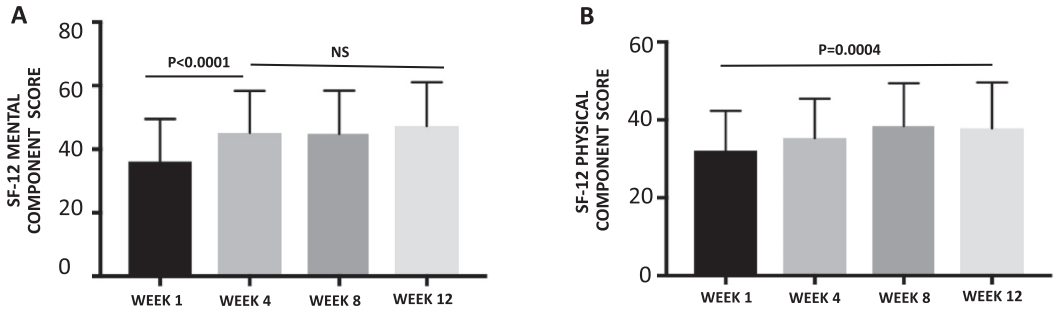

Figure 3. Mean (SD) scores on the mental (A) and physical (B) components of the Health Short Form (SF)-12 scale at all weeks. Scores at week 1 (no-treatment control) were compared with scores at weeks 4 (1 capsule/d), 8, and 12 (2 capsules/d) using 1-way ANOVA. Multiple scores at weeks 4, 8, and 12 were compared using the Tukey multiple-comparisons test. Differences were considered significant at $P < 0.05$.

mean serum level of SP was also significantly elevated in patients with FMS as compared to healthy controls (0.68 [0.31] vs 0.41 [0.31] ng/mL; $p = 0.0001$) (Figure 4B).

At the end of the study (week 12), the mean serum level of TNF was significantly reduced as compared to the beginning of the study, before treatment was started (week 1) (3.34 [3.1] vs 1.64 [1.24] pg/mL; p = 0.0003) (Figure 5A). FMS serum level of SP was significantly decreased compared to that at the beginning of the study (0.68 [0.31] vs 0.27 [0.09] ng/mL; $P < 0.0001$) (Figure 5B).

For TNF serum measurements, only 80 instead of 87 samples were included because the statistical computer software recognized 7 values as outliers that ought to be excluded.

DISCUSSION

Our findings indicate that the dietary supplement was associated with significantly improved symptoms and significantly decreased serum TNF in patients with FMS.[45] We had previously reported that TNF was elevated in the serum of patients with FMS.[17] TNF levels were also reported to be increased in the

