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Original Article

Effects of Probiotics Supplementation on General Health, Fatigue and Its Related Factors in Patients with Relapsing-Remitting Multiple Sclerosis: A Randomized, Double-Blind, Placebo-Controlled Trial

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Abstract

Introduction: Fatigue is one of the most debilitating and least understood symptoms of multiple sclerosis (MS). This study aimed to evaluate the effects of probiotics supplementation on general health, fatigue, and its related factors in patients with relapsing-remitting MS (RRMS).

Methods: In this double-blind, randomized, placebo-controlled trial, 90 RRMS patients with Expanded Disability Status Scale (EDSS) < 4 were enrolled. Participants received a multi-strain probiotics supplement (Lactocare®) or placebo for 16 weeks. The primary endpoint was the Physical, Psychological, and Social Fatigue Index derived from the Comprehensive Fatigue Assessment Battery for Multiple Sclerosis (CFAB-MS). Secondary outcomes included general health assessed by visual analogue scales (VAS) and other CFAB-MS domains, including pain, mood, stress, sleep, anxiety, nutrition, mobility, environment, and fatigue management.

Results: Out of 90 randomized patients, 60 completed the trial (29 in the probiotics group and 31 in the placebo group). Probiotics supplementation did not result in statistically significant differences in Physical, Psychological, and Social Fatigue Index (Effect size (r) = -0.087; *P*-value = 0.50) or most secondary outcomes between groups ($P > 0.05$). Analysis of covariance (ANCOVA), with baseline values score as a covariate was not associated with a significant outcome (*P*-value > 0.05). After applying the Benjamini–Hochberg correction for multiple comparisons, no effect remained statistically significant.

Conclusion: The findings suggest that routine use of multi-strain probiotic supplementation for fatigue management in patients with RRMS and EDSS < 4 cannot be recommended. Future randomized trials with longer follow-up, dietary control, and integrated microbiome and inflammatory biomarker assessments are warranted.

Introduction

Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system (CNS) characterized by inflammation, demyelination, gliosis, and neuronal loss.[1]The diverse clinical presentations of MS are attributed to the location of CNS lesions.[2] MS typically manifests during the most productive years of life, often coinciding with career development and family formation, thereby exerting a substantial impact on individuals, their families, and society.[3,4] Among its various clinical phenotypes, relapsing-remitting MS (RRMS) is the most common form.[5] Fatigue is the most frequently reported symptom, affecting up to 90% of individuals with MS. Despite its prevalence, fatigue remains one of the least defined and inconsistently characterized symptoms of the disease.[6] Consequently, fatigue management is a critical clinical challenge in MS care.[7,8] Fatigue in MS may be classified as either primary or secondary. Primary fatigue results directly from MS-related pathophysiological mechanisms. In contrast, secondary fatigue can stem from associated conditions.[9,10] Although the precise etiology of MS-related fatigue remains unclear, it is understood to be multifactorial, involving both psychological and physiological components.[11] Recent evidence indicates that MS-related fatigue arises from a complex interaction of psychological, neurological, and systemic factors, and is closely associated with symptoms

such as depression, anxiety, pain, sleep disturbances, and spasticity.[12-15]

One major contributor to MS-related fatigue is inflammation. Pro-inflammatory cytokines such as Tumor Necrosis Factor-alpha (TNF-α), Interleukin (IL)-1, and IL-6 can cross the blood brain barrier, initiating neuroimmune responses that exacerbate fatigue.[16,17] Chronic disease-related fatigue is often linked to a pro-inflammatory state, and multi-nutrient anti-inflammatory interventions may be more effective than single-agent approaches.[18] This has prompted an investigation into interventions with systemic anti-inflammatory effects, such as probiotics, as potential treatments for MS-related fatigue. Probiotics modulate systemic inflammation by altering gut microbiota composition, enhancing gut barrier integrity, and regulating immune responses.[19] Several studies have reported probiotics-induced reductions in inflammatory cytokines in MS [20]. A 12-week double-blind randomized controlled trial (RCT), conducted by Rahimlou et al, reported significant reductions in C-reactive protein (CRP), TNF-α, and Interferon gamma (IFN-γ) levels following probiotics use in MS patients.[21] Tankou et al. [22] found that a two-month probiotics regimen led to an anti-inflammatory environment and a reduction in intermediate monocytes. Another six-month clinical trial showed increases in brain-derived neurotrophic factor (BDNF) levels while decreases in IL-6 levels.[23] In addition, evidence suggests the role of anti-inflammatory diets in the management of MS-related fatigue.[24, 25] Given these findings, antioxidant effects of probiotics supplementation [26], and association between oxidative stress and MS-related symptoms [13, 27], it was hypothesized that probiotic supplementation might offer similar benefits.

