MEDICAL UNIVERSITY – PLEVEN

FACULTY OF MEDICINE

DEPARTMENT OF NEUROLOGY AND NEUROSURGERY

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ASSESSMENT OF THE IMPACT OF DIETARY HABITS ON CLINICAL AND LABORATORY PARAMETERS IN PATIENTS WITH MULTIPLE SCLEROSIS

A B S T R A C T

of a dissertation

for the acquisition of an educational and scientific degree

„DOCTOR”

Field of Higher Education: 7. Health Care and sports

Professional Field: 7.1 Medicine

Doctoral Program: Neurology

Scientific supervisor:

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Official reviewers:

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Pleven, 2026

The dissertation is written on 163 standard printed pages and is illustrated with 18 figures, 28 tables, and 7 appendices.
The literature sources used comprise 282 titles, of which 15 are in Cyrillic and 267 in Latin script. Of all cited sources, 158 were published in 2022–2026, including 96 sources from the last 3 years.

The numbers of the figures and tables in the abstract correspond to the numbers of the figures and tables in the dissertation.

The author is a PhD student in an independent form of study at the Department of Neurology and Neurosurgery, Faculty of Medicine, Medical University – Pleven and works as a lecturer in the Neurology Sector of the Department of Neurology and Neurosurgery, Faculty of Medicine, Medical University – Pleven.

Four publications have been produced in connection with the dissertation.

The dissertation has been discussed and approved for public defence by the Extended Department Council of the Department of Neurology and Neurosurgery, Faculty of Medicine, Medical University – Pleven, held on 03 June 2026.

Scientific jury composed of:

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The public defence of the dissertation will take place on 30.09.2026 at 12:30 in the Ambroise Pare Hall of MU – Pleven.

The materials related to the defence are available on the website of MU−Pleven: www.mu-pleven.bg

CONTENTS

ABBREVIATIONS USED

ALA	Alpha-linolenic acid
BBB	Blood-brain barrier
BMI	Body Mass Index
BP	Blood pressure
CD20	Cluster of Differentiation 20
CRP-hs	High-sensitivity C-reactive protein
DHA	Docosahexaenoic acid
DII	Dietary Inflammatory Index
EBV	Epstein-Barr virus
EDSS	Expanded Disability Status Scale
EPA	Eicosapentaenoic acid
FDA	Food and Drug Administration
FLAIR	Fluid Attenuated Inversion Recovery
FSMC	Fatigue Scale for Motor and Cognitive Functions
FSS	Fatigue Severity Scale
GBD	Global Burden of Disease
HRQOL	Health-Related Quality of Life
IFN	Interferon
IL-6	Interleukin-6
IL-17A	Interleukin-17A
LOMS	Late-Onset Multiple Sclerosis
MAPK	Mitogen-activated protein kinases
MEDAS-14	Mediterranean Diet Adherence Screener, 14-item version
MFIS	Modified Fatigue Impact Scale
MIND	Mediterranean-DASH Intervention for Neurodegenerative Delay
MSIF	Multiple Sclerosis International Federation
MSSS	Multiple Sclerosis Severity Score
MSQOL-54	Multiple Sclerosis Quality of Life-54
MVAGNIMS	Magnetic Resonance Imaging in Multiple Sclerosis
NF-κB	Nuclear factor kappa-B
NMSS	National Multiple Sclerosis Society
NNT	Number Needed to Treat

NUTRISEP	Nutritional Status Evaluation in People with Multiple Sclerosis
POMS	Pediatric-Onset Multiple Sclerosis
PREDIMED	Prevención con Dieta Mediterránea
PUFAs	Polyunsaturated fatty acids
QoL	Quality of Life
SCFAs	Short-Chain Fatty Acids
SF-36	Short Form-36 Health Survey
TNF-α	Tumor Necrosis Factor Alpha
VAS	Visual Analogue Scale
WAVES	Wahls And Swank Dietary Interventions in MS
AMD	Adapted Mediterranean Diet
DMT	Disease-modifying therapy
SPMS	Secondary progressive multiple sclerosis
DNA	Deoxyribonucleic acid
EAE	Experimental autoimmune encephalomyelitis
BMI	Body Mass Index
REC	Research Ethics Committee
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
PUFAs	Polyunsaturated fatty acids
PPMS	Primary progressive multiple sclerosis
RRMS	Relapsing-remitting multiple sclerosis
RCT	Randomised controlled trial
MD	Mediterranean diet
CNS	Central Nervous System

INTRODUCTION

Multiple sclerosis is a chronic autoimmune demyelinating and neurodegenerative disease of the CNS that primarily affects young people of working age and is a leading cause of neurological deficit in this population. According to current epidemiological data, the disease affects more than 2.8 million people worldwide, with a trend towards increasing incidence in countries with historically lower prevalence. Despite the substantial advances in the field of DMT, MS remains a socially significant disease associated with progressive disability, impaired quality of life, and a considerable individual, family, and societal burden.

The epidemiological picture of MS in Bulgaria reflects pan-European trends. Studies by Bulgarian authors likewise record an increasing incidence and prevalence of MS in our country, with the slight differences being attributable rather to accompanying regional, ethnic, and sociodemographic factors. The prevalence of the disease in our country is around 100–130 per 100,000 population, with the actual values likely higher due to incomplete coverage and registration of individuals affected by MS (Nikolova and Milanov 2020; Nikolova 2024). The Department of Neurology at University Hospital "Dr G. Stranski" – Pleven is one of the specialised centres for the diagnosis and treatment of MS in the country, serving a considerable proportion of the patients in Northern Bulgaria. The Bulgarian school of neurology has established scientific traditions in MS research at the European and global level. The National Consensus for the Diagnosis and Treatment of MS and the Pharmacotherapeutic Guideline for MS are the principal documents defining the standards of practice in clinical care in our country (Milanov 2025; Milanov and Bozhinova 2024). Bulgarian scientists have published significant original results from fundamental clinical and epidemiological studies on the prevalence and incidence of MS in our country (Nikolova and Milanov 2020; Nikolova 2024). A series of contemporary dissertations developed by Bulgarian authors occupy a worthy place in the evidence-based medicine of MS, focusing on cognitive impairment (Genov 2015a; Kunchev 2018; Stamenova 2016), comorbidity and quality of life (Drenska 2016), cardiovascular autonomic dysfunction (Damyanov 2019), neuropsychological and genetic-immunological correlations (Trenova 2017), the validation of diagnostic scales in the Bulgarian population (Dokova et al. 2008; Klisurski et al. 2021), and the role of neutralising antibodies against interferon-beta in patients with different forms of the disease (Hristova-Chakmakova 2016). These works form a solid national scientific basis on which the present regional study draws and enriches with significant results.

Despite considerable achievements, the interrelationships among dietary patterns, body composition, and clinical and laboratory parameters in Bulgarian MS patients have not been investigated prospectively to date. The shortage of this type of data in the national body of

scientific knowledge is the rationale for formulating the scientific hypothesis and planning the research activity of the author of the present dissertation.

The focus on the relationship between chronic fatigue in MS and the nature of the food consumed is prompted by the well-known fact that about 95% of patients complain of fatigue symptoms of varying severity, which significantly limit daily activities, reduce working capacity, and impair subjective well-being, yet still remain on the periphery of research interest. Moreover, current pharmacological approaches to treating fatigue in MS are of limited efficacy, while non-pharmacological interventions, including dietary ones, await studies informed by new knowledge of the disease's molecular basis and their translation into clinical practice..

In recent years, there has been growing interest in the role of dietary behaviour as a modifiable factor in the pathogenesis, course, and symptomatology of MS. In this regard, the Mediterranean diet, comprising a high intake of plant-based foods, fish, olive oil, and moderate wine consumption, is among the potential candidates for clinically oriented studies in MS. Its confirmed anti-inflammatory, antioxidant, neuroprotective, and immunomodulatory properties point to a reliable opportunity for its inclusion as a non-pharmacological therapeutic adjunct in patients suffering from the disease. Nevertheless, data on the effects of this type of diet on clinical and laboratory parameters, fatigue, and quality of life in people with MS remain scarce, heterogeneous, and methodologically diverse.

The present dissertation, conducted as a single-centre study at the Department of Neurology of University Hospital “Dr G. Stranski” – Pleven, was designed as a prospective, longitudinal, observational study with consecutive data collection to evaluate the influence of the Mediterranean dietary regimen on the clinical and laboratory parameters in patients with RRMS under real-world clinical conditions. This is the first study in Bulgaria to integrate the nutritional status of a homogeneous group of RRMS patients with a broad spectrum of clinical and laboratory disease parameters. The study is based on the hypothesis that a higher degree of adherence to the principles of a seasonally adapted MD is associated with more favourable values for metabolic and body biomarkers, the degree of chronic fatigue, and quality of life in RRMS patients. An additional working hypothesis is that dietary interventions aimed at optimising dietary behaviour in MS provide an accessible, acceptable, safe, and clinically significant strategy in the comprehensive management of the disease.

The expected contributions of the dissertation lie in two complementary directions. At the theoretical level, the work provides original data on the relationship between the Mediterranean dietary pattern and clinical and laboratory parameters in RRMS in the Bulgarian population, filling a gap in national scientific knowledge. At the practical level, the results are expected to provide a rationale for incorporating dietary interventions into the comprehensive,

multidisciplinary approach for MS patients and to encourage the scientific community to consider specific recommendations for future national clinical guidelines.

I. BRIEF LITERATURE REVIEW

1. Therapeutic approaches and prospects in the treatment of MS

The complex, multi-component therapy of MS includes treatment of acute relapses, disease-modifying therapies (DMT), symptomatic treatment, and non-pharmacological interventions. Treatment aims to prevent or reduce relapse frequency, slow disability progression, improve quality of life, and control disease symptoms. Disease-modifying therapies in MS are classified into two main categories: moderate-efficacy therapies (*first-line*) and high-efficacy therapies (*high-efficacy, second-line*) (Figure 1). Among the promising therapeutic directions, remyelinating and cell-based therapies, neuroprotective approaches, and anti-senescence strategies stand out.

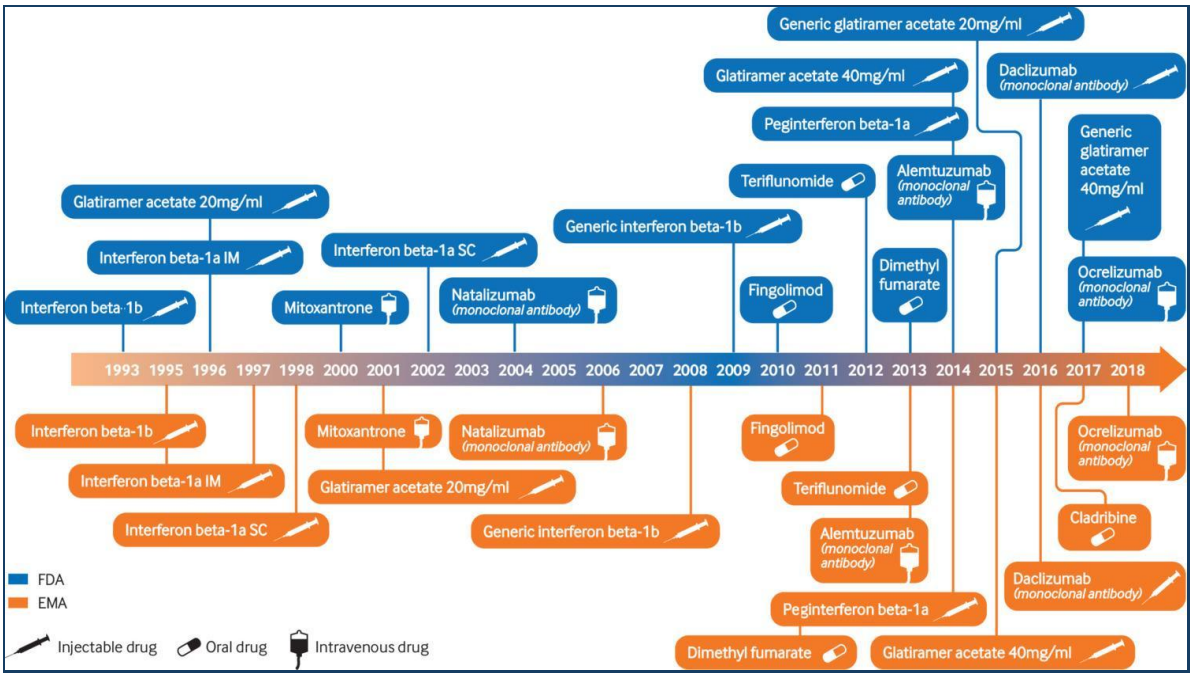

Figure 1. Timeline of the disease-modifying therapies approved by the FDA and the EMA for the period 1993–2018 (BMJ 2018;363:k4674)

The choice of therapy in MS is individualised and determined by numerous factors, including disease activity, form, severity, accompanying risks and comorbidity, reproductive plans, preferences, and access to treatment. Comorbidities in MS, such as depression, cardiovascular disease, diabetes mellitus, anxiety, and others, considerably complicate the therapeutic approach. Treatment decisions in the presence of comorbidities require an integrated approach that includes changes in dietary habits and lifestyle as an integral component of multidisciplinary care (Marrie et al. 2023). The therapeutic standard in Bulgaria is defined by the National Consensus for the Diagnosis and Treatment of MS (Milanov (ed.)

2025, 2026) and the Pharmacotherapeutic Guideline for the Treatment of Neurological Diseases (Milanov and Bozhinova 2024).

Dietary interventions are regarded as an accessible and safe form of “microbiome therapy” with beneficial effects on the gut microbiome and associated immunological effects in MS (Duscha et al. 2020; Sanchez et al. 2020; Ross et al. 2024; Yadav SK et al. 2023). The growing importance of multimodal non-pharmacological interventions in MS is reflected in a 2025 study examining the effects of a combination of non-pharmacological approaches on cardiometabolic markers in progressive MS. The authors found that combining an appropriate dietary regimen with physical activity and stress management is associated with improvements in cardiometabolic parameters in progressive MS and is a valuable addition to pharmacological treatment (Martinez AS et al. 2025). The present dissertation fits within this research paradigm, evaluating the effects of the Mediterranean diet on clinically significant parameters in MS patients in real-world clinical practice.

2. Mediterranean diet: characteristics, components, mechanisms of action, and assessment of adherence

The Mediterranean diet is a traditional dietary pattern characteristic of the peoples inhabiting the coastal regions of the Mediterranean Sea. It is characterised by a rich variety of plant-based foods, moderate intake of fish and seafood, moderate to low intake of dairy products and poultry, low intake of red meat and processed meats, moderate consumption of red wine with meals, and the use of extra virgin olive oil as the main fat (Figure 2). In 2010, UNESCO recognised the MD as an element of the intangible cultural heritage of humanity. Contemporary concepts of the MD go beyond dietary characteristics to encompass broader lifestyle habits, such as regular physical activity, convivial meals, and respect for seasonality and local production.