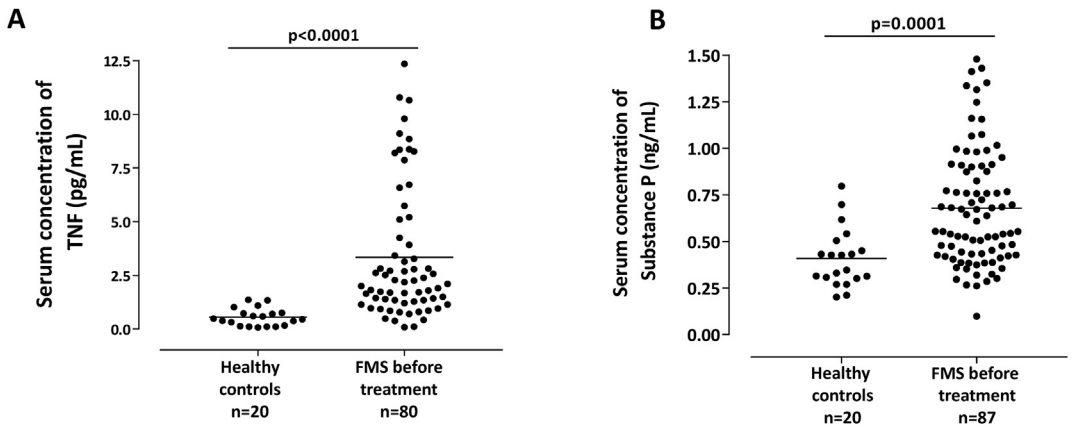

Figure 4. Week-1 serum concentrations of tumor necrosis factor (TNF) (A) and substance P (B) in healthy controls and in patients with fibromyalgia syndrome (FMS) at the beginning of the study. Symbols represent individual data points; lines represent the means in each group. Comparisons were evaluated using the Mann–Whitney *U* test. Differences were considered significant at $P < 0.05$.

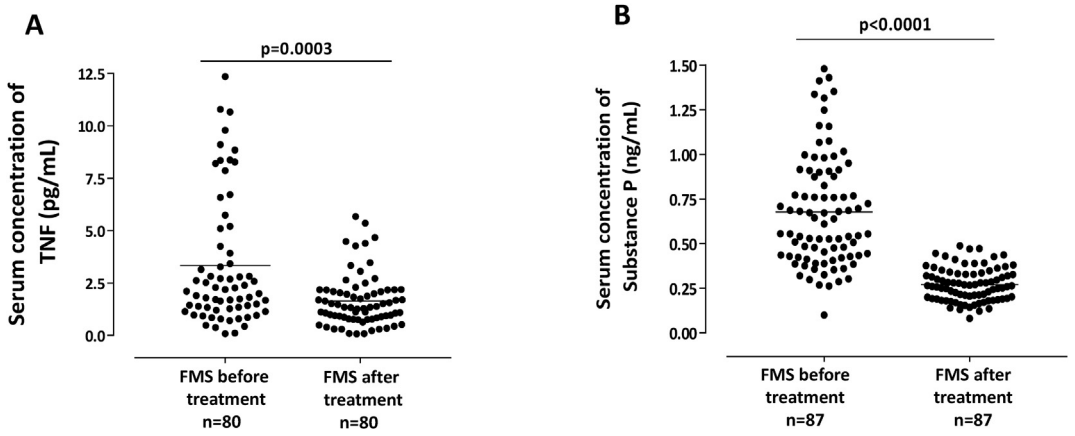

Figure 5. Serum concentrations of tumor necrosis factor (TNF) (A) and substance P (B) in patients with fibromyalgia syndrome (FMS) at weeks 1 (before treatment) and 12 (after treatment). Symbols represent individual data points; lines represent the means in each group. Comparisons were evaluated using the Mann–Whitney *U* test. Results were considered significant at $P < 0.05$.

plasma of patients with FMS.[46] TNF is up-regulated both locally and in the spinal cord in persistent pain.[47] Even though TNF is secreted by many immune cells, it is interesting that brain mast cells can synthesize and secrete TNF.[48] Moreover, mast cells are the only immune cells that store preformed TNF and can secrete it rapidly.[49–51] This is the first time to our knowledge that any intervention has been found to reduce serum levels of SP in any disease, specifically in patients with FMS, as we show that with this supplement.

SP was characterized from the rat brain[52] and is implicated in the pathogenesis of inflammation.[53–58] We had previously shown that SP is elevated in the serum of patients with FMS.[17] Elevated levels of SP had also been reported in the CSF of patients with

FMS.[10] It is interesting that serum levels of SP are elevated in patients with mastocytosis,[59] who also experience fatigue[26,60] and are also often diagnosed with FMS.[4,61] SP can stimulate the secretion of TNF from mast cells in rodents[62–64] and humans.[21,22,27,65]

