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Dietary Supplements in Combination with Conventional Medicine among People with Multiple Sclerosis

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Abstract: Despite recent advances in immune-modulatory drugs, pharmacological therapies have been proven ineffective in severe presentations of multiple sclerosis (MS), including secondary progressive MS. At present, therapeutic interventions' performance is primarily focused on ameliorating symptoms to improve the patient's quality of life. Among complementary treatments, nutrition has been considered a decisive factor to control symptoms and enhance the wellness of MS patients. Although no special diets are associated with MS, the impact of diet and dietary supplements on the course of progressive forms of the disease have been studied during the last years. Fatigue is among the most common and disabling symptoms reported by MS patients. Fatigue has been defined in the Multiple Sclerosis Council for Clinical Practice Guidelines (MSCCPG, 1998) as a "subjective lack of physical and/or mental energy that the individual perceives as an interference with habitual and desired activities". This study aimed to compare the psychometric functioning of the "Fatigue Severity Scale" (FSS) and the "Modified Fatigue Impact Scale" (MFIS) in our sample of people with MS. Specifically, during chronic treatment, the change in these two parameters with two vitamin-rich dietary supplements (Citozym® and Ergozym®) was evaluated. The impact of these nutritional supplements revealed differences in antioxidant and anti-inflammatory parameters between treatment groups with subsequent improvement in fatigue. In conclusion, the results obtained have confirmed the effectiveness of complementary nutritional therapies, evaluated essentially based on hematological biomarkers, through which it is possible to act on disability to improve the quality of life of MS patients.

Keywords: Multiple Sclerosis, fatigue, dietary supplements, vitamins, folic acids.

1. Introduction

Multiple sclerosis (MS) and acute disseminated encephalitis, acute necrotic haemorrhagic encephalitis, and myelinopathy represent a set of diseases characterized by demyelination areas in the central nervous system, with an inflammatory response and a consequent neurological course of great importance, leading to severe disability [1]. Modifiable factors, including sunlight exposure (vitamin D), obesity at a young age, and dietary intake, have been associated with the pathogenesis of the disease process [2]. However, dietary patterns in people with MS have not been extensively studied. Recently, it has been reported that oxidative stress (OS) can play a role in demyelization and axonal damage in patients with MS, both of which can lead to cellular damage through the action of oxidizing cellular components, including lipids, proteins and the DNA. Myelin destruction plays the primary role in disability in MS patients, and myelin sheaths are highly sensitive to OS [3].

There is a balance between oxidative damage and antioxidant protection in normal aerobic cells. Insufficient antioxidant protection or excessive production of reactive oxygen species (ROS) generates a condition known as OS, which is believed to play an important role in MS [4].

Currently no effective clinical indications for applying dietary supplements as a complementary treatment against MS symptoms are available. Examining the therapeutic effects of a range of nutritional supplements suitable for controlling cell metabolism in relapsing and progressive MS forms, can be an interesting approach to complement

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the limited range of treatments available [5]. Similarly, the impact of dietary intervention on inflammation can be enhanced by supplements with intense antioxidant activities. Despite current therapies aimed at improving the disease, poor quality of life in MS patients remains a significant problem, and fatigue is one of the common and disabling symptoms [6]. Patients with MS have significantly reduced quality of life compared with the general population. Some studies have reported that reduced quality of life may be partly the consequence of neurological disability. Fatigue represents the most frequent and disabling symptom among patients with MS, markedly interfering with daily life. Primary fatigue's pathophysiology remains unknown, but undoubtedly, inflammation-related, OS and immunologic factors play a central role. The treatment of this symptom to improve the quality of life presently remains a challenge.

Disability, depression, and fatigue are independent predictors of life quality in people with MS [6]. Fatigue is one of the most common symptoms of multiple sclerosis and may even be the first sign of the disease. Fatigue affects between 75 and 95% of patients; it is not strictly correlated with gender, race, and education. It is considered by 55-75% of patients as one of the most disabling symptoms [7]. However, its diagnosis is not easy because it represents a subjective state, difficult to describe and understood by others. It interferes with the person's physical and mental activities with MS and contributes to worsening the difficulties already present, negatively affecting life quality. Fatigue can have a psychological impact on the person, especially when fatigue and lack of strength make it harder to perform even the simplest tasks.