Despite the growing body of evidence for the benefits of the MD in MS, the international guidelines for the treatment of MS still do not include specific dietary recommendations, including the MD. The main reason is the insufficient number of well-designed randomised controlled trials involving dietary interventions (Evans et al. 2019; Fitzgerald et al. 2020; Harirchian et al. 2022). Leading researchers in the field consider it necessary to encourage MS patients to adhere to the MD or an analogous anti-inflammatory dietary pattern within the multidisciplinary disease-management plan (Stoiloudis et al. 2022; Rosso et al. 2025; Virgilio et al. 2026; Kabakibo et al. 2026).

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Figure 2. Food pyramid of the MD (Translated from: https://mediterra.bg)

II. AIM AND OBJECTIVES

AIM

The aim of the present dissertation is to investigate and evaluate the influence of a Mediterranean diet, seasonally adapted to Bulgarian conditions, on fatigue, body composition, and clinical and laboratory parameters in patients with relapsing-remitting multiple sclerosis, followed under real-world clinical conditions.

OBJECTIVES

1. To analyse the clinical and demographic profile of the RRMS patients included in the study with respect to sex, age, education, employment, place of residence, marital status, disease duration, degree of neurological deficit (EDSS), administered disease-modifying therapy, and main comorbidities.
2. To determine the degree of adherence to AMD using the validated MEDAS questionnaire and to analyse the sociodemographic and clinical determinants of the dietary regimen followed.
3. To assess the change in fatigue parameters, measured with the FSMC and MFIS instruments, after three months of adherence to AMD.
4. To analyse the body composition of the studied patients (percentage of body and visceral fat, muscle and bone mass, body water, metabolic rate, and BMI) by bioelectrical impedance analysis (Tanita InnerScan) and to assess the dynamics of these parameters under adherence to AMD.
5. To investigate patients' serum lipid levels (total cholesterol, LDL, HDL, triglycerides) and CRP, and to analyse their dynamics and relationships with adherence to AMD.
6. To investigate the serum levels of IL-17A in a subgroup of patients and to analyse its dynamics as a marker of Th17-mediated neuroinflammation.
7. To determine the serum levels of EPA and DHA before the start and after the end of the diet and to investigate their association with adherence to AMD and the clinical parameters.
8. To investigate the statistical relationship between the degree of adherence to AMD (MEDAS) and the severity of fatigue (FSMC and MFIS) at the two planned visits, with analysis of dose-dependence.
9. To investigate the correlation between the MEDAS scores and the degree of neurological disability (EDSS).

10. To investigate the statistical relationship between the MEDAS scores and the changes in the bioimpedance parameters, lipid profile, CRP, and omega-3 profile (EPA, DHA).
11. To compare the degree of adherence to AMD and the associated clinical parameters for fatigue, EDSS, body composition, and biomarkers in subgroups by sex, education, marital status, employment, disease duration, and degree of disability by EDSS.

III. MATERIALS AND METHODS

1. Design and programme of the study

Design: A prospective, longitudinal, observational, single-arm, single-centre, open clinical study with two time-point assessments (Visit 1 – baseline; and Visit 2 – after a three-month dietary intervention). Each participant served as their own control by comparing parameters between the two visits (before-and-after design).

Observation period: The study was conducted from 2020–2025, with gradual enrolment of RRMS patients and coverage across different seasons.

Setting: Department of Neurology at University Hospital „Dr Georgi Stranski“ – Pleven. The laboratory tests were performed at the Central Clinical Laboratory of University Hospital “Dr Georgi Stranski” – Pleven and the Sector „Biology“ of the Department of Anatomy, Histology, Cytology and Biology of MU–Pleven.

Programme: The study was carried out in the following sequential stages:

- Selection and analysis of current literature data, scientific medical evidence, and up-to-date management guidelines for MS patients.
- Selection of a nutritional regimen suitable for the purposes of the study and its adaptation to the seasonal conditions in Bulgaria.
- Selection of validated assessment scales adequate for the purpose of the study.
- Participation in a research project on the influence of an adapted Mediterranean diet on the symptoms of chronic fatigue, serum levels of omega-3 PUFAs, and interleukin-17 (IL-17) in RRMS patients undergoing disease-modifying therapy (DMT).
- Drafting of participant selection criteria, specification of the diagnostic instruments, and preparation of ethics documents for evaluation by the Research Ethics Committee (REC) at MU – Pleven.
- Recruitment of patients according to established inclusion and exclusion criteria from the general flow of MS patients attending the Department of Neurology of University Hospital “Dr G. Stranski” – Pleven and Diagnostic and Consultation Center – Pleven for routine monitoring of ongoing therapy and assessment of clinical status.

- Informing the recruited participants about the nature of the dietary recommendations, providing a purpose-designed AMD brochure and a sample diary for the regular recording of the foods consumed.
- Familiarising the patients with the nature of the study and the non-pharmacological intervention used, explaining their rights, obligations, and the methods for protection of personal data, discussing their willingness to cooperate during the observation stages, and obtaining written informed consent.
- Planning the analytical stages and the duration of the study, preparing the research documents, and creating an electronic database with an alphanumeric code for anonymising the personal data.
- Drawing venous blood twice from each participant for the testing of general laboratory parameters (lipid profile, CRP-hs) and serum levels of EPA, DHA, and IL-17A.
- Bioimpedance assessment of the patients' body composition before and after the dietary intervention with Tanita InnerScan.
- * Regular communication with patients through outpatient examinations, telephone consultations, and email for additional clarification and advice on adherence to AMD.
- Baseline and final assessment of the dynamics of the studied parameters during the dietary intervention.
- Statistical processing and stepwise publication of the study results.

2. MATERIALS

2.1. Ethical aspects of the study

All investigations in the present work comply with the UN Universal Declaration of Human Rights and the Declaration of the World Medical Association of Helsinki (latest revision 2013) on the ethical principles for biomedical research involving human subjects. The protection of participants' rights in the processing of their personal data was carried out in accordance with the latest amendments and supplements to the Personal Data Protection Act of the Republic of Bulgaria and Regulation (EU) 2016/679 (GDPR).

The ethical aspects of the research activity related to the dissertation were evaluated positively by the Research Ethics Committee at MU – Pleven (REC) and approved by Decision Ref. No. 636-REC/11.06.2020.

Each participant received detailed oral and written information about the aim, methods, duration, expected benefits, and potential risks of the study, and signed an Informed Consent to participate. Participants were informed of their right to withdraw from the study at any time

without this affecting standard medical care. To anonymise the personal data, an alphanumeric code was generated and used in the Patient Chart and in the study's electronic database.

2.2. Clinical cohort

The subjects of the study were patients of the Department of Neurology at University Hospital "Dr G. Stranski" – Pleven, totalling n=76, enrolled consecutively *(consecutive sampling)* from the general flow of RRMS patients, in accordance with the formulated selection criteria. The cohort comprised mainly patients from the Pleven region and Central Northern and Northwestern Bulgaria for the period 2020–2025.

Participants were enrolled non-randomly, on an open basis, in the order of hospitalisation in the department or outpatient attendance for a follow-up examination at the Neurology Office of the Diagnostic and Consultation Center – Pleven. The clinical cohort included patients with a diagnosis of RRMS who met the inclusion criteria, were informed in writing about the nature of the study, provided written consent to participate, and expressed willingness and ability to follow the dietary regimen recommended by the investigator.

Inclusion criteria:

- Diagnosis of RRMS confirmed in accordance with the revised McDonald diagnostic criteria (2017, 2024) by a specialist physician in neurology.
- Age ≥ 18 years.
- Stable clinical condition, with no active relapse at the time of enrolment (≥ 30 days from the last relapse).
- Degree of disability on the Expanded Disability Status Scale (EDSS) ≤ 6.5.
- Stable DMT during the last three months before enrolment, or no indications for DMT.
- Preserved cognitive and communicative capacity to understand the dietary recommendations, complete the questionnaires, and carry out the study procedures.
- Signed written informed consent to participate in the study.
- Willingness and ability to follow the recommended dietary regimen for 12 weeks.
- Ability to attend the two planned visits (V1 – baseline and V2 – final).

Exclusion criteria:

- Other forms of MS (primary progressive, secondary progressive, clinically isolated syndrome).
- Active MS relapse or administration of pulse corticosteroid therapy in the last 30 days before enrolment.
- EDSS > 6,5.
- Pregnancy, breastfeeding, or planning a pregnancy during the study period.

- A diagnosed severe comorbidity requiring a specific dietary regimen incompatible with AMD (e.g. advanced renal failure, advanced-stage liver failure, active oncological disease, severe digestive diseases – coeliac disease, Crohn's disease in an active phase).
- Clinically significant allergies or intolerances to key components of AMD (fish, nuts, olive oil, legumes).
- Psychiatric disorders that impair the ability to give informed consent or follow dietary recommendations.
- Active abuse of alcohol or narcotic substances.
- Refusal to sign informed consent or withdrawal of consent during the study.
- Inability to follow AMD for socioeconomic or other individual reasons.

2.3. Biological material

Biological materials were collected from each participant at the two planned visits:

- Venous blood – by a standard procedure through peripheral venepuncture, under fasting conditions of at least 12 hours. The blood was drawn into appropriate vacuum tubes for the respective analyses: serum tubes for biochemical parameters (lipid profile), EDTA tubes for haematological tests, and specialised tubes for storing serum samples for the testing of EPA, DHA, and IL-17A. The serum samples intended for the testing of omega-3 PUFAs and IL-17A were frozen at −20°C until the time of analysis.

2.4. Diagnostic instruments and equipment

Laboratory equipment:

- A biochemistry analyser for testing the lipid profile (total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides) and high-sensitivity C-reactive protein (CRP-hs) at the Central Clinical Laboratory of University Hospital „Dr G. Stranski" – Pleven.
- Enzyme-linked immunosorbent assay (ELISA) for testing the serum levels of human IL-17A, EPA, and DHA with an ELISA Kit (Abbexa, UK) at the Sector "Biology" of MU – Pleven.

Bioimpedance anthropometry: Body composition was assessed with a Tanita InnerScan bioimpedance analyser, by means of which the following parameters were measured: body mass index (BMI, kg/m²); body fat (BF %); visceral fat (VF %); body water (BW %); muscle mass (MM, kg); bone mass (BM, kg); basal metabolism (BaM, kcal); metabolic age (MA, years). Measurements were performed in the morning, under fasting conditions, with an empty bladder, with no preceding intense physical activity in the last 24 hours, under standardised conditions (body temperature, hydration, removal of metal objects).

Standardised assessment scales and questionnaires:

The study used validated assessment instruments that have been translated, adapted, and approved for clinical use in Bulgarian patients and are freely available for research purposes; these are presented in detail in the subsection "Diagnostic methods".

3. METHODS

The diagnosis and treatment of the disease, the follow-up, and the assessment of clinical status were performed by a team of specialist physicians in Neurology from the Department of Neurology of University Hospital "Dr Georgi Stranski" – Pleven, together with the author of the dissertation.

3.1. Clinical methods

- Diagnostic interview using a Patient Chart approved by the REC, with 16 parameters including demographic data (sex, age, education, employment, marital status, place of residence), diagnosis, family and medication history, allergies, harmful habits (smoking, alcohol use), comorbidity, accompanying DMT, paraclinical tests, dietary adherence, bioimpedance anthropometry parameters, and the time-point results from Visit 1 and Visit 2.
- Anthropometric measurements – height, weight, BMI.
- Vital signs – arterial pressure, pulse, and respiratory rate, measured by a standardised method. Blood pressure was assessed categorically in accordance with the guidelines of the European Society of Cardiology ESC/ESH 2018 (Williams B et al., Eur Heart J 2018).
- Bioimpedance assessment of body composition with a Tanita InnerScan device.

3.2. Diagnostic methods

- Expanded Disability Status Scale (EDSS) – for assessing the degree of neurological disability;
- Fatigue Scale for Motor and Cognitive Functions (FSMC) – for the differentiated assessment of motor and cognitive fatigue;
- Modified Fatigue Impact Scale (MFIS) – for assessing the impact of fatigue on daily functioning;
- Mediterranean Diet Adherence Screener, 14-item version (MEDAS-14) – for assessing adherence to the Mediterranean diet;

3.3. Laboratory methods: Total cholesterol (mmol/L), LDL cholesterol (mmol/L), HDL cholesterol (mmol/L), triglycerides (mmol/L), C-reactive protein (mg/L), IL-17A (pg/mL), EPA (μg/mL), and DHA (μg/mL).

3.4. Medication methods:

During the study, the patients continued their usual therapy in accordance with the international and national standards of practice for RRMS, with no intervention by the investigator. Symptomatic therapy was prescribed to control comorbidities.

3.5. Non-medication methods:

Adapted Mediterranean diet (AMD). The patients were provided with a specially developed AMD brochure containing a visual food pyramid, a list of recommended and undesirable foods, sample menus for each season, and practical advice on preparing dishes. In addition, a sample diary (food diary) was provided for the regular recording of the foods consumed.

3.6. Methods for statistical processing and analysis

The statistical processing of the data was performed using the statistical software packages IBM SPSS Statistics (V21.0) and MS Excel (Office 365). The adopted level of statistical significance was $p < 0.05$ (two-sided).

Descriptive methods: Qualitative (categorical) variables were presented as absolute frequencies (n) and relative proportions (%). Quantitative (interval) variables were tested for normality using the Shapiro-Wilk test and were presented as mean and standard deviation (mean ± SD) for a normal distribution, or as median and interquartile range (Me, IQR, 25th–75th percentile) for a non-normal distribution. The range (R) of the values is additionally presented.

Comparative tests:

- Wilcoxon test for related samples (Wilcoxon Signed-Rank Test) – for assessing the changes between Visit 1 and Visit 2 in related (paired) measurements with non-normally distributed data.
- Mann-Whitney U test – for comparing quantitative variables between two independent groups (e.g. sex differences) with non-normally distributed data.
- Kruskal-Wallis Test – for comparing quantitative variables between three or more independent groups (e.g. the three MEDAS groups – low, medium, and high degree of adherence).
- Post-hoc analysis with the Mann-Whitney U test and Bonferroni correction (adjusted $\alpha = 0.017$ for three pairwise comparisons) – for identifying the specific between-group differences when the Kruskal-Wallis Test was significant.
- Fisher's exact test – for comparing qualitative variables in small samples.
- Pearson's χ^2 test – for comparing qualitative variables in larger samples.

Correlation analysis: Spearman correlation analysis (Spearman's rank correlation, r) – for assessing the relationships between quantitative variables (MEDAS, EDSS, FSMC, MFIS, biochemical parameters), applied owing to the non-normal distribution of the clinical scales

and the robustness of the method to extreme values. The correlation coefficient was interpreted according to the established criteria of Cohen (1988): $|r| < 0.30$ – weak correlation; $0.30 \leq |r| < 0.50$ – moderate correlation; $|r| \geq 0.50$ – strong correlation.

Binary Logistic Regression. Assessment of the independent contribution of demographic and clinical predictors to binary clinically significant outcomes by binary logistic regression analysis. Univariate and multivariate Odds Ratios (OR / aOR) with 95% confidence intervals (CI) were calculated.