Despite growing interest in the neurological and psychological benefits of probiotics, their therapeutic potential in MS remains underexplored. Studies have examined the impact of probiotics therapy on MS, reporting favorable clinical and metabolic outcomes. Clinical trials have also reported a reduction in pro-inflammatory cytokines (e.g., TNF-α and IL-6), improvements in oxidative stress markers, and enhanced immune function.[20, 23, 28, 29] A recent meta-analysis reported that probiotic supplementation improved mental health outcomes in MS patients, but noted that the included studies were of limited quality, underscoring the need for larger and more rigorous randomized controlled trials.[30-32]Limited evidence is available regarding the effects of probiotics on fatigue in patients with MS. One randomized controlled trial reported that Saccharomyces boulardii supplementation reduced fatigue severity. [33] Therefore, to address this gap, the present randomized controlled trial aimed to evaluate the effects of probiotic supplementation on general health, fatigue, and its related factors in patients with RRMS.

Methods

Study Design

This research was conducted as a parallel, randomized, double-blind (participants and outcome assessors were not aware of the assigned intervention), placebo-controlled trial aimed at assessing the effects of probiotics supplementation on fatigue and its related factors in patients with RRMS. The study adhered to the Consolidated Standards of Reporting Trials (CONSORT) guidelines in its reporting [34]. The allocation ratio for this trial was 1:1.

Participants

Participants were recruited from a university-affiliated MS clinic. The details of the eligibility criteria, as well as the recruitment strategy, are presented elsewhere [35]. In brief, eligible participants were individuals diagnosed with RRMS based on the 2017 McDonald criteria [36], with Expanded Disability Status Scale (EDSS) < 4, and reporting fatigue at baseline based on self-report. Fatigue severity was subsequently assessed using Comprehensive Fatigue Assessment Battery for MS (CFAB-MS), at baseline and follow-up and was included among the study outcomes.

Intervention

The intervention group received a multi-strain probiotics supplement (Lactocare®) containing 2×10^9 colony-forming units (CFUs) of *Lactobacillus casei*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Lactobacillus bulgaricus*, *Bifidobacterium breve*, *Bifidobacterium longum*, *Streptococcus thermophilus*, and the prebiotic fructo-oligosaccharide, manufactured by Zist Khamtir (Tehran, Iran). The placebo group received identical capsules (in shape, size, and odor) filled with starch. Participants were instructed to take two capsules daily for four months (16 weeks). For allocation concealment, the study capsules were placed in sequentially numbered, opaque, sealed envelopes, which were opened only after participant enrolment. The envelopes were provided to the study groups according to the allocation sequence.

Outcome Measures

The primary outcome was Physical, Psychological, and Social Fatigue Index assessed using the CFAB-MS. The questionnaire includes 133 items across 13 domains. It incorporates visual analog scales (VAS) to evaluate general health as well as assessment of fatigue and its related factors, including pain, sleep, stress, mood, anxiety, nutrition, mobility, environment, and fatigue management strategies [15]. The Persian version of CFAB-MS has demonstrated acceptable validity ($P < 0.05$) and moderate to high internal consistency. Validity analysis showed strong correlations between CFAB-MS subscale scores and the corresponding validated instruments: the fatigue score correlated strongly with the Modified Fatigue Impact Scale (MFIS, Cronbach's alpha: 0.863); the anxiety and mood scores with the Hospital Anxiety and Depression Scale (HADS, Cronbach's alpha: 0.701 for anxiety and 0.470 for mood); the sleep scores with the Pittsburgh Sleep Quality Index (PSQI, Cronbach's alpha: 0.497); the pain score with the Short-Form McGill Pain Questionnaire (SF-MPQ, Cronbach's alpha: 0.381); and mobility index with the Multiple Sclerosis Walking Scale (MSWS-12, Cronbach's alpha: 0.602).