Effective treatment options for fatigue remain limited. In this observational study was noted that, among the multiple symptoms of volunteers with MS, fatigue was one of the most painful complaints that patients may experience throughout their lives. The pathogenesis of fatigue and its primary causes remains obscure, and there are currently no adequate therapies. One of the main obstacles to understanding this symptom was the absence of a measurement method universally accepted, that could quantify an experience properly, often disabling and elusive. Without a measurement, progress in fatigue therapy remained limited. Currently, this limitation has been overcome employing two assessment methods [8], such as the psychometric functioning of "Fatigue Severity Scale" (FSS) and the "Modified Fatigue Impact Scale" (MFIS), which is part of the Multiple Inventory Quality of Life (MSQLI), a battery consisting of 10 individual scales providing a quality of life measure that is both generic and MS-specific [9].

Several factors could contribute to fatigue development and/or exacerbation, and this requires careful work-up to search for all possible underlying causes, such as sleep disorders, endocrine dysfunction and mood disorders, to name a few. Central fatigue is described as fatigue not from the muscle itself but rather from the central nervous system (CNS) and the transmission of signals from the brain to the muscle [6]. Therefore, central fatigue is related to the brain and spinal cord [10].

Nutrition is considered a possible factor in the pathogenesis of MS. Current studies in nutritional interventions suggest that diet may be regarded as a complementary treatment to control disease progression [11]. Although several observational studies have demonstrated a relationship between specific dietary patterns and the prevalence of MS, very few have shown a correlation of the diet with fatigue and quality of life, and even fewer have correlated nutritional intake with biological markers in people with MS. Low levels of specific micronutrients, including vitamins D, B12, and A, have also been shown to contribute to the pathogenesis of MS, and found diets lower in folate and magnesium to correlate to increased fatigue in MS [5]. The purpose of this observational study was to relate the intake of two nutritional supplements containing vitamins and folic acid, among other numerous micro-nutrients, to fatigue in patients with MS.

2. Materials and Methods

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This observational study was conducted on 60 MS patients to determine the therapeutic and protective effects of two commercial dietary supplements called Citozym® and Ergozym® ,(Citozeatec Italy-FDA registration 12932524008 Pin n. bfJ3h263). The two nutritional supplements contain vitamins C, B5, D, B9 (Citozym®), A,B5, B2,B3,B6,B9,B12 (Ergozym®). A total of 60 volunteers with a "Kurtzke Extended disability status score" (EDSS) of less than 6 were recruited for the study, with a definitive diagnosis of relapsing-remitting form of MS. The EDSS is widely used to measure and assess the clinical characteristics of multiple sclerosis patients. It is also a widely accepted tool in clinical trials, for example, to assess the effect of treatments on disease progression [12]. The total EDSS score is determined by two factors: walking ability and scores for eight functional systems. A subscale is used that assesses the functional status of certain functional systems that are variably affected by the disease. 30 volunteers were treated for 70 days according to an experimental nutritional protocol (see supplemental material), and 30 were treated using a placebo preparation made with distilled water, honey, and permitted food pigments, with the same color as that of Citozym® and Ergozym® and considered as a negative control. The age ranged from 30 to 50 years, and the sex was predominantly female (the number of women with multiple sclerosis is almost three times that of men). Demographic and anthropometric characteristics expressed as mean and standard deviation were collected from the patients' medical history (**Table I**).