Effect size analysis: Cohen's d – for determining the effect size in related samples. Post-hoc statistical power analysis – for assessing the adequacy of the sample size to detect the observed effects at a significance level of $\alpha = 0.05$.

Epidemiological measures: Number Needed to Treat (NNT) – for assessing the clinical effectiveness of the intervention with respect to clinically significant improvement in fatigue; Absolute Risk Reduction (ARR) – for calculating the absolute reduction in risk.

Graphical presentation of the results: The results are presented in tables and graphs (bar, pie, scatter plots with regression lines), produced using MS Excel and the graphical capabilities of IBM SPSS Statistics.

Significance level: Statistical significance was adopted at $p < 0.05$ (two-sided test). The degrees of significance in the tables and graphs are denoted as follows: ns – non-significant ($p \geq 0.05$); $p < 0.05$; $p < 0.01$; $p < 0.001$.

IV. RESULTS

1. Sociodemographic and clinical characteristics

The study included 76 RRMS patients followed at the Department of Neurology of University Hospital "Dr Georgi Stranski" – Pleven, of whom 42 were women (55.3%) and 34 men (44.7%). The full demographic and clinical characteristics of the studied group are presented in Table 1 and Figure 3.

Table 1. Demographic and clinical characteristics of the studied group (n=76)

Parameter	Value
Sex – men / women n (%)	34 (44.7%) / 42 (55.3%)
Age (years)	45.5 ± 7.7 (R 23–64)
Disease onset (years)	36.0 ± 9.3
MS duration (years)	9.5 ± 7.6
Number of relapses	5.5 ± 3.9
EDSS	3.52 ± 1.38

Non-smokers n (%)	56 (73.7%)
No alcohol use n (%)	51 (67.1%)
Family history of MS n (%)	5 (6.6%)
On DMT n (%)	62 (81.6%)
Education – primary / secondary / higher n (%)	17 (22.4%) / 43 (56.6%) / 16 (21.1%)
MEDAS score	6.78 ± 3.08 (Me 6, R 2–13)
MEDAS — low / medium / high	25 (32.9%) / 34 (44.7%) / 17 (22.4%)
Comorbidities n (%)	27 (35.5%)

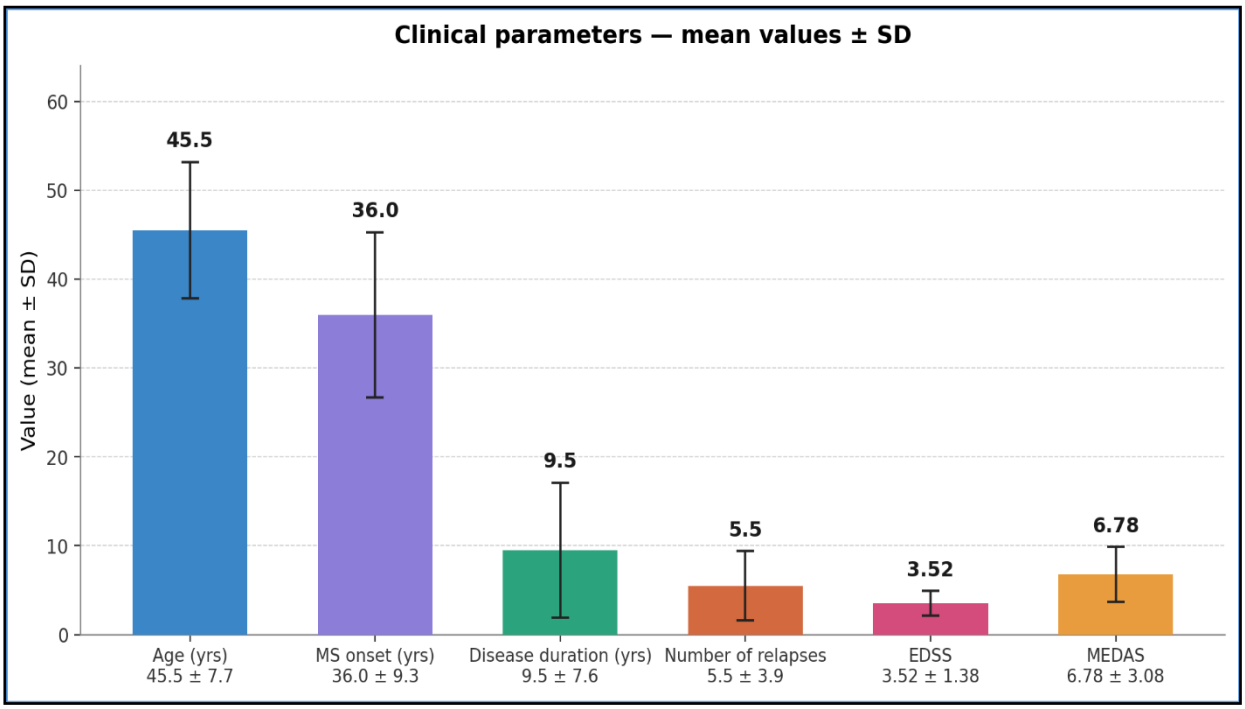

Figure 3. Distribution of patients by age, MEDAS, and clinical parameters

Distribution by sex, age, and sociodemographic parameters

The analysis of the study sample yielded the following data, presented in detail in Table 2, Figure 4, and Figure 5.

- Distribution by sex: 42 women (55.3%) / 34 men (44.7%).
- Distribution by age group: the predominant group was 46–55 years (n=40, 52.6%); 36–45 years (n=24, 31.6%), ≤35 years (n=9 patients, 11.8%), and only three patients (3.9%) ≥ 55 years, which reflects the typical age structure in RRMS.
- Mean age: 45.5 ± 7.7 years (R 23–64 years).
- Mean age at disease onset: 36.0 ± 9.3 years.

- Marital status: 46 patients (60.5%) – married; 30 (39.5%) – unmarried. Of these, 44 patients (57.9%) had children.
- Place of residence: 54 patients (71.1%) live in a town, and 22 (28.9%) – in a village.

Table 2. Sociodemographic characteristics of the studied group (n=76)

Parameter	**Category**	**N (%)**
Sex	Men	34 (44.7%)
	Women	42 (55.3%)
Age group	≤35 yrs	9 (11.8%)
	36–45 yrs	24 (31.6%)
	46–55 yrs	40 (52.6%)
	>55 yrs	3 (3.9%)
Marital status	Unmarried	30 (39.5%)
	Married	46 (60.5%)
Children	Without children	32 (42.1%)
	With children	44 (57.9%)
Place of residence	Village	22 (28.9%)
	Town	54 (71.1%)
Education	Primary	17 (22.4%)
	Secondary	43 (56.6%)
	Higher	16 (21.1%)
Employment	Unemployed	16 (21.1%)
	Employed	50 (65.8%)
	Retired	2 (2.6%)
	Disabled	8 (10.5%)

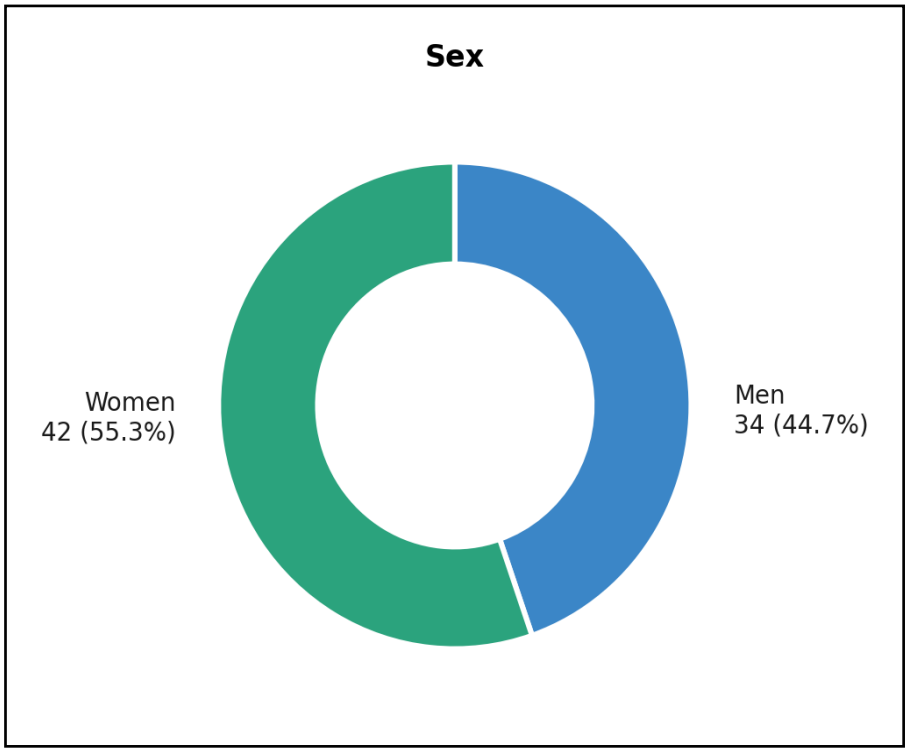

Figure 4. Distribution of patients by sex

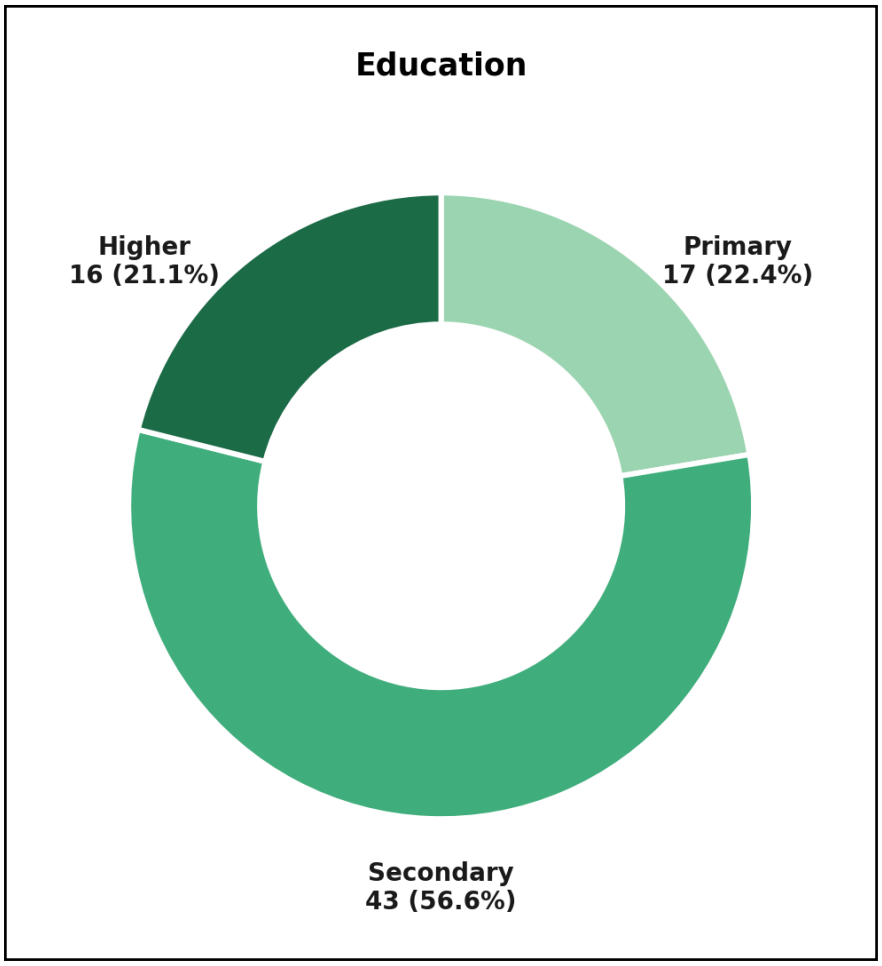

Figure 5. Distribution of patients by level of education

Characteristics of the disease

The mean MS duration in the studied patients was 9.5 ± 7.6 years. The mean number of relapses was 5.5 ± 3.9

The distribution into groups based on disease duration showed an even representation:

- Duration up to 5 years: 29 (38.2%);
- Duration 6–10 years: 18 (23.7%);
- Duration ≥ 10 years: 29 (38.2%).

The mean value of the degree of disability, assessed by EDSS, was 3.52 ± 1.38 (Table 3). The distribution by severity showed neurological deficits of EDSS 2.5–4 in 44 patients (57.9%), EDSS 0–2 in 15 patients (19.7%), EDSS 4.5–6 in 12 patients (15.8%), and EDSS >6 in 5 patients (6.6%).

Comorbidities

Comorbidities were identified in 27 patients (35.5%), while 49 patients (64.5%) had no registered comorbidities. The most common accompanying condition was arterial hypertension (n=14, 18.4%), followed by hypothyroidism (n=6, 7.9%), iron-deficiency anaemia (n=4, 5.3%), diabetes mellitus (n=3, 3.9%), and Hashimoto's thyroiditis (n=2, 2.6%). The distribution of the most common comorbidities is presented in Table 4.

Table 3. Clinical characteristics of the disease (n=76)

Parameter	Category	N (%)
MS duration	0–5 yrs	29 (38.2%)
	6–10 yrs	18 (23.7%)
	>10 yrs	29 (38.2%)
EDSS category	0–2	15 (19.7%)
	2.5–4	44 (57.9%)
	4.5–6	12 (15.8%)
	>6	5 (6.6%)
Family history	No	71 (93.4%)
	Yes	5 (6.6%)
DMT	Without DMT	14 (18.4%)
	On DMT	62 (81.6%)

Table 4. Comorbidities in the studied group (n=76)

Comorbidity	N (%)
No comorbidities	49 (64.5%)
Arterial hypertension	14 (18.4%)

Hypothyroidism	6 (7.9%)
Iron-deficiency anaemia	4 (5.3%)
Diabetes mellitus	3 (3.9%)
Hashimoto's thyroiditis	2 (2.6%)

Risk factors and lifestyle

Of all the studied patients, 56 (73.7%) were non-smokers and 20 (26.3%) – active smokers. Regarding alcohol consumption, 51 (67.1%) did not consume alcohol, 20 (26.3%) consumed it moderately, 4 (5.3%) – averagely, and 1 (1.3%) – regularly (Figure 5). The distribution of patients by family history, DMT, and harmful habits is illustrated in Figure 6.

Adherence to AMD (MEDAS)

The mean MEDAS score was 6.78 ± 3.08 (Me 6, R 2–13). The distribution into groups based on adherence to AMD, illustrated in Figure 7, was as follows:

- Low degree (0–5): 25 patients (32.9%);
- Medium degree (6–9): 34 (44.7%);
- High degree (10–14): 17 (22.4%).

The analysis of MEDAS by sociodemographic groups revealed significant differences. Patients with higher education had the highest mean MEDAS score (9.19 ± 3.31), significantly higher than those of patients with primary education (5.24 ± 3.13) and secondary education (6.49 ± 2.44). Employed patients had a higher MEDAS score (7.46 ± 3.13) than unemployed patients (5.31 ± 2.55) and disabled patients (5.12 ± 2.42). No significant difference in MEDAS was observed between men (6.62 ± 3.17) and women (6.90 ± 3.03). These data are presented in Table 5.