Sample Size

The required sample size was estimated using G*Power software (version 3.1.9.2), assuming 80% power, a two-sided type I error (α) of 0.05, and an allocation ratio of 1:1. The effect size was derived from the reported EDSS results of the previously published randomized controlled trial by Ebrahim Kouchaki et al.‘ [37] which was subsequently retracted. This study was used solely as a reference for sample size estimation and not as evidence supporting the efficacy of the intervention. Based on these assumptions, a minimum of 24 participants per group (48 participants in total) was required. Because the present study used individual-level randomization, no cluster-related design effect was applied (design effect = 1). To allow for potential loss to follow-up and to increase the statistical power of the study, the target sample size was increased to 90 participants (45 per group).

Randomization and Blinding

Ninety patients were randomly assigned to the intervention and placebo groups in a 1:1 ratio using a random number table. The randomization sequence was generated by a researcher who was not involved in participant recruitment, allocation, intervention administration, or outcome assessment. Simple randomization was used without block or stratified randomization. After eligibility was confirmed and informed consent was obtained, a separate researcher implemented the allocation sequence and assigned participants to the intervention or placebo group. The researcher responsible for outcome assessment and data collection was blinded to participants' group assignments throughout the study, thereby maintaining the double-blind design.

Data Collection and Statistical Analysis

A per-protocol analysis was conducted using IBM SPSS Statistics version 23 for MacOS. All outcome variables were measured both before and after the intervention. This pre–post comparison enabled adjustment for baseline values and reduced the impact of individual-level confounders. Statistical tests were selected based on data type and distribution, which was assessed using the Shapiro-Wilk test. Considering the lack of normal distribution, the Mann-Whitney U-test was used for between groups comparisons. For the adjusted analysis of post-intervention values, an analysis of covariance (ANCOVA) model was fitted, with post-intervention values score as the dependent variable, treatment group as the primary independent variable, and baseline values score as a covariate. To control for multiple testing, the Benjamini–Hochberg procedure was applied to reduce the risk of type I error. In addition, effect sizes for between-group comparisons were calculated as $r = Z/\sqrt{N}$ based on the Mann–Whitney test statistics. Intragroup comparisons were made using the Wilcoxon signed-rank test. All statistical tests were two-tailed, with a significance level of P-value < 0.05.

Results

A total of 122 patients were assessed for eligibility, of whom 32 were not included (18 did not meet the inclusion criteria and 14 declined to participate). The remaining 90 patients were randomized equally to the probiotic and placebo groups. During follow-up, 30 participants did not complete the study because of loss to follow-up, disease relapse, gastrointestinal side effects, or personal reasons, as detailed in Figure 1. Ultimately, 29 patients in the probiotics group and 31 in the placebo group completed the trial and were included in the final per-protocol analysis. No additional participants who completed the study were excluded from the analysis. Regarding safety, disease relapse occurred in two participants in the probiotics group and one participant in the placebo group. Gastrointestinal side effects led to treatment discontinuation in two participants in the probiotics group and one participant in the placebo group. Constipation was the only reported adverse event, and no serious safety concerns or intervention-related adverse events were identified.

Patient Recruitment and Data Collection

The details of the baseline data have been reported previously. [35] In brief, 29 participants (including 21 females) in the probiotics group and 31 patients (including 25 females) in the placebo group completed the trial. Mean ages were 33.66 (SD 8.32) and 31.23 (SD 8.85) in the probiotics and placebo groups. Medians of education were 12 [5] and 14 [4] years, and disease durations were 48 [63] and 60 [93] months in the probiotics and placebo groups.

Effect of Probiotics Supplementation on General Summary of Health

No statistically significant differences were found between the two groups across any other components of the general health summary ($P > 0.05$). A significant within-group improvement in the VAS for fatigue was observed in the intervention group ($P = 0.04$) (Table 1).