Table I

Demographic and Anthropometric Characteristics of Participants

Average age (years)	42 (± 10)
Height (cm.)	170 (± 5.37)
Weight (Kg.)	65 (± 10.37)
BMI	24.5 (± 2.76)
Duration of MS (years)	14 (±3.00)

Values expressed as an average ± Standard Deviation

Fatigue symptoms were quantified using the psychometric tests FSS and MFIS [13]. Items on the MFIS can be aggregated into three subscales (physical, cognitive, and psychosocial) and a total MFIS score. All items are scaled so that higher scores indicate a more significant impact of fatigue on a person's activities. In this experimental trial, only the physical and total MFIS scores were considered. Written informed consent was obtained from all study participants. The study was reviewed and approved by the board of the "CRSC" Research Institute, under protocol No. "AB2745P28" on 25 January 2020 as part of the "Dietary Supplementation Project". Patients with secondary or primary progressive MS, pregnancy, corticosteroid treatment, or who simultaneously suffered from another chronic disease such as rheumatic disease, severe heart disease, malignant cancers, and other neurologic and inflammatory diseases were excluded. Patients were advised not to discontinue their routine medications. Written informed consent was completed before the study for all patients. No specific blood tests are available for the diagnosis of MS. However, after the patient's objective examination and history, a series of blood tests were prescribed with a view to a differential diagnosis, the purpose of which was not to confirm the presence of MS but to check the inflammatory status and OS levels to preclude the presence of diseases whose symptoms might overlap with those of MS.

2.1 Processing and analysis of blood samples.

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Venous blood sampling was performed according to "WHO Guidelines on Drawing Blood, 2010". Whole blood was immediately centrifuged at 500×*g* for 20 min after collection. Venous blood samples (5 mL) were collected at the beginning and weekly during the treatment. Serum was separated and aliquots were stored at -80°C. The total antioxidant status (TAS), superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) activities were determined by previously reported method [14, 15]. Several routine blood parameters were monitored (blood glucose, total cholesterol, triglycerides, total lipids, albumin, creatinine, retinol-binding protein (RBP), and C-reactive protein (CRP). Measurement was accomplished through the CBC test using Cell Counter Sysmex XP-300 model (Sysmex, Kobe, Japan) and chemiluminescence microparticle immunoassay/Abbott biochemical method using Abbott IMX kits with Abbott IMx® unit (Abbott Diagnostics, Lake Forest, IL, USA).

2.2 Statistical analysis

The statistical analysis was performed using SPSS software (ver. 14.0; SPSS Inc., Chicago, IL). The data were expressed as average ± standard deviation (SD). The differences in clinical and biochemical variables between pre and post within each intervention group with normal distribution were analyzed using the coupled t-test. The statistical significance was defined as $p < 0.05$.

3. Results

Enzyme oxidation activities (**Table II**) were assessed from blood samples taken from treated and placebo-treated patients. Tests performed to monitor patients' oxidative status following treatment showed increased total antioxidant status (TAS) values (from 0.32±0.20 to 0.89±0.30 U/mg protein). All enzymatic parameters related to the antioxidative status by treatment with the mentioned nutritional supplementation were found increased: SOD (from 3.84±1.80 to 6.43±0.60), GPx (from 2.95±0.56 to 4.95±0.67) and Cat (from 2.34±1.37 to 5.24±1.27), with values, expressed as U/mg protein **(Table II).**

Table II

Monitoring the oxidative state of volunteers

	Control	Treated
TAS (U/mg protein)	0.32 (± 0.20)	0.89 (± 0.30)
SOD (U/mg protein)	3.84 (± 1.80)	6.43 (± 0.60)
GPx (U/mg protein)	2.95 (± 0.56)	4.95 (± 0.67)
Cat (U/mg protein)	2.34 (± 1.37)	5.24 (± 1.27)

Values expressed as an average ± Standard Deviation

Hematological parameters showed increased levels of blood glucose (from 84.52±10.37 to 94.61±6.30 g/dL), total cholesterol (from 182.70±42.88 to 232.84±52.22 mg/dL), triglycerides (from 104.75±52.72 to 112.73±32.24 mg/dL), total lipids (from 632.82±130.42 to 732.86±100.20 mg/dL), albumin (from 4.45±0.52 to 7.45±0.32 g/L), creatinine (from 0.35±0.02 to 0.83±0.25), and RBP (from 2.12±1.50 to 5.82±1.92 mg/dL), while CRP (from 18.45±9.53 to 9.22±6.42 mg/dL) appeared decreased (**Table III**).