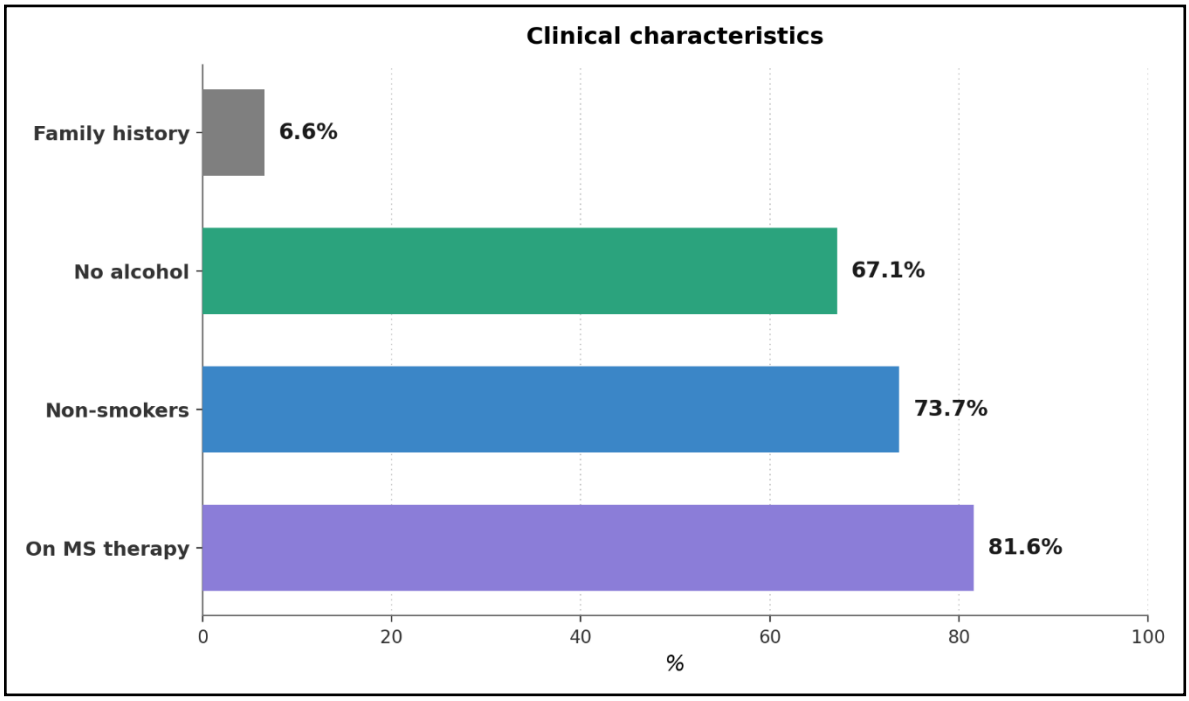

Figure 6. Distribution of patients by family history, DMT, and harmful habits

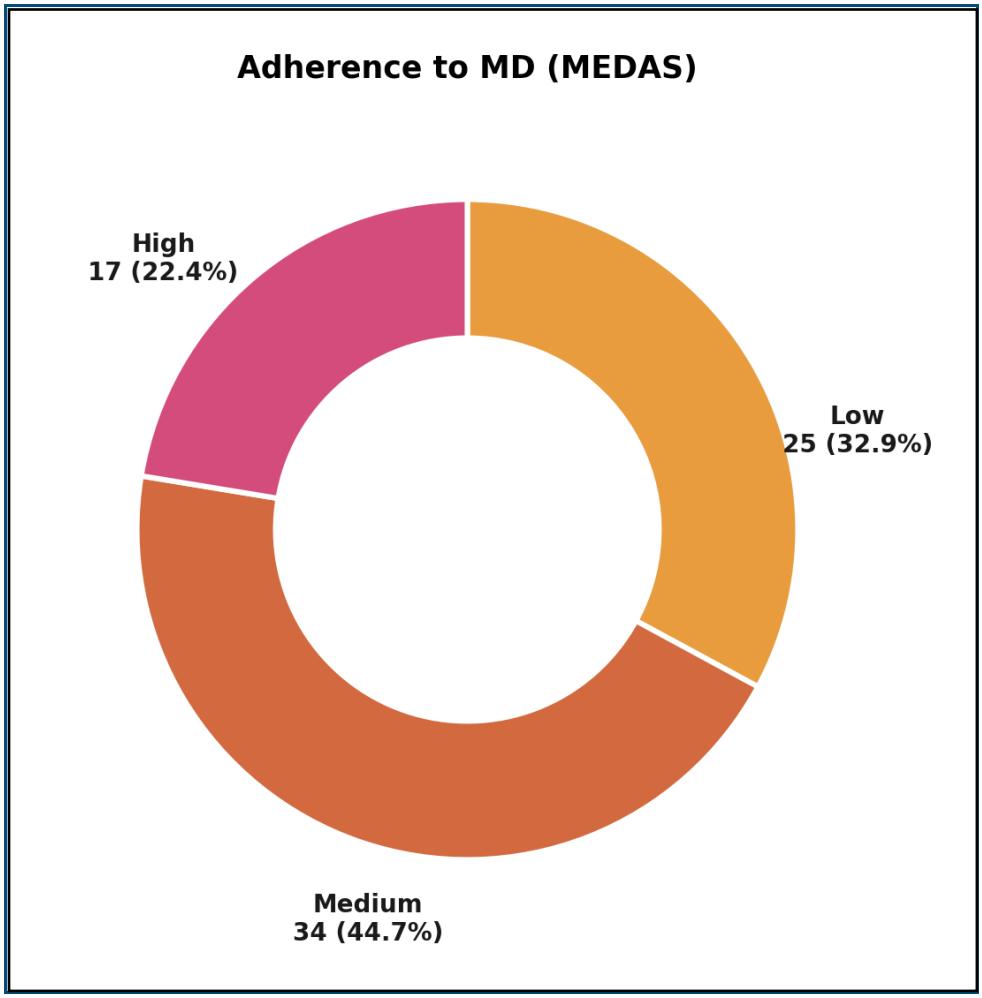

Figure 7. Distribution by degree of adherence to AMD (MEDAS)

Table 5. MEDAS score by sociodemographic groups (n=76)

Group	N (%)	MEDAS (mean ± SD)
Sex		
Men	34 (44.7%)	6.62 ± 3.17
Women	42 (55.3%)	6.90 ± 3.03
Education		
Primary	17 (22.4%)	5.24 ± 3.13
Secondary	43 (56.6%)	6.49 ± 2.44
Higher	16 (21.1%)	9.19 ± 3.31
Employment		
Unemployed	16 (21.1%)	5.31 ± 2.55
Employed	50 (65.8%)	7.46 ± 3.13
Retired	2 (2.6%)	8.00 ± 2.83
Disabled	8 (10.5%)	5.12 ± 2.42
Place of residence		
Village	22 (28.9%)	6.14 ± 3.63
Town	54 (71.1%)	7.04 ± 2.81

2. Comparative analysis of the clinical parameters between the two visits (Wilcoxon Signed-Rank Test)

To assess changes in clinical and laboratory parameters between the baseline visit (V1) and the final visit (V2), the Wilcoxon test for related samples was used. The results are presented in Table 6, Figure 8, and Figure 9. The analysis revealed a statistically significant improvement in nine of the twenty studied parameters.

Fatigue, assessed with FSMC, decreased significantly from 52.91 ± 10.79 to 48.79 ± 10.82 points ($p<0.001$), with 80.3% of patients demonstrating improvement.

Fatigue, measured with the MFIS, decreased from 39.34 ± 13.50 to 35.12 ± 13.73 points ($p<0.001$), with documented improvement in 84.2% of the patients studied – the highest percentage among all parameters.

Significant decreases were observed in the following parameters: BMI ($p<0.001$), body fat – from 27.60% to 26.73% ($p<0.001$), and visceral fat – from 5.99% to 5.69% ($p<0.001$). Body water increased from 47.37% to 49.28% ($p<0.001$), and muscle mass – from 44.75 to 45.62 kg ($p=0.002$). Among the biomarkers, IL-17A decreased significantly in the 15 studied patients – from 118.58 pg/mL to 17.63 pg/mL ($p<0.001$), while DHA increased significantly

(p=0.003). No significant changes were found in the lipid profile, CRP, EPA, pulse, or blood pressure.

The distribution of fatigue by category between the two visits, illustrating the direction and magnitude of the changes, is presented in Table 7 and Table 8. When fatigue was assessed with FSMC (Table 7), at V1 patients with moderate fatigue predominated (44.7%), whereas at V2 the mild-fatigue category prevailed (40.8%). The proportion of patients with severe fatigue decreased more than twofold (from 15.8% to 6.6%), and the proportion with no fatigue doubled (from 11.8% to 23.7%).

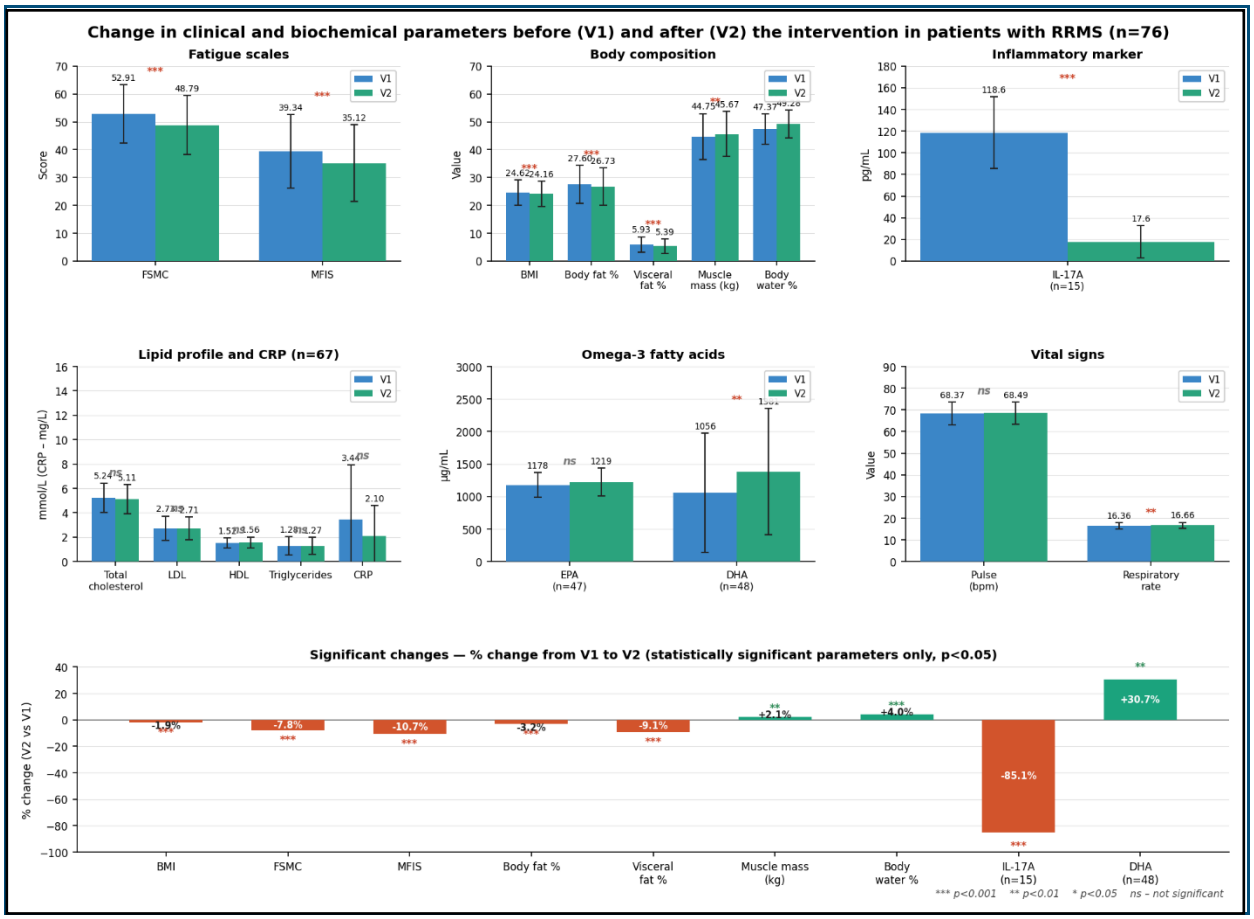

Figure 8. Change in clinical and biochemical parameters before (V1) and after (V2) the dietary intervention in RRMS patients (n=76).

*SD – standard deviation; *** p<0.001; ** p<0.01; ns – non-significant*

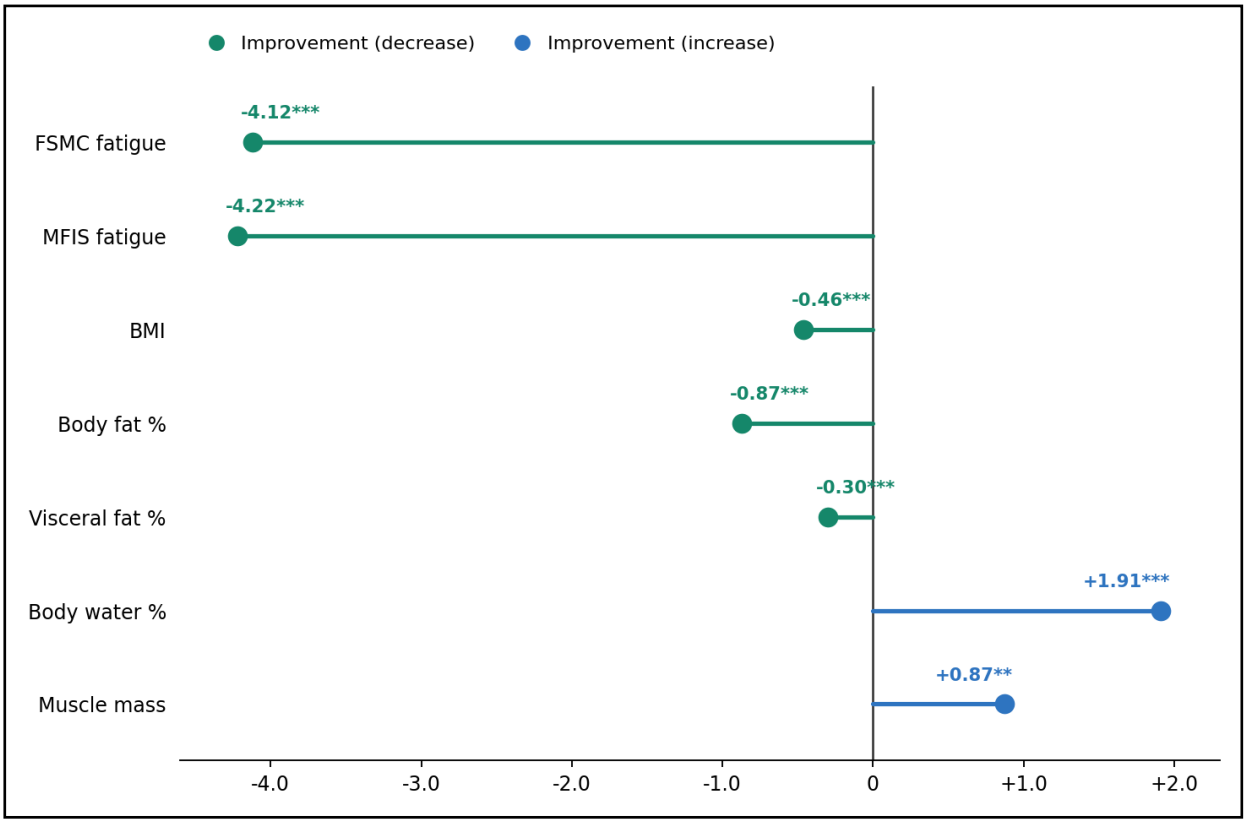

Figure 9. Changes in the significant clinical parameters between Visit 1 and Visit 2 *(Wilcoxon Signed-Rank Test, n=76). ** p<0.01; *** p<0.001.*

On the MFIS scale, at V1, more than half of patients (56.6%) fell into the "moderate to significant" fatigue category, whereas at V2, their proportion decreased significantly to 35.5%. At the second visit, the percentage of patients in the severe-fatigue category fell, and that in the minimal-fatigue category doubled. The observed changes are clinically significant and reflect an improvement in subjective fatigue, which, according to current literature, favourably affects daily functioning and QoL (Table 8).