Effect of Probiotics Supplementation on Fatigue and Its Related Factors

Intergroup comparisons revealed no statistically significant differences in most outcome measures, including physical, psychological, and social fatigue index, depression, stress, anxiety, sleep, nutrition, and mobility indices ($P > 0.05$). Within-group analyses showed significant improvements in stress ($P = 0.01$) and anxiety and worry ($P = 0.01$) in the placebo group. Conversely, the probiotics group showed a significant within-group improvement only in the nutrition index ($P = 0.02$), with no other statistically significant changes observed (Table 2). Importantly, after applying the Benjamini–Hochberg correction for multiple comparisons, none of the observed effects remained statistically significant.

Discussion

This study investigated the effects of probiotics supplementation on general health, fatigue, and its related factors in RRMS patients with EDSS < 4. Overall, probiotics supplementation showed limited effects on the investigated

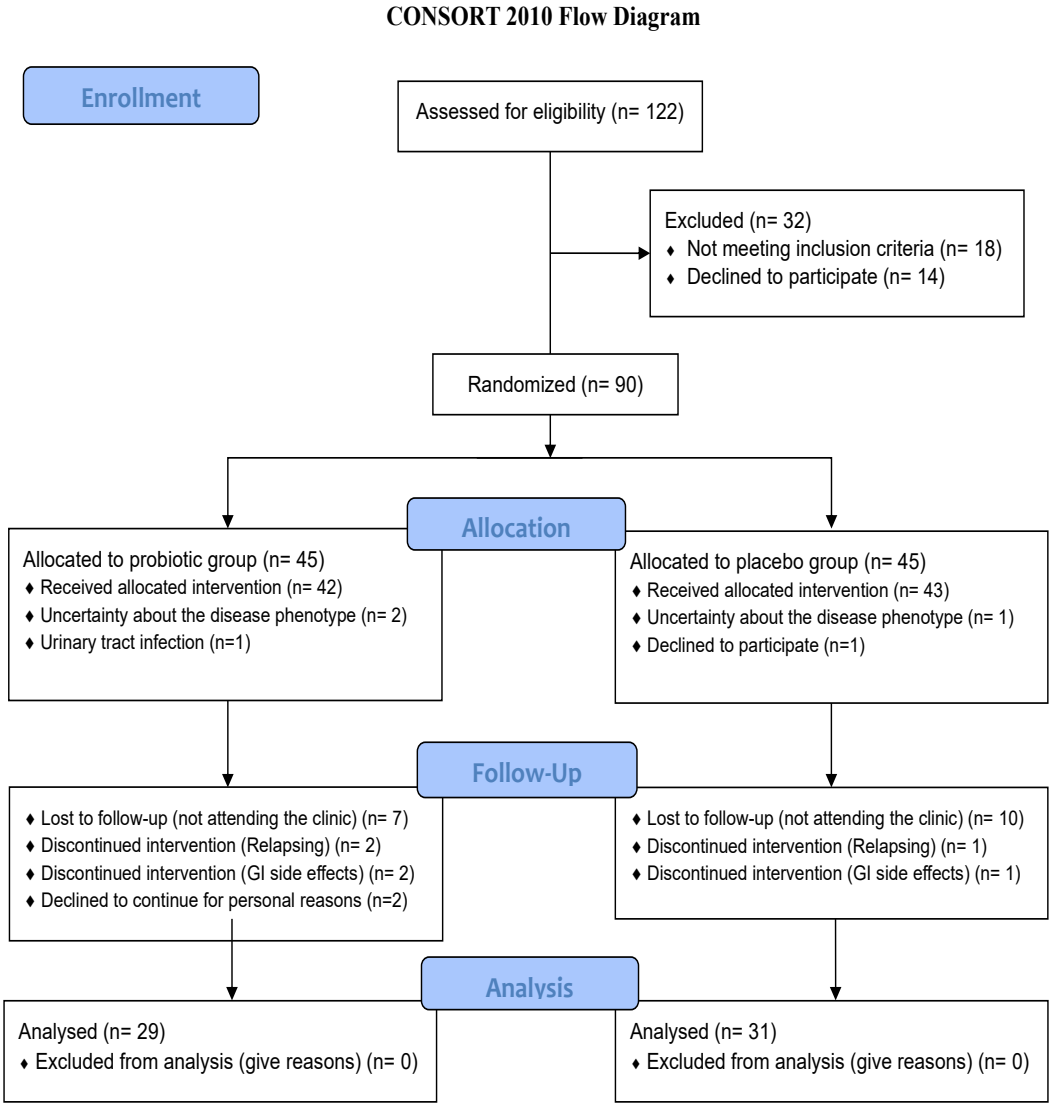

Figure 1. CONSORT flow diagram

health measures. The findings of this RCT revealed no significant improvement in the measured outcomes, including general health, fatigue, pain, sleep, stress, mood, anxiety, nutrition, mobility, environment, and fatigue management methods with four-month supplementation with a seven-strain probiotics product (Lactocare®).