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Table III

Hematological Parameters

	Control	Treated
Glycemia (mg/dL)	84.52 (± 10.37)	94.61 (± 6.30)
Total Cholesterol (mg/dL)	182.70 (± 42.88)	232.84 (± 52.22)
Triglycerides (mg/dL)	104.75 (± 52.72)	112.73 (± 32.24)
Total Lipids (mg/dL)	632.82 (± 130.42)	732.86 (± 100.20)
Albumin (g/L)	4.45 (± 0.52)	7.45 (± 0.32)
Creatinine (mg/dL)	0.35 (± 0.02)	0.83 (±0.25)
RBP (mg/dL)	2.12 (± 1.50)	5.82 (± 1.92)
CRP (mg/L)	18.45 (± 9.53)	9.22 (± 6.42)

Values expressed as average ± Standard Deviation

The symptom of fatigue was examined to assess treatment effectiveness with the two nutritional supplements, using the FSS and MFIS tests. As with the FSS, a higher score in the MFIS means that fatigue has a more significant impact on the patient's life. FSS is a self-report scale of nine items about fatigue, its severity and how it affects certain activities. Responses are rated on a seven-point scale where 1 = strongly disagree and 7 = strongly agree [16]. Thus, the minimum possible score is 9, and the maximum is 63. The FSS test administered, to patients, to assess the impact of fatigue in their daily activities was performed at intervals during the administration of the two nutritional supplements combined with the usual daily diet. Comparing the values obtained for the FSS in treated patients to the control allowed highlighting a significant reduction of the symptom fatigue of about 68% during the 70 days of observation (**Figure 1**).The significance of these reductions indicates a clear improvement in the symptom of fatigue.

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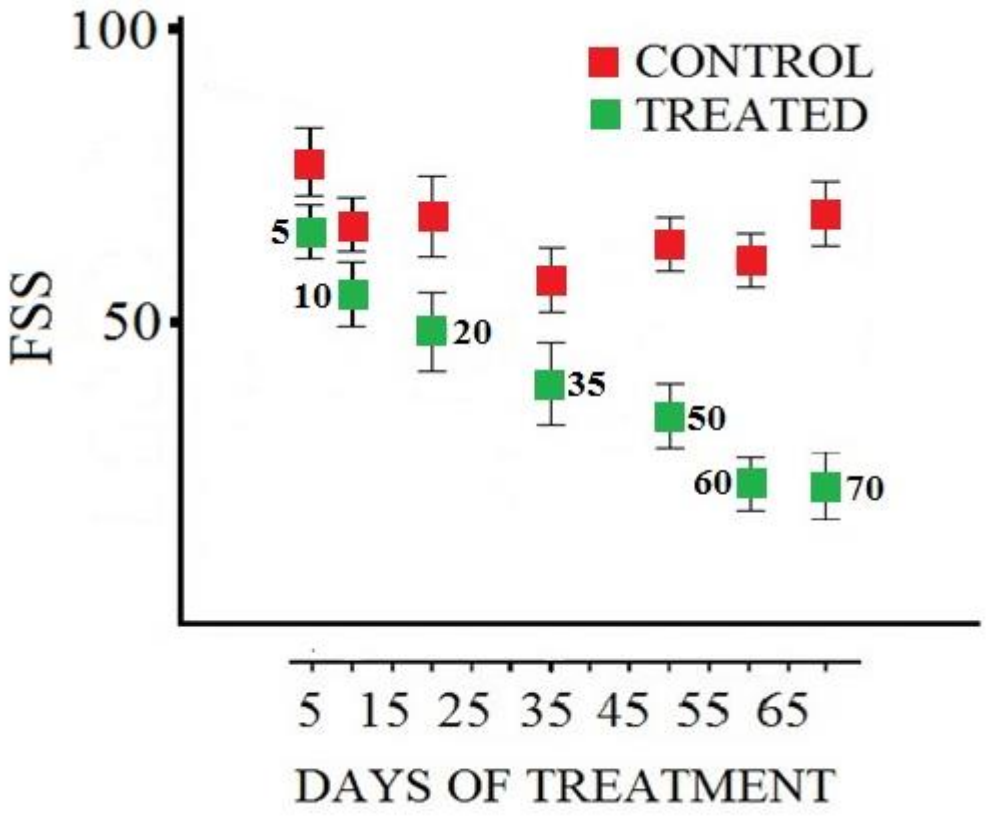