Table 6. Comparison of the clinical parameters between V1 and V2 (Wilcoxon Signed-Rank Test, n=76)

Parameter	**n**	**V1 (Mean ± SD)**	**V2 (Mean ± SD)**	**Δ**	**W p-value**	**sig.**	**Improved %**
BMI	76	24.62 ± 4.61	24.16 ± 4.47	-0,462	0,000099	***	67,10%
FSMC	76	52.91 ± 10.79	48.79 ± 10.82	-4,118	0,000000089	***	80,30%
MFIS	76	39.34 ± 13.50	35.12 ± 13.73	-4,224	0,0000000046	***	84,20%
Chol	67	5.24 ± 1.19	5.11 ± 1.01	-0,131	0,0528	ns	61,20%
LDL	67	2.73 ± 1.03	2.71 ± 0.97	-0,015	0,8636	ns	44,80%
HDL	67	1.52 ± 0.44	1.56 ± 0.51	0,036	0,2718	ns	52,20%

TGL	67	1.28 ± 0.70	1.27 ± 0.72	-0,01	0,492	ns	56,70%
CRP	67	3.44 ± 7.36	2.10 ± 2.22	-1,348	0,1741	ns	55,20%
IL-17A	15	118.58 ± 33.64	17.63 ± 15.63	-100,949	0,000653	***	
EPA	47	1178.96 ± 196.78	1219.46 ± 220.41	40,498	0,1017	ns	
DHA	48	1056.88 ± 922.98	1381.00 ± 967.78	324,121	0,00275	**	
Pulse	76	68.37 ± 5.44	68.49 ± 5.19	0,118	0,6933	ns	
RR	76	16.36 ± 0.76	16.66 ± 0.74	0,303	0,00222	**	
BF %	76	27.60 ± 6.85	26.73 ± 6.72	-0,87	0,000023	***	68,40%
BM	76	2.75 ± 0.54	2.74 ± 0.53	-0,015	0,1424	ns	
VF %	76	5.99 ± 3.17	5.69 ± 2.89	-0,305	0,000575	***	39,50%
BW %	76	47.37 ± 5.04	49.28 ± 4.87	1,914	0,0000000046	***	77,60%
MM	76	44.75 ± 8.35	45.62 ± 8.22	0,872	0,00219	**	65,80%
BaM (kcal)	76	1423.37 ± 302.28	1437.86 ± 278.70	14,487	0,3098	ns	
MA	76	44.45 ± 11.98	44.18 ± 11.99	-0,263	0,01494	*	67,10%

*W p-value – Wilcoxon p-value; Δ – difference; sig. – statistical significance; ns – non-significant; * p<0.05; ** p<0.01; *** p<0.001.* V1 – baseline visit; V2 – final visit; RR – respiratory rate; BF – body fat; BM – bone mass; VF – visceral fat; BW – body water; MM – muscle mass; BaM – basal metabolism; MA – metabolic age.

Table 7. Fatigue categories by FSMC during V1 and V2 (n=76)

FSMC	V1 n (%)	V2 n (%)	Direction
No fatigue (<43)	9 (11.8%)	18 (23.7%)	↑
Mild (43–52)	21 (27.6%)	31 (40.8%)	↑
Moderate (53–62)	34 (44.7%)	22 (28.9%)	↓
Severe (≥63)	12 (15.8%)	5 (6.6%)	↓

Table 8. Fatigue categories by MFIS during V1 and V2 (n=76)

MFIS	V1 n (%)	V2 n (%)	Direction
Minimal (0–20)	8 (10.5%)	16 (21.1%)	↑
Mild to moderate (21–38)	21 (27.6%)	32 (42.1%)	↑
Moderate to significant (39–58)	43 (56.6%)	27 (35.5%)	↓
Severe (≥59)	4 (5.3%)	1 (1.3%)	↓

The in-depth analysis of the two scales showed a clear shift in the distribution towards the milder fatigue categories between the two visits. This finding is consistent with the

quantitative data from the Wilcoxon test and additionally confirms the clinical significance of the established changes.

3. Distribution of MEDAS by sociodemographic parameters and clinical groups

MEDAS by sociodemographic parameters

The analysis of the distribution of the MEDAS score by sociodemographic groups revealed significant and clinically interesting associations, presented in Table 9.

Regarding marital status, a statistically significant difference was observed, with married patients having a higher MEDAS score (7.35 ± 3.21) than unmarried patients (5.90 ± 2.67; p=0.041). This finding may reflect the more favourable conditions for healthy eating in a family environment, including shared food preparation, a more regular eating routine, and mutual support for a healthy lifestyle.

A trend towards a relationship between education and the degree of adherence to the recommended dietary regimen was noted. Patients with higher education had a higher MEDAS (9.19 ± 3.31) than those with primary (5.24 ± 3.13) or secondary education (6.49 ± 2.44), reflecting the influence of health literacy on dietary behaviour. Employed patients (7.46 ± 3.13) had a higher MEDAS compared with the unemployed (5.31 ± 2.55) and the disabled (5.12 ± 2.42), which may be due to better socioeconomic conditions.

No significant differences in MEDAS were found by sex (p=0.659), presence of children (p=0.743), smoking (p=0.541), comorbidities (p=0.282), or disease duration (p=0.653).

MEDAS by clinical parameters of the disease

Particularly significant is the association between MEDAS and the degree of disability (Table 10). The Kruskal-Wallis analysis by EDSS categories revealed statistically significant differences (H=9.220, p=0.027). Patients with the lowest EDSS values (0–2) had the highest mean MEDAS (8.87 ± 3.38), whereas those with a higher degree of disability by EDSS (4.5–6 and >6) demonstrated the lowest values (5.17 ± 2.37 and 5.60 ± 2.19, respectively). This inverse relationship between MEDAS and EDSS is clinically significant and suggests that more severe disability is associated with lower adherence to the principles of the MD, probably owing to limited functional capacity for independent food preparation and active adherence to specific dietary recommendations.

No significant differences in MEDAS were found by MS duration (p=0.653), age (p=0.201), or presence of comorbidities (p=0.282).

Table 9. MEDAS score by sociodemographic groups (n=76)

Group	N	MEDAS Mean ± SD	p
Sex			0.659

Men	34	6.62 ± 3.17	
Women	42	6.90 ± 3.03	
Marital status			0.041*
Unmarried	30	5.90 ± 2.67	
Married	46	7.35 ± 3.21	
Education			–
Primary	17	5.24 ± 3.13	
Secondary	43	6.49 ± 2.44	
Higher	16	9.19 ± 3.31	
Employment			–
Unemployed	16	5.31 ± 2.55	
Employed	50	7.46 ± 3.13	
Retired	2	8.00 ± 2.83	
Disabled	8	5.12 ± 2.42	
Place of residence			–
Village	22	6.14 ± 3.63	
Town	54	7.04 ± 2.81	
Smoking			0.541
Non-smokers	56	6.89 ± 2.95	
Smokers	20	6.45 ± 3.46	
Children			0.743
Without children	32	6.66 ± 2.96	
With children	44	6.86 ± 3.19	
DMT			0.263
Without DMT	14	5.86 ± 2.63	
On DMT	62	6.98 ± 3.15	

*Mann-Whitney U Test; * $p<0.05$*

Table 10. MEDAS score by EDSS categories, age groups, and disease duration (n=76)

Group	N	MEDAS Mean ± SD	p
EDSS category			0.027*
0–2	15	8.87 ± 3.38	
2.5–4	44	6.64 ± 2.91	
4.5–6	12	5.17 ± 2.37	
>6	5	5.60 ± 2.19	

MS duration			0.653
0–5 yrs	29	6.90 ± 3.04	
6–10 yrs	18	6.17 ± 3.17	
>10 yrs	29	7.03 ± 3.11	
Age group			0.201
≤35 yrs	9	7.67 ± 3.24	
36–45 yrs	24	7.54 ± 2.96	
46–55 yrs	40	6.03 ± 3.04	
>55 yrs	3	8.00 ± 2.65	

*Kruskal-Wallis Test; * $p<0.05$*

4. Correlation analysis: MEDAS and clinical parameters (Spearman)

Correlations with the absolute values

To establish the relationship between the degree of adherence to AMD and the clinical characteristics of the studied patients, a Spearman correlation analysis was performed between the MEDAS score (assessed after the end of the dietary intervention) and the clinical and biochemical parameters from the two visits (n=76). The results are presented in Table 11.

A statistically significant negative correlation was found between the MEDAS score and the EDSS score ($r = -0.350$, $p = 0.002$), indicating that patients with a lower degree of disability showed better adherence to the diet.

Regarding fatigue, a significant inverse correlation was found with MFIS at V1 ($r = -0.384$, $p = 0.001$) and at V2 ($r = -0.472$, $p < 0.0001$), as well as with FSMC at V2 ($r = -0.351$, $p = 0.002$). The correlation with baseline MFIS values suggests that patients with better baseline functional status achieved greater adherence to the dietary regimen. The correlation with the parameters after the dietary intervention reflects the association between the degree of dietary adherence and the reduction in fatigue at the end of the study.

A significant inverse correlation was also found with the total cholesterol levels – at V1 ($r = -0.331$, $p = 0.006$) and at V2 ($r = -0.275$, $p = 0.024$), which may reflect both better baseline dietary habits in patients with a higher MEDAS score and the lipid-modulating effect of the Mediterranean diet.

No significant correlation was found with BMI, LDL, HDL, CRP, body and visceral fat, age, disease duration, or number of exacerbations ($p > 0.05$ for all).

Table 11. Correlations of MEDAS with clinical parameters (Spearman, n=76)

Parameter	N	Spearman r	p-value	sig.
EDSS	76	-0,3503	0,00192	**

FSMC V1	76	-0,1822	0,1151	ns
FSMC V2	76	-0,3508	0,00189	**
MFIS V1	76	-0,3838	0,000621	***
MFIS V2	76	-0,4725	0,000016	***
BMI V1	76	-0,0416	0,721	ns
BMI V2	76	-0,1103	0,343	ns
Chol V1	67	-0,3305	0,00631	**
Chol V2	67	-0,2752	0,0242	*
LDL V1	67	-0,0702	0,573	ns
HDL V1	67	-0,1344	0,278	ns
CRP V1	68	0,0316	0,798	ns
BF % V1	76	-0,0465	0,69	ns
VF % V1	76	-0,0428	0,714	ns
Age	76	-0,2167	0,06	ns (trend)
Duration	76	0,0264	0,821	ns
Number of relapses	76	-0,1454	0,21	ns

*ns — non-significant; * $p<0.05$; ** $p<0.01$; *** $p<0.001$*

Some limitations should be considered when interpreting the results of the correlation analysis. Spearman correlation analysis establishes statistical associations but does not permit causal inference; therefore, the relationships between the MEDAS score and the clinical parameters cannot be interpreted as evidence of a direct causal effect of the diet. Since the MEDAS score was assessed only once, at the end of the intervention, there is no baseline quantitative assessment of dietary behaviour, which does not allow a distinction between patients with a real change in dietary habits and those who, in general, consumed foods with compositions close to the MD. The presence of "confounding" factors that may affect the final assessment cannot be excluded either. Not least, the assessment of dietary behaviour via MEDAS is based on self-report and may include overestimation of adherence due to socially desirable responses.

Alongside the noted limitations, the present analysis has several methodological advantages. The use of the non-parametric Spearman method is justified by the nature of the data – clinical scales such as EDSS and fatigue assessment instruments rarely follow a normal distribution, and Spearman's correlation is robust to extreme values and does not require assumptions about the variables' distributions. The sample of 76 RRMS patients is relatively large for a study in this specific population. The longitudinal design with two time-point assessments allows comparison of parameters before and after the intervention, which enriches the interpretation of correlations with the MEDAS score. The multidimensional nature of the

analysis provides a comprehensive picture of the associations between dietary behaviour and the patients' clinical status. The simultaneous inclusion of two validated fatigue assessment instruments (FSMC and MFIS) further strengthens the reliability of the findings, since convergent results from the two instruments reduce the likelihood of chance associations. The analysis was conducted in a research context and aimed to generate hypotheses for future studies with a more rigorous design, providing a methodologically sound framework for interpreting correlation data in the absence of randomisation.

Correlations with the changes between visits (Δ parameters)

The correlation analysis between the MEDAS score and changes in parameters between the two visits revealed additional significant associations, as presented in Table 12. A higher MEDAS score was significantly associated with a greater reduction in fatigue by FSMC (r=−0.326, p=0.004) and MFIS (r=−0.283, p=0.013) between the two visits. A significant inverse correlation was found between MEDAS and the change in BMI (r=−0.274, p=0.017) and visceral fat (r=−0.343, p=0.002), suggesting that better adherence to the diet predicts a more pronounced improvement in anthropometric parameters. No significant correlations were found with the changes in the lipid profile, CRP, or the other body composition parameters.