SP may be secreted in response to physiologic or psychological stresses, which may explain how stress exacerbates FMS symptoms.[66,67] There is evidence of elevated levels of corticotropin-releasing hormone (CRH) in the cerebrospinal fluid of patients with FMS and a correlation with pain.[20] We reported increased serum levels of CRH, secreted under stress,[21] in patients with FMS.[17] We had also reported that SP induces the expression CRH receptor-1 on mast cells,[68] the activation of which induces selective release of vascular endothelial growth factor,[69] permitting inflammation. CRH-positive nerve endings are plentiful in the median eminence of the hypothalamus, where mast cells are most plentiful.[35] Mast cells derive from bone marrow progenitors and mature in tissues depending on microenvironmental conditions.[70] Mast cells are crucial for the development of allergic reactions,[26] but are also implicated in immunity[71] and inflammation.[27]

The mechanism of action of the supplement on clinical improvement in patients with FMS, as well as of the reductions in TNF and SP, is not presently known. The supplement was previously reported to reduce liver carcinogenesis[40] and pancreatic cancer.[72] The polydeoxyribonucleotide content of salmon has been reported to have antiinflammatory activity possibly via the activation of the adenosine A_{2A} receptor.[39]

We suggest that a more plausible mechanism of action of the supplement may be related to the salmon milt content of the polyamines spermidine and spermine.[73] For instance, the amount of spermine in salmon was reported to be 5.96–6.04 mg/100 g weight, and in mackerel, 22.1–26.9 mg/100 g weight.[73] These natural cationic molecules are produced by ornithine decarboxylase[74] and have been reported to have immunomodulatory[75] and antiinflammatory[76] activity. Spermine may also have direct inhibitory activity on SP. For instance, it has been reported that spermine can bind to SP and inactivate it.[77] Polyamines may also be conformationally linked to SP via transglutaminase[77] and switch its binding capacity from the proinflammatory neurokinin-1 to neurokinin-3 receptors.[78,79] Moreover, spermine was reported to be a negative regulator of mouse liver[80] and skin[81] macrophage activation. In addition, we reported that oxidized polyamines can inhibit mast cells,[82] the secretory granules of which were reported to contain spermine,[83] where it regulates their ability to secrete proinflammatory mediators.[84]

Another possibility is that the milt content of the supplement may contain the cytokine interleukin (IL)-37, which was identified in human seminal plasma.[86] IL-37 has been reported to have antiinflammatory properties.[85,87,88,89] However, serum levels of IL-37 have not yet been measured in patients with FMS.

Limitations

In this open-label study, the clinical assessment was subjective. This study needs to be repeated in a double-blind, randomized fashion. Although we hypothesize that the beneficial effects of the supplement may be due to spermine, the main active ingredient of the supplement is not presently known. The control serum was purchased and was not obtained and stored by the investigators involved in this study. The demographics of the donors were generally similar to those of the patients. Such purchased control serum samples from the same company were used previously in a study in patients with FMS.[17] Serum TNF and SP were measured in a blinded fashion and constitute crucial objective findings.

CONCLUSIONS

Our findings indicate that use of this supplement may result in significant clinical benefit in patients with FMS. Even though SP receptor antagonists have been developed, this is the first time to our knowledge that any molecule has been reported to be associated with significantly reduced serum SP levels. Consequently, this supplement or its main active ingredient may be developed as a novel treatment approach to FMS or other neuroinflammatory conditions.

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Drs. Tsilioni and Pipis contributed equally to, but in different aspects of, this study.