Figure 1. Changes in FSS values in control subjects and those treated with nutritional supplementation as described in the text. *p>0.05*

As is well known, the MIFS scale is divided into three subscales: physical, cognitive and psychosocial [17]. To highlight the fatigue aspect, the physical subscale was compared with the total MIFS score. From **Figure 2** it is possible to observe how much the physical score has affected the total MIFS score. Fatigue reduction reached and remained during the 70-day treatment period approximately 46% lower than control, similarly to that observed for the total MIFS score reduction (35%). This data suggests how much the symptom fatigue can influence the psychosocial and cognitive aspect of MS patients.

4. Discussion

This study explored the effects of a multivitamin nutritional treatment, in patients with a definitive diagnosis of relapsing-remitting form of MS, on a prevalent symptom of this pathology: fatigue. It was possible to show a significant temporal improvement in the quality of life of treated subjects. Despite recent advances in developing immunomodulatory drugs, therapies have proven ineffective in severe MS presentations, including secondary progressive MS. Therapeutic interventions to improve patients' quality of life have focused primarily on symptom control. Among complementary treatments, nutrition has been considered a decisive factor to control symptoms and improve the well-being of MS patients [18]. Although there are no particular diets associated with MS, the impact of diet and dietary supplements on the course of progressive forms of the disease has been studied in recent years [19]. Current research has demonstrated a correlation between deficiency of vitamins A, B12, B6, C , D and folic acid in the diet and the development and exacerbation of MS symptoms [20]. Most MS patients have long-term vitamin D

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deficiency, characterized by low bone mass and high fracture rates [21]. Notably, it has been suggested that increased serum concentrations of vitamin D, a potent immune-modulator, may reduce the risk of MS [22]. Experimental evidence has also shown that serum vitamin D concentrations are lower during MS relapses than in remission and are associated with a greater degree of disability [11]. Additional evidence reports that circulating vitamin D can be considered a biomarker of MS and recommends vitamin D administration for therapeutic purposes [23]. Vitamin D shows significant anti-inflammatory effects. Some studies have reported a possible association between OS markers and vitamin D level [24], concluding that vitamin D supplementation could reduce the levels of OS markers [25]. The antioxidant effect of vitamin D seems to have a protective role on neurons and could alleviate MS progression [26].

Vitamin A supplementation as a therapeutic possibility for MS has been investigated [27] for its proven benefits at a cellular level [28, 29] . Several pieces of evidence suggest that inadequate levels of vitamin A result in the organism's inability to maintain the normal balance of the T-cell subgroups,[30] as well as a negative correlation between serum vitamin A and the development of the disease; because the plasmatic level of vitamin A is lower in patients with MS.[31,32] A cohort study suggested an inverse association of vitamin A levels in serum and the activity of relapsing-remitting MS employing magnetic resonance imaging.[33].