Environmental factors, including alterations in the gut microbiome, have been implicated in MS pathogenesis.[23] Growing evidence highlights the microbiota-gut-brain axis as a key player in neurodegenerative diseases (e.g., MS, Parkinson's disease, and Alzheimer's disease). Gut dysbiosis triggers systemic inflammation through aging- and diet-driven immune dysregulation. For instance, *Akkermansia muciniphila* promotes inflammation, while anti-inflammatory species like *Parabacteroides distasonis* are depleted. [38] In MS specifically, fecal microbiota transplantation studies reveal impaired IL-10 production by Treg cells and shifts in microbial communities (e.g., reduced *Butyricicoccus*), exacerbating neuroinflammation. Additionally, bacterial antigen mimicry—such as *Acinetobacter* and *Pseudomonas* resembling myelin proteins—may drive B-cell and IgA-mediated CNS autoimmunity, further supported by heightened immune responses to *Clostridium perfringens* toxins in MS patients. [39, 40] A recent study with a two-tiered strategy, comparing gut microbial profiles in a cohort of 81 monozygotic twins discordant for MS, identified over 50 differently abundant taxa, including the *Anaerotruncus colihominis*, *Eisenbergiella tayi*, along with newly identified taxa, such as *Copromonas* and *Acutalibacter.* [41] Although no clinical benefits were observed in this trial, existing literature suggests that probiotics may modulate fatigue through biological mechanisms, particularly immune regulation and the gut-brain axis. For instance, previous studies using *Saccharomyces boulardii* have shown reductions in pain and fatigue—measured by the VAS and Fatigue Severity Scale—as well as improvements in quality of life and psychosomatic function, possibly due to reduced inflammatory and oxidative biomarkers. [33] The difference in probiotics strains used across studies could explain the inconsistencies in outcomes. However, our trial observed only modest improvements in fatigue and nutrition in the probiotics group. These findings highlight the complex interplay between physiological and psychological factors

Table 1. Effect of Probiotic Supplementation on General Summary of Health

Measure (Units, Statistics)	Probiotic Group (n = 29)	Placebo Group (n = 31)	Effect size (r), *P*-value	*P*-value for ANCOVA
Fatigue (Before, Median [IQR])	80 [40]	65 [60]	-0.184, 0.15	-
Fatigue (After, Median [IQR])	70 [50]	50 [75]	-0.076, 0.56	0.72
Fatigue Change (Median [IQR])	-5 [35]	-5 [30]	-0.091, 0.48	-
Within-Group Comparison (*P*-value)	0.04*	0.43	-	-
Pain (Before, Median [IQR])	45 [73]	20 [45]	-0.203, 0.12	-
Pain (After, Median [IQR])	45 [68]	10 [45]	-0.267, 0.04*	0.19
Pain Change (Median [IQR])	0 [10]	0 [20]	-0.134, 0.30	-
Within-Group Comparison (*P*-value)	0.27	0.81	-	-
Depression (Before, Median [IQR])	45 [65]	20 [45]	-0.206, 0.11	-
Depression (After, Median [IQR])	30 [58]	15 [55]	-0.134, 0.30	0.79
Depression Change (Median [IQR])	0 [30]	0 [15]	-0.147, 0.25	-
Within-Group Comparison (*P*-value)	0.56	0.34	-	-
Stress (Before, Median [IQR])	75 [58]	70 [50]	-0.028, 0.83	-
Stress (After, Median [IQR])	55 [63]	50 [70]	-0.051, 0.69	0.71
Stress Change (Median [IQR])	0 [22.5]	0 [25]	-0.037, 0.78	-
Within-Group Comparison (*P*-value)	0.06	0.11	-	-
Anxiety and Worry (Before, Median [IQR])	60 [60]	75 [55]	-0.135, 0.30	-
Anxiety and Worry (After, Median [IQR])	50 [58]	60 [60]	-0.044, 0.73	0.90
Anxiety and Worry Change (Median [IQR])	-5 [22.5]	-5 [25]	-0.049, 0.70	-
Within-Group Comparison (*P*-value)	0.34	0.16	-	-
Sleep Problems (Before, Median [IQR])	25 [73]	20 [75]	-0.078, 0.55	-
Sleep Problems (After, Median [IQR])	15 [58]	20 [45]	-0.016, 0.90	0.84
Sleep Problems Change (Median [IQR])	-5 [12.5]	0 [15]	-0.089, 0.49	-
Within-Group Comparison (*P*-value)	0.10	0.34	-	-
Nutrition Status (Before, Median [IQR])	15 [45]	40 [40]	-0.218, 0.09	-
Nutrition Status (After, Median [IQR])	15 [43]	40 [55]	-0.163, 0.20	0.55
Nutrition Status Change (Median [IQR])	0 [30]	0 [15]	-0.007, 0.96	-
Within-Group Comparison (*P*-value)	0.92	0.80	-	-
Walking Disability (Before, Median [IQR])	15 [50]	15 [30]	-0.103, 0.42	-
Walking Disability (After, Median [IQR])	10 [33]	10 [40]	-0.081, 0.53	0.92
Walking Disability Change (Median [IQR])	0 [17.5]	0 [10]	-0.040, 0.76	-
Within-Group Comparison (*P*-value)	0.82	0.88	-	-