CONFLICTS OF INTEREST

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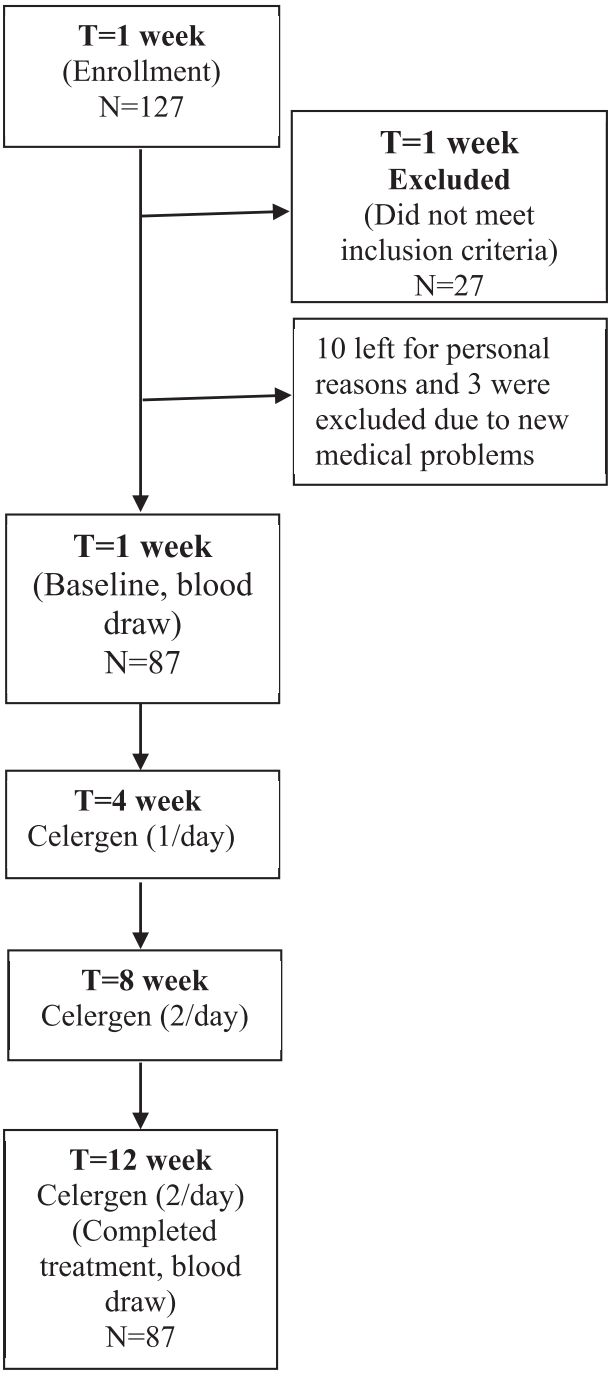
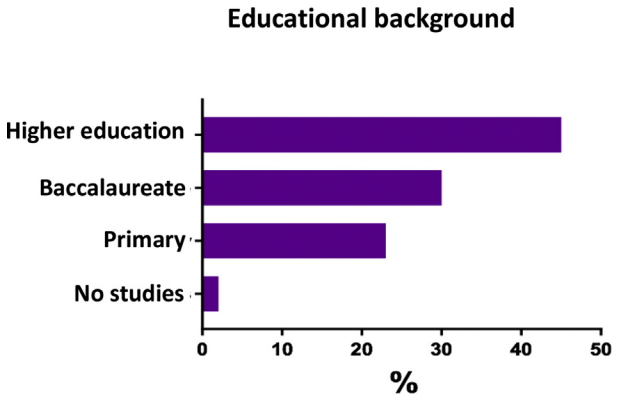
APPENDIX A. SUPPLEMENTARY DATA

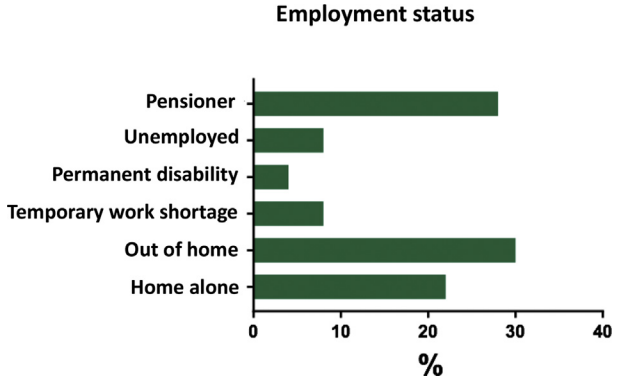
The following is the supplementary data related to this article:



Supplementary Fig. S1. Participants Flow Chart.



Supplementary Fig. S2. Educational background.



Supplementary Fig. S3. Employment status.