Table 12. Correlations of MEDAS with the changes V2−V1 (Spearman, n=76)

Parameter **Δ Change variable**	**n**	**Spearman r**	**p-value**	**sig.**
ΔFSMC	76	−0.3263	0.00402	**
ΔMFIS	76	−0.2826	0.01337	*
ΔBMI	76	−0.274	0.01662	*
ΔVisceral fat	76	−0.3426	0.00245	**
ΔChol	67	+0.0842	0.498	ns
ΔLDL	67	−0.0344	0.782	ns
ΔHDL	67	−0.0013	0.991	ns
ΔTGL	67	−0.0284	0.820	ns
ΔCRP	67	+0.0427	0.731	ns
ΔBody fat %	76	−0.1496	0.197	ns
ΔWater %	76	+0.1429	0.218	ns
ΔMuscle mass	76	+0.1809	0.118	ns

*Δ change variable – difference between the variables; Spearman r – Spearman's rank correlation coefficient; ns – non-significant; * p<0.05; ** p<0.01; *** p<0.001*

Visualisation of the MEDAS – EDSS and MEDAS – MFIS V2 correlations

The scatter plot analysis clearly illustrates the negative correlations between the MEDAS score and the two key clinical parameters. A correlation was recorded between MEDAS and EDSS (r=−0.350, p=0.002, n=76), showing a trend towards an increase in the MEDAS score in patients with a lower degree of disability by EDSS (Figure 10), and a strong correlation between MEDAS and MFIS at V2 (r=−0.473, p<0.001, n=76) (Figure 11). Patients with higher dietary adherence demonstrated significantly lower fatigue scores at the three-month follow-up. The scatter of points around the regression line reflects the individual variability characteristic of clinical studies in MS.

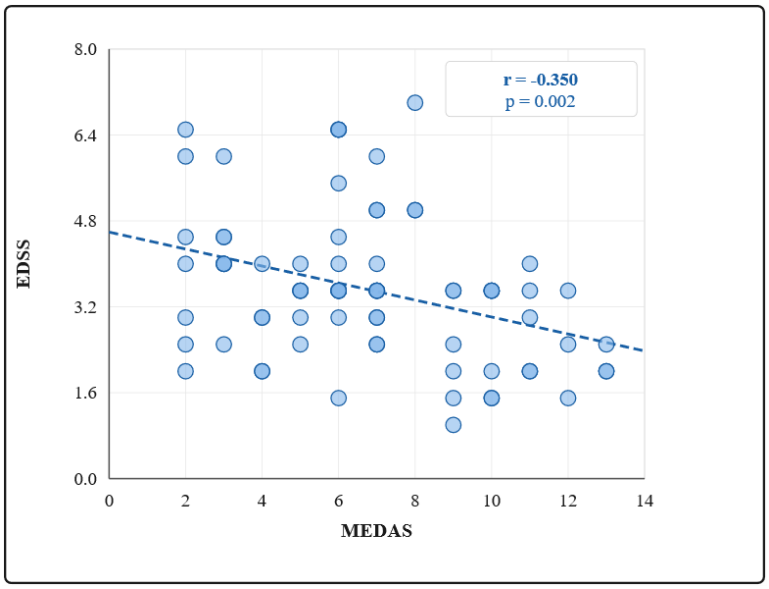

Figure 10. Scatter plot with regression line of the correlation between MEDAS and EDSS (r=−0.350, p=0.002, n=76)

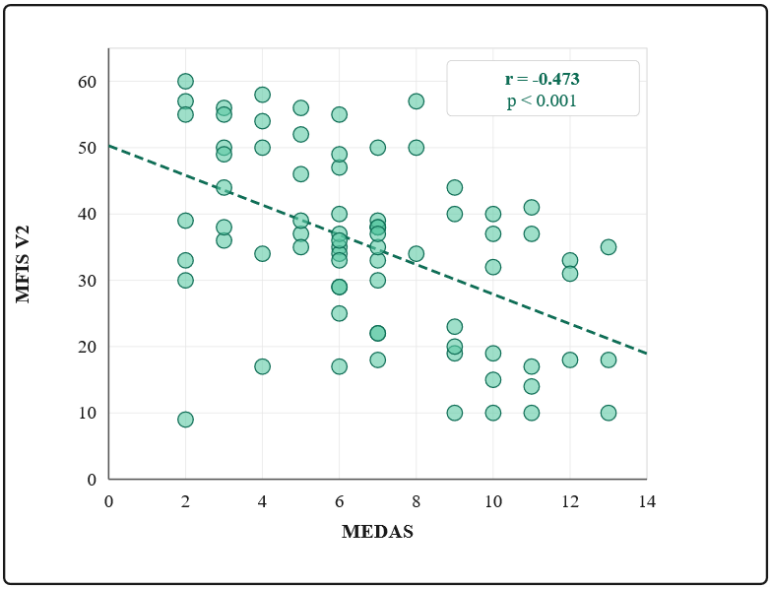

Figure 11. Scatter plot with regression line of the correlation between MEDAS and MFIS V2 (r=−0.473, p<0.001, n=76)

5. Extended analysis by MEDAS groups – Kruskal-Wallis and post-hoc test

Kruskal-Wallis analysis

To assess differences in clinical parameters across the three groups by level of adherence to the recommended diet, the Kruskal-Wallis Test was applied. The groups were formed on the MEDAS score (mean ± SD): low (0–5, n=25), medium (6–9, n=34), and high (10–14, n=17). The results are presented in Table 13.

Statistically significant differences between the groups were found for four of the parameters: EDSS (H=10.883, p=0.004), FSMC at V2 (H=11.959, p=0.003), MFIS at V1 (H=12.615, p=0.002), and MFIS at V2 (H=18.629, p<0.001). No significant differences were found in BMI, lipid profile, CRP, or body composition parameters among the three groups, suggesting that adherence to AMD primarily affects neurological and functional parameters rather than metabolic parameters in the short term.

Table 13. Clinical parameters by MEDAS groups (Kruskal-Wallis Test, n=76)

Parameter	Low (n=25) Mean±SD	Medium (n=34) Mean±SD	High (n=17) Mean±SD	p	sig.
EDSS	3.68±1.22	3.87±1.51	2.59±0.85	0.004	**
FSMC V1	57.28±12.58	52.26±6.95	47.76±12.24	0.074	ns
FSMC V2	54.68±10.50	47.85±6.43	42.00±13.87	0.003	**
MFIS V1	46.32±13.29	38.76±10.81	30.24±13.54	0.002	**
MFIS V2	43.56±13.01	34.21±11.34	24.53±11.48	<0.001	***
BMI V1	24.13±3.73	25.63±5.18	23.34±4.35	0.488	ns
Chol V1	5.64±1.15	5.08±1.07	4.92±1.35	0.069	ns
Chol V2	5.38±0.94	5.14±0.99	4.64±1.05	0.076	ns
LDL V1	2.82±1.13	2.60±0.92	2.79±1.07	0.864	ns
HDL V1	1.60±0.44	1.49±0.47	1.46±0.41	0.465	ns
CRP V1	3.00±4.04	3.41±9.37	3.99±7.44	0.455	ns
BF % V1	26.57±5.97	29.35±7.12	25.61±7.03	0.202	ns
VF % V1	5.78±2.32	6.60±3.59	5.03±3.39	0.298	ns
BW % V1	47.28±5.11	46.32±4.79	49.59±4.99	0.077	ns
MM V1	43.38±7.72	44.96±9.02	46.34±7.98	0.501	ns
DHA V1	1009.78±879.77	1179.88±1053.35	799.66±777.43	0.670	ns

*ns — non-significant; ** p<0.01; *** p<0.001*

Post-hoc analysis (Mann-Whitney U with Bonferroni correction)

To identify the specific between-group differences, a post-hoc analysis was performed using pairwise comparisons with the Mann-Whitney U test and a Bonferroni correction (adjusted alpha = 0.017). The results are presented in Table 14.

The post-hoc analysis revealed clinically important differences between the groups. For EDSS, the significant differences were between low and high MEDAS (p=0.003) and between medium and high MEDAS (p=0.003), whereas low and medium MEDAS did not differ significantly (p=0.710). The analysis identified better adherence to AMD among patients with a lower degree of disability, as recorded at V1 (MEDAS 10–14, EDSS 2.59). For MFIS at V2, significant differences were found among the three groups: low *vs* medium (p=0.004), low *vs* high (p<0.001), and medium *vs* high (p=0.011). This is the only parameter with full three-step dose-dependent significance, in which each step of dietary adherence is associated with a clinically significant reduction in fatigue at V2 (43.56 → 34.21 → 24.53 points). These results provide the most convincing evidence in the present study of a dose-dependent effect of MD on fatigue in RRMS. For FSMC at V2 and MFIS at V1, significant differences were found between the low and medium groups and the low and high groups but were absent between the medium and high groups. This suggests the presence of a threshold effect, in which the transition from low to at least medium adherence to the diet yields measurable clinical improvement, whereas the additional transition to high adherence adds no statistically significant difference on these scales.

Table 14. Post-hoc analysis of the significant parameters by MEDAS groups (Mann-Whitney U + Bonferroni correction)

Parameter	Low vs Medium	Low vs High	Medium vs High
EDSS	p=0.710 ns	p=0.003 *	p=0.003 *
FSMC V2	p=0.005 *	p=0.004 *	p=0.164 ns
MFIS V1	p=0.013 *	p=0.002 *	p=0.043 ns
MFIS V2	**p=0.004 ***	**p<0.001 ***	**p=0.011 ***

*Significant at p<0.017 (Bonferroni correction); ns — non-significant; * significant*

6. Sex differences in clinical parameters (Mann-Whitney U Test)

The analysis of sex differences was performed using the Mann-Whitney U test for independent samples. The results are presented in Table 15 and Table 16.

Demographic and clinical parameters by sex

No statistically significant sex differences were found with respect to age (men: 45.0 ± 7.6 yrs; women: 46.0 ± 7.8 yrs; p=0.645), disease duration (9.4 ± 8.4 yrs versus 9.6 ± 7.0 yrs; p=0.619), number of relapses (5.7 ± 4.4 versus 5.3 ± 3.6; p=0.883), degree of disability by EDSS (3.54 ± 1.56 versus 3.50 ± 1.23; p=0.979), and MEDAS scores (6.62 ± 3.17 versus 6.90

± 3.03; p=0.659), which attests to a good sex distribution with respect to the main clinical parameters.

The distribution by EDSS categories is presented in Table 15. Men more often fell into the lower-degree disability category (EDSS 0–2): m/w 10 (29.4%) versus 5 (11.9%), whereas in women the higher scores predominated (EDSS 2.5–4): w/m 29 (69.0%) versus 15 (44.1%).

Table 15. EDSS and MEDAS categories by sex (n=76)

Parameter	Category	Men n (%)	Women n (%)
EDSS	0–2	10 (29.4%)	5 (11.9%)
	2.5–4	15 (44.1%)	29 (69.0%)
	4.5–6	6 (17.6%)	6 (14.3%)
	>6	3 (8.8%)	2 (4.8%)
MEDAS	Low (0–5)	14 (41.2%)	11 (26.2%)
	Medium (6–9)	12 (35.3%)	22 (52.4%)
	High (10–14)	8 (23.5%)	9 (21.4%)

Body composition and anthropometric parameters by sex

Significant sex differences were found in the body composition parameters. Men had a significantly higher BMI at V1 (25.41 ± 3.10 versus 23.99 ± 5.49 kg/m²; p=0.019) and V2 (24.91 ± 3.11 versus 23.56 ± 5.28 kg/m²; p=0.031), higher muscle mass (49.21 ± 8.65 versus 41.14 ± 6.11 kg; p<0.001), and a higher body-water percentage (48.74 ± 4.52% versus 46.25 ± 5.21%; p=0.017). Women had a significantly higher body-fat percentage at V1 (29.73 ± 7.68% versus 24.96 ± 4.51%; p=0.003) and V2 (29.02 ± 7.36% versus 23.90 ± 4.51%; p=0.002), reflecting the characteristic sex-specific differences in the distribution of adipose tissue. Visceral fat was significantly higher in men at V1 (7.42 ± 3.11% versus 4.87 ± 2.82%; p<0.001) and V2 (6.88 ± 2.78% versus 4.73 ± 2.64%; p=0.001).

Fatigue and biochemical parameters by sex

No significant sex differences were found with respect to fatigue by FSMC (V1: 51.88 ± 12.56 versus 53.74 ± 9.18; p=0.211) and MFIS (V1: 39.24 ± 14.23 versus 39.43 ± 13.06; p=0.754), nor at V2 (p=0.790 and p=0.766, respectively). In women, significantly higher levels of HDL cholesterol were observed (1.63 ± 0.47 versus 1.37 ± 0.36 mmol/L; p=0.032), consistent with known sex-specific differences in the lipid profile. An interesting finding in women was the significantly higher DHA levels at V1 (1289.81 ± 998.26 versus 720.38 ± 768.66 µg/mL; p=0.005), which may reflect differences in dietary behaviour or in the metabolism of omega-3 PUFAs (Table 16).

Table 16. Sex differences in the clinical and anthropometric parameters (Mann-Whitney U Test, n=76)

Parameter	Men (Mean ± SD)	Women (Mean ± SD)	p	sig.
Age (yrs)	45.0 ± 7.6	46.0 ± 7.8	0.645	ns
MS duration (yrs)	9.4 ± 8.4	9.6 ± 7.0	0.619	ns
Number of relapses (n)	5.7 ± 4.4	5.3 ± 3.6	0.883	ns
EDSS	3.54 ± 1.56	3.50 ± 1.23	0.979	ns
MEDAS	6.62 ± 3.17	6.90 ± 3.03	0.659	ns
BMI V1 (kg/m²)	25.41 ± 3.10	23.99 ± 5.49	0.019	*
BMI V2 (kg/m²)	24.91 ± 3.11	23.56 ± 5.28	0.031	*
FSMC V1	51.88 ± 12.56	53.74 ± 9.18	0.211	ns
FSMC V2	48.29 ± 12.97	49.19 ± 8.84	0.790	ns
MFIS V1	39.24 ± 14.23	39.43 ± 13.06	0.754	ns
MFIS V2	34.79 ± 14.28	35.38 ± 13.43	0.766	ns
HDL V1 (mmol/L)	1.37 ± 0.36	1.63 ± 0.47	0.032	*
BF% V1	24.96 ± 4.51	29.73 ± 7.68	0.003	**
BF% V2	23.90 ± 4.51	29.02 ± 7.36	0.002	**
VF% V1	7.42 ± 3.11	4.87 ± 2.82	<0.001	***
VF% V2	6.88 ± 2.78	4.73 ± 2.64	0.001	**
BW% V1	48.74 ± 4.52	46.25 ± 5.21	0.017	*
MM V1 (kg)	49.21 ± 8.65	41.14 ± 6.11	<0.001	***
DHA V1 (µg/mL)	720.38 ± 768.66	1289.81 ± 998.26	0.005	**
EPA V1 (µg/mL)	1237.85 ± 157.73	1188.98 ± 201.39	0.409	ns

*ns — non-significant; * p<0.05; ** p<0.01; *** p<0.001*

7. Analysis of body composition

Changes in the body composition parameters between the two visits

Body composition was assessed by bioimpedance analysis (Tanita) at V1 and V2. The results of the comparative analysis are presented in Table 17.