* Statistically significant ($P < 0.05$); VAS: Visual Analogue Scale, Values are presented as median (IQR: interquartile range). Lower VAS scores indicate better health status (i.e., lower symptom burden). Negative change values indicate improvement from baseline. *P*-values represent between-group comparisons of change from baseline using the Mann–Whitney U test. Within-group comparisons (Wilcoxon signed-rank test) and analysis of covariance (ANCOVA) model, with post-intervention values score as the dependent variable, treatment group as the primary independent variable, and baseline values score as a covariate, are also presented in the table.

in clinical interventions.

Beyond MS, multiple RCTs have explored the impact of probiotics on fatigue in various inflammatory and chronic conditions. In one RCT, high-dose probiotics significantly decreased fatigue in patients with irritable bowel syndrome. [42] Probiotics supplementation has also shown encouraging results in reducing fatigue and inflammatory responses in athletes, [43] and early evidence suggests possible benefits in managing cancer-related fatigue. [44] In an examination of patients with chronic fatigue syndrome, probiotics microorganisms that produce lactic acid were reported to enhance neurocognitive performance; [35] however, there were no discernible improvements in fatigue levels. In addition, probiotics administration significantly decreased fatigue in a study of colorectal cancer survivors. [45] Also, a multi-strain probiotics intervention over six months significantly reduced fatigue and enhanced quality of life in individuals with post-infectious fatigue. [46] Moreover, a study on post-COVID-19 myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) showed that administering a symbiotic supplement reduced post-exercise malaise and enhanced markers of brain metabolism. [47] On the other hand, probiotics supplementation did not significantly enhance fatigue or quality of life in a randomized trial of patients with spondylarthritis. [48] In the present study, probiotics supplementation was not associated with significant improvement in fatigue and its related factors in RRMS patients with EDSS < 4. These disparities underscore