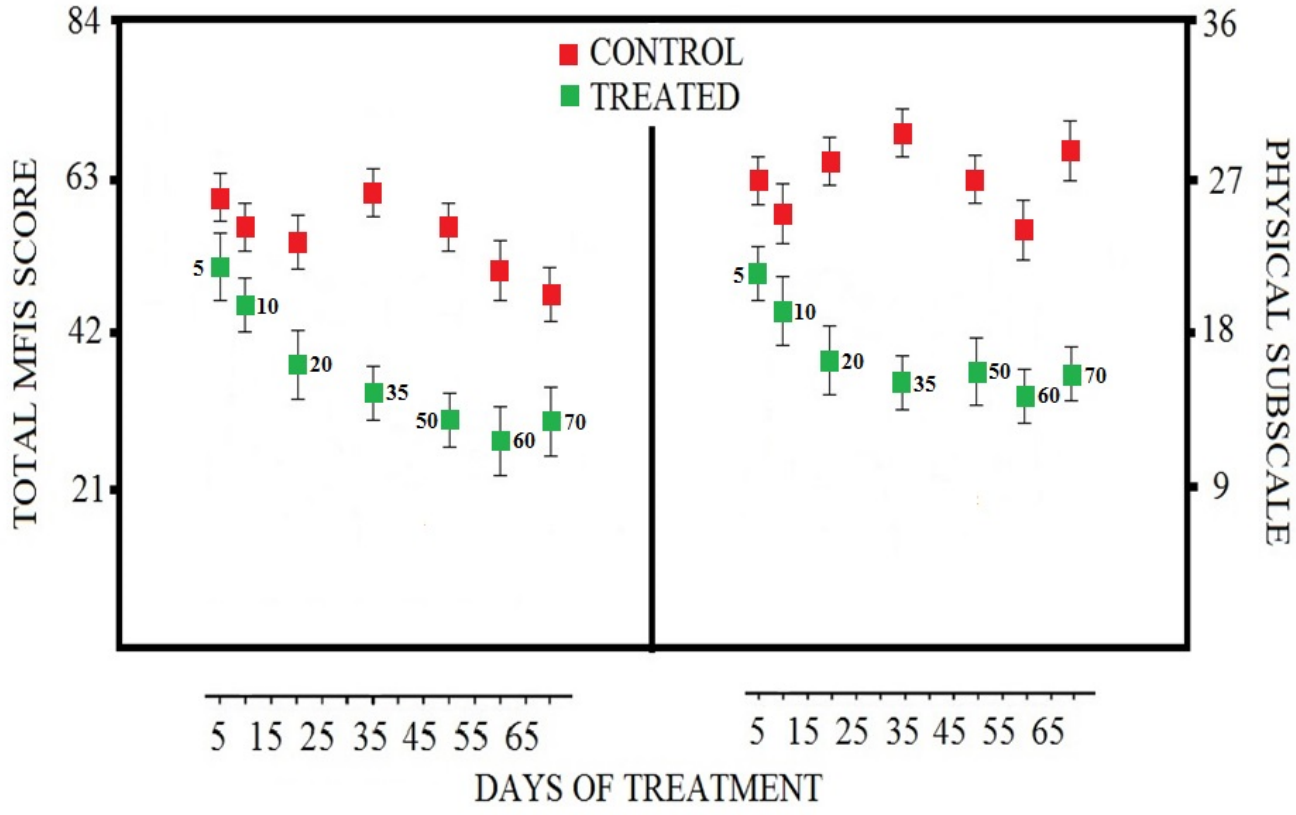

Figure 2. (A) Changes in MFIS score in control subjects and those treated with nutritional supplementation as described in the text. *p>0.05.* (B) Influence of physical subscale on total MIFS score. *p>0.05*.

Vitamin B12 deficiency leads to low availability of methionine and S-adenosyl-methionine, both of which are necessary for myelin synthesis. A meta-analysis study conducted on MS patients to find a relationship between vitamin B12, B6, and other micronutrients, showed a possible link between the appearance of the first neurological signs of MS and serum levels of vitamin B12, B6, and folic acid. Indeed, increasing the intake of vitamin B12, B6, and

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folate in immunotherapy treatments has shown promising MS patients results [34]. The explanation for the role of vitamins B12, B6, and folate in MS has recently been highlighted. Several studies have established that myelin replacement requires the natural function of the "folate-vitamin B12-methylation" pathway, which is vital to provide methyl groups for myelin regeneration. [34].

Several pieces of evidence confirm that inflammation is involved in all stages of MS development and progression [36], markers of inflammation, such as CRP, may provide additional information about the biological status of the lesion. It was observed that patients enrolled for the trial initially had high serum CRP levels. CPR values gradually decreased during the two nutritional supplements' administration, compared with the control, to reach significantly lower values. This decrease could be in response to the reported effect of the anti-inflammatory properties of Citozym® and Ergozym® [37]. Based on recent investigations suggesting that physical activity reduces CRP levels [38], the reduction of these parameter values, observed in the treated group of patients, compared with the control group, could be related to the marked improvement in the symptom fatigue, which would allow for greater muscle motility, improving the quality of life of MS patients.