Statistically significant improvements were observed in four of the seven parameters studied. The body-fat percentage decreased significantly from 27.60 ± 6.85% to 26.73 ± 6.72% (Δ = -0.87 %; p < 0.001), with improvement observed in 68.4% of patients., with improvement recorded in 68.4% of patients. Visceral fat decreased from 5.99 ± 3.20% to 5.71 ± 2.91% (Δ=−0.28%; p=0.001), and the proportion of patients with above-normal visceral fat (≥9%) decreased from 18.7% (n=14) to 15.8% (n=12). Body water increased significantly from 47.37

± 5.04% to 49.28 ± 4.87% (Δ=+1.91%; p<0.001), with 77.6% of patients showing improvement, the highest percentage of increase among the studied bioimpedance parameters. n 65.8% of patients, muscle mass increased from 44.75 ± 8.35 to 45.62 ± 8.22 kg (Δ = +0.87 kg; p = 0.002). No significant changes were found in bone mass (p=0.142) or basal metabolism (p=0.310).

Table 17. Body composition parameters (Tanita) at V1 and V2 (Wilcoxon Signed-Rank Test, n=76)

Parameter	V1 Mean±SD	V2 Mean±SD	Δ	p	sig.	% improved
BF %	27.60±6.85	26.73±6.72	−0.87	<0.001	***	68.4%
VF %	5.99±3.20	5.71±2.91	−0.28	0.001	***	38.7%
BW %	47.37±5.04	49.28±4.87	+1.91	<0.001	***	77.6%
MM (kg)	44.75±8.35	45.62±8.22	+0.87	0.002	**	65.8%
BM (kg)	2.75±0.54	2.74±0.53	−0.02	0.142	ns	13.2%
BaM (kcal)	1423.37±302.28	1437.86±278.70	+14.49	0.310	ns	47.4%
Metabolic age	44.45±11.98	44.18±11.99	−0.26	0.015	*	32.9%

*ns – non-significant; * p<0.05; ** p<0.01; *** p<0.001*

Distribution by reference values

The range of body fat was 9.0–44.8% (Me 26.4%, IQR 22.5–30.9%) at V1 and 10.0–41.2% (Me 25.6%, IQR 21.2–31.5%) at V2, with a trend towards lower median values. For visceral fat, the range at V1 was 1.0–15.5% (Me 5.0%, IQR 3.5–7.8%), with 14/76 patients (18.4%) having above-normal values (≥9%), while at V2 this proportion decreased to 12/76 (15.8%) (Me 5.5%, IQR 3.5–7.1%). Regarding body water, the range at V1 was 38.6–62.1% (Me 47.7%, IQR 44.2–50.6%), and at V2 – 39.1–60.7% (Me 49.8%, IQR 45.4–52.6%), reflecting a trend towards improvement in hydration status. The range of muscle mass at V1 was 25.8–73.4 kg (Me 43.3 kg, IQR 40.6–48.4 kg), and at V2 it was 27.9–75.6 kg (Me 44.4 kg, IQR 40.5–49.5 kg).

Correlations of body composition with MEDAS, EDSS, and fatigue

No significant correlations were found between the body composition parameters at V1 and MEDAS, EDSS, or MFIS at V1. A significant correlation was found between MEDAS and the change in visceral fat (ΔVF; r = −0.343, p = 0.002), supporting the thesis that adherence to AMD exerts a distinct influence on the dynamics of visceral adipose tissue during follow-up. Significant improvements were recorded in 5 of the 7 bioimpedance parameters – body fat, visceral fat, body water, muscle mass, and metabolic age, at p < 0.05 (Table 17). The highest proportion of patients with individual improvement in a favourable direction was recorded for body water (77.6%), followed by body fat (68.4%) and muscle mass (65.8%). The number of

patients with above-normal visceral fat (≥9%) decreased from 14/76 (18.4%) at V1 to 12/76 (15.8%) at V2.

8. Analysis of serum biomarkers (lipid profile, CRP, IL-17A, omega-3 PUFAs)

Lipid profile

The lipid profile was tested in 67 (88.2%) patients (29 men/38 women) at both visits (Table 18). No statistically significant changes were found in any of the lipid profile parameters between V1 and V2 (Wilcoxon signed-rank test).

The correlation analysis revealed a significant negative correlation between MEDAS and total cholesterol at V1 ($r = -0.330$, $p = 0.006$), suggesting that better adherence to AMD is associated with lower baseline cholesterol levels. Regarding the MEDAS groups, patients with low MEDAS had the highest mean cholesterol at V1 (5.49 mmol/L), followed by those with high MEDAS (5.14 mmol/L) and medium MEDAS (4.40 mmol/L); the difference between the groups was statistically significant (Kruskal-Wallis, $p = 0.012$). Total cholesterol showed a trend toward a decrease (Δ = -0.13 mmol/L) that did not reach statistical significance ($p = 0.053$). In comparative terms, LDL remained practically unchanged ($\Delta = -0.02$ mmol/L; $p = 0.864$), and triglycerides were stable ($\Delta = -0.01$ mmol/L; $p = 0.492$). Overall, a minimal increase was recorded in HDL ($\Delta = +0.04$ mmol/L; $p = 0.272$), but it should be noted that the number of patients with reduced HDL (<1.0 mmol/L in men, <1.2 mmol/L in women) increased from 10 (14.9%) at V1 to 14 (20.9%) at V2. The analysis by sex showed that this increase was due entirely to men, in whom the proportion of reduced HDL rose from 8.8% (n=3) to 20.6% (n=7) at V2, whereas in women it remained unchanged at 16.7% (n=7) at both visits. The trend towards decreased HDL in men, although not statistically significant, warrants attention during longer-term follow-up, since low HDL is an independent risk factor for cardiovascular disease.

Table 18. Lipid profile at V1 and V2 (Wilcoxon Signed-Rank Test, n=67)

Parameter	V1 Mean±SD	V2 Mean±SD	V1 Me	B1 IQR	V2 Me	B2 IQR	Δ	p	sig.
Chol	5.24±1.19	5.11±1.01	5.23	4.38–6.02	5.08	4.42–5.79	−0.13	0.053	ns
LDL	2.73±1.03	2.71±0.97	2.48	2.05–3.45	2.43	1.94–3.63	−0.02	0.864	ns
HDL	1.52±0.44	1.56±0.51	1.45	1.23–1.87	1.45	1.19–1.91	+0.04	0.272	ns
TGL	1.28±0.70	1.27±0.72	1.03	0.81–1.56	1.07	0.83–1.51	−0.01	0.492	ns

Mean ± SD – mean ± standard deviation; Me – median; IQR – interquartile range (25th–75th percentile). Wilcoxon Signed-Rank Test, n=67.

C-reactive protein

C-reactive protein was tested in 68 patients. At V1, the mean value was 3.40 ± 7.31 mg/L (median 0.95 mg/L, IQR 0.42–3.15), and at V2 – 2.10 ± 2.22 mg/L (Me 1.22 mg/L, IQR 0.60–2.73). Despite the observed absolute decrease in the mean values, the difference did not reach statistical significance (Wilcoxon p=0.174). A clinically significant finding is that the proportion of patients with elevated CRP (>5.0 mg/L) decreased by 7.3 percentage points – from 17.6% (n=12) at V1 to 10.3% (n=7) at V2, despite the absence of statistical significance in the paired analysis. No significant correlation was found between MEDAS and CRP (r=+0.032, p=0.798).

Interleukin-17A

IL-17A was tested in 30 patients (V1) and 15 patients (V2) due to limited material availability. The paired analysis was performed on 15 patients with documented data at both visits (Table 19). At V1, the mean value was 118.58 ± 33.64 pg/mL (Me 116.04 pg/mL), and at V2 – 17.63 ± 15.63 pg/mL (Me 18.21 pg/mL). The established decrease in IL-17A was substantial and statistically significant (Wilcoxon p<0.001) in all studied patients. The absolute change (Δ = −100.95 pg/mL) represents a reduction of more than 85% relative to the baseline values. Despite the small size of the subgroup, this finding is clinically significant and suggests a pronounced anti-inflammatory effect of the dietary intervention on Th17-mediated inflammation.

Omega-3 PUFAs (EPA and DHA)

Of the omega-3 PUFAs, EPA was tested in 47 patients and DHA – in 48 (Table 19). The EPA results showed a non-significant trend toward an increase (1179.0 ± 196.8 versus 1219.5 ± 220.4 μg/mL; Δ = +40.5 μg/mL; p = 0.102). For DHA, a significant increase was observed from 1056.9 ± 923.0 to 1381.0 ± 967.8 μg/mL (Δ = +324.1 μg/mL; p = 0.003), with a 60.5% increase in the median value (796.5 versus 1278.5 μg/mL). This finding is clinically significant and may reflect changes in dietary behaviour and in the consumption of fish and seafood during the course of follow-up.

Table 19. CRP, IL-17A, and omega-3 parameters at V1 and V2

Parameter	N	V1 Mean±SD	V2 Mean±SD	V1 Me	V2 Me	p	sig.
CRP (mg/L)	68	3.40±7.31	2.10±2.22	0.95	1.22	0.174	ns
IL-17A (pg/mL)	15	118.58±33.64	17.63±15.63	116.04	18.21	<0.001	***
EPA (μg/mL)	47	1179.0±196.8	1219.5±220.4	1221.0	1285.0	0.102	ns
DHA (μg/mL)	48	1056.9±923.0	1381.0±967.8	796.5	1278.5	0.003	**

*ns – non-significant; ** p<0.01; *** p<0.001*

Among the changes in body composition, particularly significant are the trend towards a decrease in total cholesterol (p=0.053), the pronounced, significant decrease in IL-17A (−85%, p<0.001) in all patients in the studied subgroup, and the significant increase in DHA (Me +60%, p=0.003).

9. Analysis of vital signs: blood pressure, pulse, and respiratory rate

Blood pressure

Blood pressure (BP) was assessed categorically at both visits in all RRMS patients (n=76). Given the main study period of 2020–2025, the diagnostic thresholds for office-measured BP were aligned with the guidelines of the European Society of Cardiology ESC/ESH 2018 (Williams B et al., Eur Heart J 2018).

The distribution by category and the changes between V1 and V2 are presented in Table 20. At the baseline visit V1, optimal blood pressure was present in 27 patients (35.5%), normal in 30 (39.5%), high-normal in 17 (22.4%), and high (hypertension) in 2 (2.6%). At V2,, a statistically non-significant trend (Wilcoxon p=0.284) towards improvement was observed, with the proportion of patients with high-normal blood pressure decreasing from 22.4% to 19.7% and with high blood pressure decreasing from 2.6% to 1.3%.

The analysis of individual changes showed that in 63 patients (84.0%) the BP category remained unchanged, in 8 (10.7%) an improvement was observed (transition to a lower category), and in 4 (5.3%) – a worsening. The analysis by sex revealed a significant difference in the distribution at V1 – in women the proportion of patients with optimal BP was higher (50.0% versus 17.6% in men), whereas men more often fell into the high-normal BP category (35.3% versus 11.9%).

A significant positive correlation was found between EDSS and the blood-pressure category (r=+0.234, p=0.042), suggesting that a more severe degree of disability is associated with a less favourable haemodynamic profile with respect to BP. Among the MEDAS groups, patients with high adherence to AMD (82.4%) were more likely to have optimal or normal BP than those with low (76.0%) or medium (70.6%) MEDAS scores, although the difference was not statistically significant.

Table 20. Distribution by blood-pressure category (V1 and V2, n=76)

Category (mmHg)	V1 n (%)	V2 n (%)	Change
Optimal	27 (35.5%)	28 (36.8%)	+1
Normal	30 (39.5%)	31 (40.8%)	+1
High-normal	17 (22.4%)	15 (19.7%)	−2
High	2 (2.6%)	1 (1.3%)	−1

Wilcoxon p=0.284 (ns)

Pulse

The pulse rate remained stable between the two visits – 68.37 ± 5.44 bpm at V1 versus 68.49 ± 5.19 bpm at V2 (Δ = +0.12 bpm; Wilcoxon p = 0.693). The ranges at V1 and V2 were 58–85 and 59–84 bpm, respectively, with an unchanged median of 68 bpm at both sites (Table 21). A significant sex difference was observed: men had a higher pulse rate at V1 (69.9 ± 4.7 versus 67.2 ± 5.8 bpm; Mann-Whitney, p=0.012). No significant correlations were found between pulse and MEDAS (r = −0.003, p=0.976) or EDSS (r = −0.114, p = 0.326).

Respiratory rate

The respiratory rate showed a statistically significant increase between the two visits – from 16.36 ± 0.76 to 16.66 ± 0.74 breaths/min (Wilcoxon p=0.002). The median increased from 16 to 17 breaths/min, within a range of 15–18 breaths/min at V1. Despite the statistical significance, the absolute change (Δ = +0.30 breaths/min) is clinically negligible (Table 21). A weak but significant positive correlation was found between MEDAS and respiratory rate (r = +0.242, p = 0.035), which probably reflects individual physiological parameters and other confounding factors in patients with higher dietary adherence scores, rather than a pathological change.

Table 21. Pulse and respiratory rate at V1 and V2 (n=76)

Parameter	V1 Mean±SD	Me V1	V2 Mean±SD	Me V2	p	Sig.
HR	68.37±5.44	68.0	68.49±5.19	68.0	0.693	ns
RR	16.36±0.76	16.0	16.66±0.74	17.0	0.002	**

*ns — non-significant; ** p<0.01*

10. Multivariate logistic regression analysis

To assess the independent contribution of the main demographic and clinical factors to the clinically significant outcomes of the dietary intervention, a binary logistic regression analysis was applied. Univariate and multivariate Odds Ratios (OR / aOR) with 95% confidence intervals (CI) were calculated. The inclusion of predictors in the multivariate models was carried out in accordance with the rule of a minimum 8–10 events per predictor (Peduzzi et al. 1996). The results are presented in Tables 22–25.

Model 1: Improvement in fatigue by FSMC (MCID ≥ 4 points). In a multivariate analysis adjusted for MEDAS, EDSS, and age, none of the studied predictors reached statistical significance. A borderline effect was observed for female sex (aOR = 2.60; 95% CI 0.97–6.98; p = 0.057) and EDSS (univariate: OR = 0.70; 95% CI 0.49–0.99; p = 0.045), which lost significance after adjustment for the other variables. These results suggest that the FSMC

improvement occurs relatively uniformly across all patients, with no specific predictor identified. The model has moderate discriminative ability (AUC = 0.718) (Table 22).

Table 22. Logistic regression for clinically significant improvement in fatigue by FSMC (MCID ≥ 4 points)

Predictor	Univariate analysis			Multivariate analysis (Adjusted)		
	OR	95% CI	p	aOR	95% CI	p
MEDAS score (per 1 point)	1.13	0.97–1.32	0.1197	1.06	0.89–1.26	0.5046
EDSS (per 1 point)	0.70	0.49–0.99	0.0454 *	0.74	0.50–1.09	0.1329
Age (per 1 year)	0.95	0.89–1.01	0.0897	0.95	0.89–1.02	0.1386
Female sex	2.32	0.92–5.85	0.0741	2.60	0.97–6.98	0.0571
Higher education	1.20	0.40–3.65	0.7444	–	–	–

*OR – odds ratio (univariate); aOR – adjusted odds ratio (multivariate, adjusted for the other predictors); 95% CI – 95% confidence interval. ns – non-significant (p ≥ 0.05); * p < 0.05; ** p < 0.01; *** p < 0.001.*

Model 2: Improvement in fatigue by MFIS (MCID ≥ 4 points). he MEDAS score was the only statistically significant independent predictor of clinically significant improvement in fatigue (MFIS; aOR = 1.22; 95% CI 1.02–1.45; p = 0.027), indicating that each additional point of adherence to AMD is associated with a 22% higher likelihood of clinically significant improvement in fatigue. This finding remains statistically significant after adjustment for EDSS, age, and sex, confirming the independent dose-dependent effect of adherence to AMD on fatigue in RRMS (Table 23).