Table 2. Effect of Probiotic Supplementation on Fatigue and Contributing Factors

Measure (Units, Statistics)	Probiotic Group (n = 29)	Placebo Group (n = 31)	Effect size (r), *P*-value	*P*-value for ANCOVA
Physical, Psychological, and Social Fatigue Index (Before, Median [IQR]*)	28 [34]	23 [35]	-0.087, 0.50	-
Physical, Psychological, and Social Fatigue Index (After, Median [IQR])	30 [34]	18 [42]	-0.117, 0.36	0.70
Change in Fatigue Index (Median [IQR])	-1 [22.5]	-3 [12]	-0.028, 0.83	-
Within-Group Comparison (*P*-value)	0.87	0.25	-	-
Pain Index (Before, Median [IQR])	6 [16]	4 [9]	-0.033, 0.80	-
Pain Index (After, Median [IQR])	10 [16]	5 [7]	-0.091, 0.48	0.62
Change in Pain Index (Median [IQR])	0 [6.5]	0 [4]	-0.077, 0.52	-
Within-Group Comparison (*P*-value)	0.61	0.80	-	-
Sleep Problems Index (Before, Median [IQR])	12 [7]	11 [8]	-0.189, 0.14	-
Sleep Problems Index (After, Median [IQR])	13 [6]	10 [7]	-0.300, 0.02*	0.05
Change in Sleep Problems Index (Median [IQR])	-2 [8]	-1 [5]	-0.038, 0.77	-
Within-Group Comparison (*P*-value)	0.92	0.31	-	-
Stress Index (Before, Median [IQR])	20 [16]	20 [12]	-0.041, 0.75	-
Stress Index (After, Median [IQR])	16 [20]	15 [17]	-0.054, 0.67	0.24
Change in Stress Index (Median [IQR])	0 [1.5]	0 [7]	-0.183, 0.16	-
Within-Group Comparison (*P*-value)	0.56	0.01*	-	-
Mood Index (Before, Median [IQR])	15 [9]	16 [8]	-0.028, 0.83	-
Mood Index (After, Median [IQR])	15 [7]	16 [8]	-0.069, 0.59	0.37
Change in Mood Index (Median [IQR])	0 [4]	0 [3]	-0.014, 0.91	-
Within-Group Comparison (*P*-value)	0.96	0.55	-	-
Anxiety and Worry Index (Before, Median [IQR])	9 [14]	10 [11]	-0.038, 0.77	-
Anxiety and Worry Index (After, Median [IQR])	8 [14]	5 [11]	-0.129, 0.32	0.08
Change in Anxiety and Worry Index (Median [IQR])	0 [6]	-1 [5]	-0.199, 0.12	-
Within-Group Comparison (*P*-value)	0.83	0.01*	-	-
Nutrition Index (Before, Median [IQR])	5 [7]	7 [6]	-0.041, 0.75	-
Nutrition Index (After, Median [IQR])	3 [7]	5 [4]	-0.103, 0.43	0.42
Change in Nutrition Index (Median [IQR])	-1 [3.5]	-1 [3]	-0.090, 0.49	-
Within-Group Comparison (*P*-value)	0.02*	0.18	-	-
Mobility (Before, Median [IQR])	0 [4]	0 [1]	-0.147, 0.25	-
Mobility (After, Median [IQR])	1 [1]	0 [1]	-0.135, 0.30	0.90
Change in Mobility (Median [IQR])	0 [1.5]	0 [0]	-0.068, 0.60	-
Within-Group Comparison (*P*-value)	0.26	0.79	-	-
Environment (Before, Median [IQR])	0.4 [1.1]	0.8 [1.5]	-0.116, 0.37	-
Environment (After, Median [IQR])	0.6 [1.2]	0.4 [1.00]	-0.034, 0.79	0.35
Change in Environment (Median [IQR])	0 [0.72]	0 [0.5]	-0.166, 0.20	-
Within-Group Comparison (*P*-value)	0.69	0.25	-	-
Fatigue Management Index (Before, Median [IQR])	1 [5]	1 [5]	-0.015, 0.91	-
Fatigue Management Index (After, Median [IQR])	1 [5]	0 [5]	-0.030, 0.81	0.82
Change in Fatigue Management Index (Median [IQR])	0 [2]	0 [2.5]	-0.007, 0.96	-
Within-Group Comparison (*P*-value)	0.84	0.99	-	-

* IQR: Interquartile Range; ANCOVA: Analysis of Covariance.

the importance of disease-specific pathophysiology and probiotics strain selection in determining therapeutic efficacy.