It is well known that OS, as responsible for demyelination, plays a crucial role in MS, [39]. Indeed, it seems to be involved in oligodendrocyte loss, neuronal damage and myelin degeneration [40]. It has been proposed that OS's biomarker enzymes may be considered helpful in evaluating MS therapeutic treatments' outcomes [41]. Neuronal and glial cells are very sensitive to OS, and neurons appear to be particularly sensitive to vitamin C deficiency, therefore, ascorbate recycling by astrocytes and neuronal uptake, are important mechanisms in restoring or maintaining redox homeostasis under oxidative conditions.

Ascorbic acid (vitamin C) is essential for several physiological processes in the human body. It is present in high concentration in the brain, particularly in the central nervous system (CNS), promoting oligodendrocyte generation and remyelination [42]. Ascorbate recycling by astrocytes and neuronal uptake are essential mechanisms to restore or maintain redox homeostasis under oxidative conditions. Neuronal and glial cells are susceptible to OS, and neurons appear to be particularly sensitive to vitamin C deficiency. In demyelinating diseases, such as MS, myelin sheaths are damaged, and the remyelination process is somewhat hampered. Restoration of myelin sheaths requires differentiation of oligodendrocyte precursor cells into mature oligodendrocytes [42]. Since the production of reactive oxygen species is increased in inflammatory and demyelinating diseases such as MS, as a response to the failure of cellular detoxification and antioxidant mechanisms, the results presented in this report would suggest a reduction of oxidative damage operated by the nutritional therapy adopted. Concerning this, it was possible to observe that the degree of OS appeared high in placebo-treated patients and was significantly reduced following treatment with the two nutritional supplements. In fact, in control patients, a low level of activity of some of the main antioxidant enzymes, such as Cat, GPx and SOD, whose activity was increased by the nutritional treatment, was found. Since the Cat enzyme removes hydrogen peroxide produced during inflammation, this enzyme's increased activity may explain the reactivation of the detoxification process after the administration of Citozym® and Ergozym®. Serum levels of RBP, creatinine, and CRP have been proposed as potential biomarkers for neurodegenerative diseases, including amyotrophic lateral sclerosis, multiple sclerosis, and Parkinson's disease [43]. Blood creatinine levels primarily reflect the amount of muscle mass and renal function [44], and an inverse relationship between creatinine level and multiple sclerosis progression has been proposed [45]. The changes in these three parameters, observed following the inclusion of the two nutritional supplements in MS subjects' daily diet, allowed us to follow their blood levels' normalization.

5. Conclusions

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In conclusion, the reported exploratory study suggests that the administration of some key cellular metabolism components as part of nutritional treatment may have a beneficial effect on MS-associated fatigue. However, further studies including larger cohorts of patients will be needed to confirm diet's role in this disabling symptom in MS. Although sample size limits the statistical significance of this study and the interpretation of results, the comparison between placebo and supplemented groups allows the assessment of relevant trends and set the basis for further investigation. The effectiveness of complementary and alternative nutritional therapies should also rely on developing feasible non-invasive markers that allow the quantification and the implementation of disability progression scales to improve MS patients' quality of life.

Competing Interests

The authors declare that they have the following competing interests to disclose in connection with this article: F.P., D.M., and L.S. have served as scientific advisors to "CRSC". B.S., I.B. and S.A. have no competing interests to disclose in connection with this article. F.A. is the president of the Scientific Association "ARSS" who offered his contribution as a nutritionist. However, no significant competing interests could have influenced the results of this work.

Author Contributions:

Conceptualization, Simone Beninati; Formal analysis, Ilaria Borromeo and Simone Beninati; Funding acquisition, Francesco Antonelli; Investigation, Anna Shevchenko, Manfred Doepp and Stefano Lenzi; Methodology, Ilaria Borromeo and Simone Beninati; Supervision, Pasquale Ferorelli; Writing – original draft, Simone Beninati; Writing – review & editing, Simone Beninati.

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