The gut-brain axis and the role of psychobiotics as modulators of mental health have also been extensively studied. A systematic review by Vaghef-Mehrabany et al. highlighted positive outcomes in depression management, particularly from probiotics, though results for prebiotics and symbiotics were inconsistent. [49, 50] Similarly, a meta-analysis by Sikorska et al. involving 20 RCTs found that certain probiotic strains reduced depressive symptoms and modulated biomarkers such as BDNF, despite an unclear mechanism. [51] Likewise, another meta-analysis by Rahmannia et al, [52] comprising 12 studies, found that symbiotics and probiotics have mixed results across different measurement scales, indicating the need for

cautious interpretation. Furthermore, a systematic meta-analysis performed by Zhao et al. showed possible anxiety symptom relief of interventions that alter the gut microbes (including probiotics, prebiotics, and symbiotics). [53] Nevertheless, our findings showed no improvement in mental health parameters, indices of depression, mood, anxiety, and worries among probiotics recipients with RRMS, which can also be attributed to the low level of depression of the participants and the exclusion of participants with diagnosed depression.

The present study had some limitations that should be considered in interpreting the results. A small sample size is a considerable limitation of this study. Moreover, due to participant drop-out over the course of the study, one of the main limitations of this study is the attrition bias. Overall, 30 of the 90 randomized participants (33.3%) did not complete the study, including 16 of 45 (35.6%) in the probiotic group and 14 of 45 (31.1%) in the placebo group. The dropout rates were similar between the two groups. However, because the analysis was performed on participants who completed the study, attrition bias cannot be ruled out. Baseline characteristics of completers and non-completers were not compared, so the possible impact of attrition on the results remains uncertain. Furthermore, dietary intake, a crucial factor influencing probiotics efficacy and gut microbiota composition, was not controlled for, which could have confounded the results. [54, 55] The other significant limitation of our investigation was the lack of assessment of changes in intestinal microbial profiles in fecal samples. Our trial did not include microbiota composition analysis, which could have provided insight into individual responses to probiotics therapy. Additionally, evaluating the gene expression of various inflammatory factors, particularly transforming growth factor-1 (TGF-1) and Forkhead box P3 (FOXP3), may improve the accuracy of the results. [21] The generalizability of the findings of this study is limited to RRMS patients with EDSS < 4; therefore, it cannot be extended to severely disabled patients and/or patients with progressive forms of the disease. Additionally, the homogeneity regarding the ethnicity and setting of the study, which was limited to a single clinic in Tabriz, Iran, may affect the external validity of the findings in this study. Poor reliability in sleep, mood, and pain (Cronbach's alpha < 0.6) measures in CFAB-MS is another limitation which should be acknowledged, too.

This study has several important strengths. First, it was conducted as a parallel, randomized, double-blind, placebo-controlled trial, and we tried to follow CONSORT guidelines carefully, which increases the reliability of our results. Second, we included a relatively homogeneous group of patients with RRMS and low disability (EDSS < 4), so the findings are less affected by differences in disease stage and severity. Third, we used a clearly defined multi-strain probiotics (Lactocare®) with a known dose and composition over a 16-week period, which makes the intervention easier to reproduce in future studies. Another strength is the use of the CFAB-MS, which allowed us to assess fatigue in a multidimensional and MS- specific way. Finally, we did not only look at fatigue severity, but also at several related factors such as pain, sleep, stress, mood, anxiety, nutrition, mobility, and fatigue management strategies, which gives a more complete picture of the possible effects of probiotics supplementation in RRMS.

Conclusion

This study found no significant improvement with probiotics supplementation on general health, fatigue, pain, sleep, stress, mood, anxiety, nutrition, mobility, environment, and fatigue management in RRMS patients with EDSS < 4. Future studies should specifically investigate whether the absence of a detectable effect on fatigue in the present trial is related to baseline fatigue characteristics, adherence to the intervention, or heterogeneity in individual responses to probiotics. Studies incorporating dietary intake and relevant clinical or biological measures may also help identify potential sources of response heterogeneity and clarify which patients, if any, are more likely to benefit from probiotic supplementation.

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Authors' Contribution

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Competing Interests

The authors declare that they have no competing interests.

Data Availability Statement

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethical Approval

The study protocol was approved by the Ethics Committee of Tabriz University of Medical Sciences (approval code: IR.TBZMED.REC.1404.214). Written informed consent was obtained from all participants. The trial was registered in the Iranian Registry of Clinical Trials (IRCT20220713055465N1; registration date: August 30, 2022). Participants were recruited between January 2023 and

January 2024. Trial registration was completed before enrolment of the first participant. The full study protocol is available from the corresponding author upon reasonable request.

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