Table 23. Logistic regression for clinically significant improvement in fatigue by MFIS (MCID ≥ 4 points)

Predictor	Univariate analysis			Multivariate analysis (Adjusted)		
	OR	95% CI	p	aOR	95% CI	p
MEDAS score (per 1 point)	1.21	1.03–1.42	0.0213 *	1.22	1.02–1.45	0.0274 *
EDSS (per 1 point)	0.87	0.63–1.22	0.4183	1.00	0.69–1.43	0.9827
Age (per 1 year)	0.99	0.94–1.05	0.8164	1.01	0.95–1.08	0.7556
Female sex	0.96	0.38–2.37	0.9222	0.89	0.34–2.30	0.8041
Higher education	2.06	0.64–6.65	0.2275	–	–	–

*n = 76, number of events = 42 (55.3%); ns – non-significant (p ≥ 0.05); * p < 0.05; ** p < 0.01; *** p < 0.001.*

Model 3: High adherence to AMD (MEDAS ≥ 10 points).

This model demonstrated the strongest discriminative ability (AUC = 0.843; pseudo-R^2 = 0.283; LR test $p < 0.001$) and identified two independent predictors. Higher education was the strongest predictor of high adherence to AMD – patients with higher education were 8.75 times more likely to have high adherence than those with lower education (aOR = 8.75; 95% CI 2.25–34.00; p = 0.002), independently of EDSS. The degree of disability was a significant inverse predictor – each additional EDSS point reduced the chance of high adherence by approximately 53% (aOR = 0.47; 95% CI 0.24–0.90; p = 0.022). Univariately, employment was also a significant predictor (OR = 5.14; 95% CI 1.08–24.58; p = 0.040), but was not included in the multivariate model owing to the 10 EPV constraint (n = 17 events). Marital status and age did not emerge as independent predictors (Table 24).

Table 24. Logistic regression for high adherence to AMD (MEDAS ≥ 10 points)

Predictor	Univariate analysis			Multivariate analysis (Adjusted)		
	OR	95% CI	p	aOR	95% CI	p
Higher education	12.62	3.50–45.51	0.0001 ***	8.75	2.25–34.00	0.0017 **
Marital status (married)	2.56	0.75–8.79	0.1350	–	–	–
Employed	5.14	1.08–24.58	0.0402 *	–	–	–
EDSS (per 1 point)	0.40	0.22–0.73	0.0030 **	0.47	0.24–0.90	0.0225 *
Age (per 1 year)	0.96	0.90–1.03	0.2644	–	–	–

*n = 76, number of events = 17 (22.4%); ns – non-significant (p ≥ 0.05); * p < 0.05; ** p < 0.01; *** p < 0.001.*

A comparative visualisation of the univariate and multivariate Odds Ratios for all potential predictors in Model 3 is presented in Figure 14.

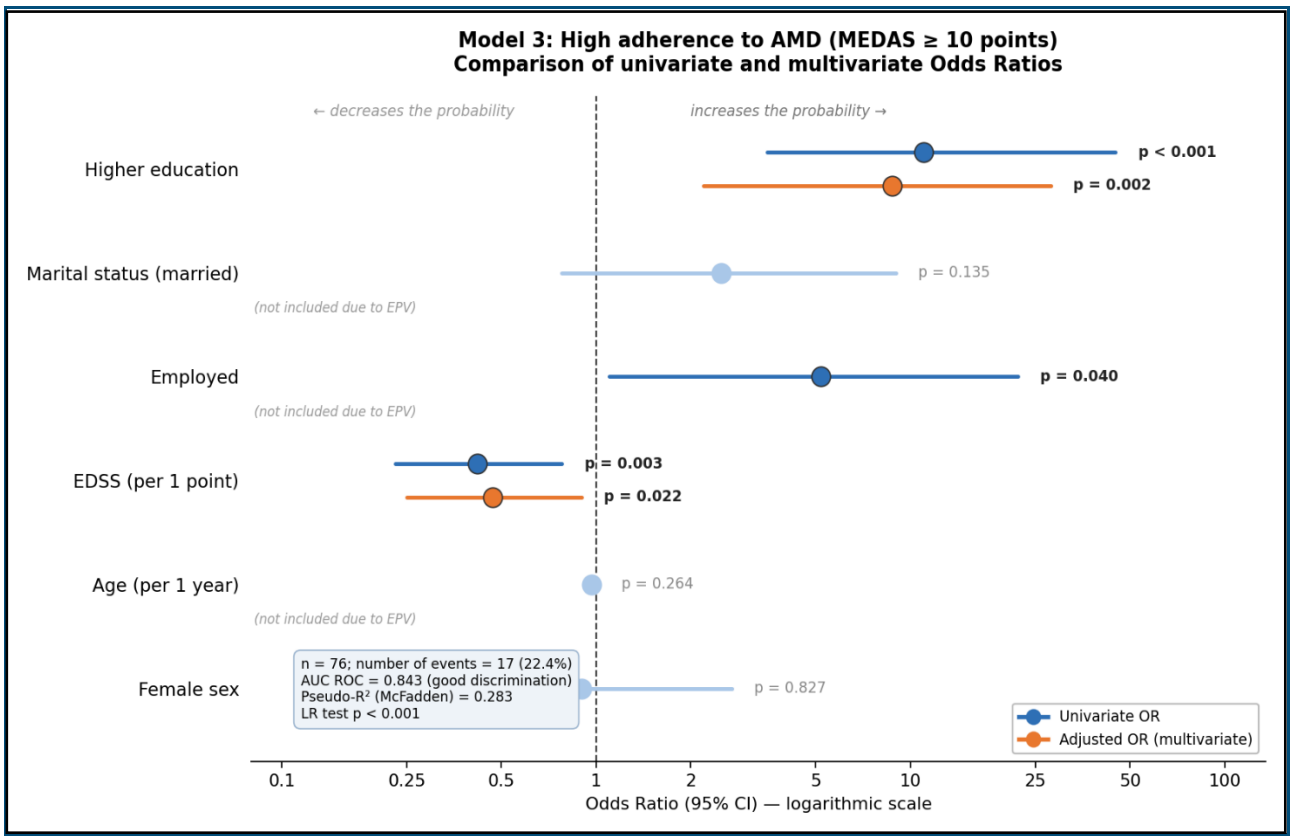

Figure 14. Forest plot of Model 3: High adherence to AMD, MEDAS ≥ 10 points, with a comparison of the univariate and multivariate Odds Ratios.

OR – odds ratio (univariate); aOR – adjusted odds ratio (multivariate); 95% CI – 95% confidence interval; EPV – events per variable; AUC – area under the curve; LR test – likelihood ratio test.

The detailed forest plot of Model 3 presents, in parallel, the univariate Odds Ratios (blue, top row) and the multivariate-adjusted Odds Ratios (orange, bottom row) for each of the six potential predictors of high adherence to AMD (the p-values are indicated next to the corresponding points). Owing to the constraint of the rule of a minimum of 8–10 events per predictor (EPV) (Peduzzi et al. 1996), with n = 17 events in this model, four of the variables (marital status, employment, age, female sex) were analysed univariately and were not included in the multivariate model. Higher education demonstrated a pronounced effect size in both analyses (univariate OR = 12.62; multivariate aOR = 8.75), and EDSS emerged as a significant inverse predictor (multivariate aOR = 0.47). The key model statistics – AUC = 0.843 (good discrimination) and pseudo-R^2 = 0.283, LR test $p < 0.001$ – are also presented in Figure 14.

Model 4: Reduction in visceral fat.

The multivariate analysis identified three independent predictors. A higher MEDAS score remained significantly associated with a greater likelihood of a reduction in visceral fat (aOR = 1.24 per point; 95% CI 1.04–1.49; p = 0.018), confirming the dose-dependent effect of adherence to AMD on the anthropometric parameters. A higher baseline BMI was also a

significant predictor (aOR = 1.12; 95% CI 1.00–1.26; p = 0.049), which may reflect both a greater potential for improvement and regression to the mean in patients with a higher baseline BMI. Female sex was a strong inverse predictor (aOR = 0.27; 95% CI 0.09–0.78; p = 0.015) – men were 3.7 times more likely to have a reduction in visceral fat, which is consistent with the known sex-specific differences in the distribution of adipose tissue and the higher baseline visceral fat in men (Table 25).

Table 25. Logistic regression for reduction in visceral fat

Predictor	Univariate analysis			Multivariate analysis		
	OR	**95% CI**	**p**	**aOR**	**95% CI**	**p**
MEDAS score (per 1 point)	1.18	1.00–1.38	0.0530	1.24	1.04–1.49	0.0179 *
Baseline BMI (per 1 kg/m²)	1.12	1.00–1.25	0.0466 *	1.12	1.00–1.26	0.0490 *
Female sex	0.30	0.11–0.78	0.0139 *	0.27	0.09–0.78	0.0153 *
Age (per 1 year)	0.98	0.93–1.05	0.6138	–	–	–
EDSS (per 1 point)	0.87	0.61–1.23	0.4214	–	–	–

*n = 75, number of events = 29 (38.7%); ns – non-significant (p ≥ 0.05); * p < 0.05; ** p < 0.01; *** p < 0.001.*

The overall picture of the established independent predictors across the four regression models is presented in summary form in Figure 15, which simultaneously visualises the adjusted Odds Ratios (aORs) with 95% confidence intervals for all models.

The discriminative ability of the four logistic regression models is presented graphically through Receiver Operating Characteristic (ROC) curves in Figure 16.

In summary, the multivariate logistic regression analysis confirms and enriches the findings of the correlation analysis. Higher adherence to AMD (MEDAS) was an independent predictor of clinically significant improvement in fatigue on the MFIS and of reductions in visceral fat, even after adjustment for other clinical and demographic variables. At the same time, higher education and a lower degree of disability (EDSS) emerged as the strongest independent determinants of high adherence to the dietary regimen, which has direct implications for the development of individualised dietary support strategies in the Bulgarian RRMS patient population.

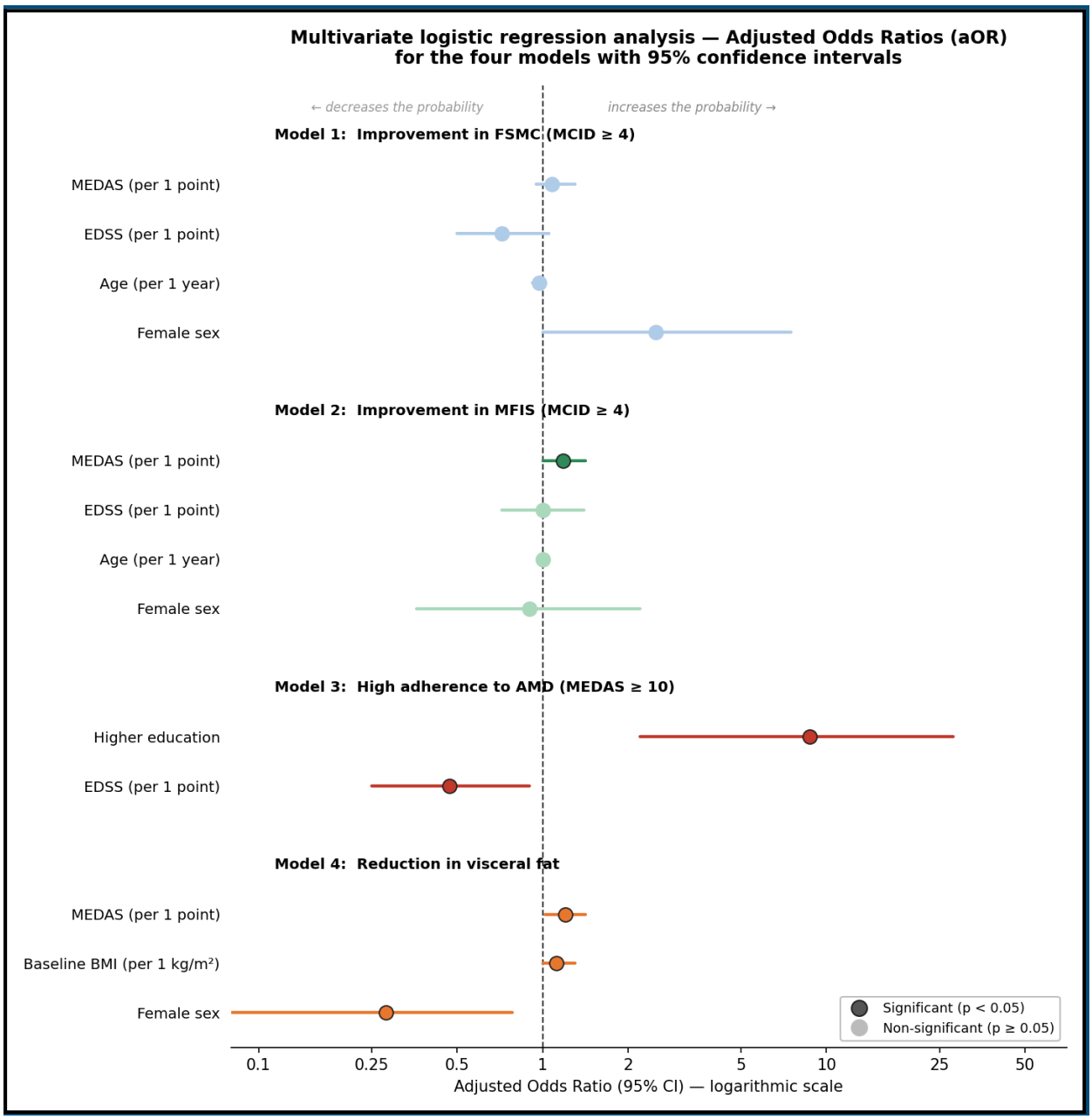

Figure 15. Forest plot of the four logistic regression models – adjusted Odds Ratios (aOR), 95% CI for the predictors in the four multivariate logistic regression models.

The points represent the OR, and the horizontal lines – the 95% CI. The statistically significant predictors are in a more saturated colour and with a larger point marker. The vertical dashed line (OR = 1) marks the absence of an effect; values to the left of the line indicate a decreased probability of the outcome; values to the right – an increase. The emphasised points and lines denote statistically significant predictors ($p < 0.05$); the lighter ones – non-significant ($p \geq 0.05$). The horizontal axis is presented on a logarithmic scale. Colour coding: blue – Model 1 (FSMC); green – Model 2 (MFIS); red – Model 3 (High adherence to AMD); orange – Model 4 (Visceral fat).

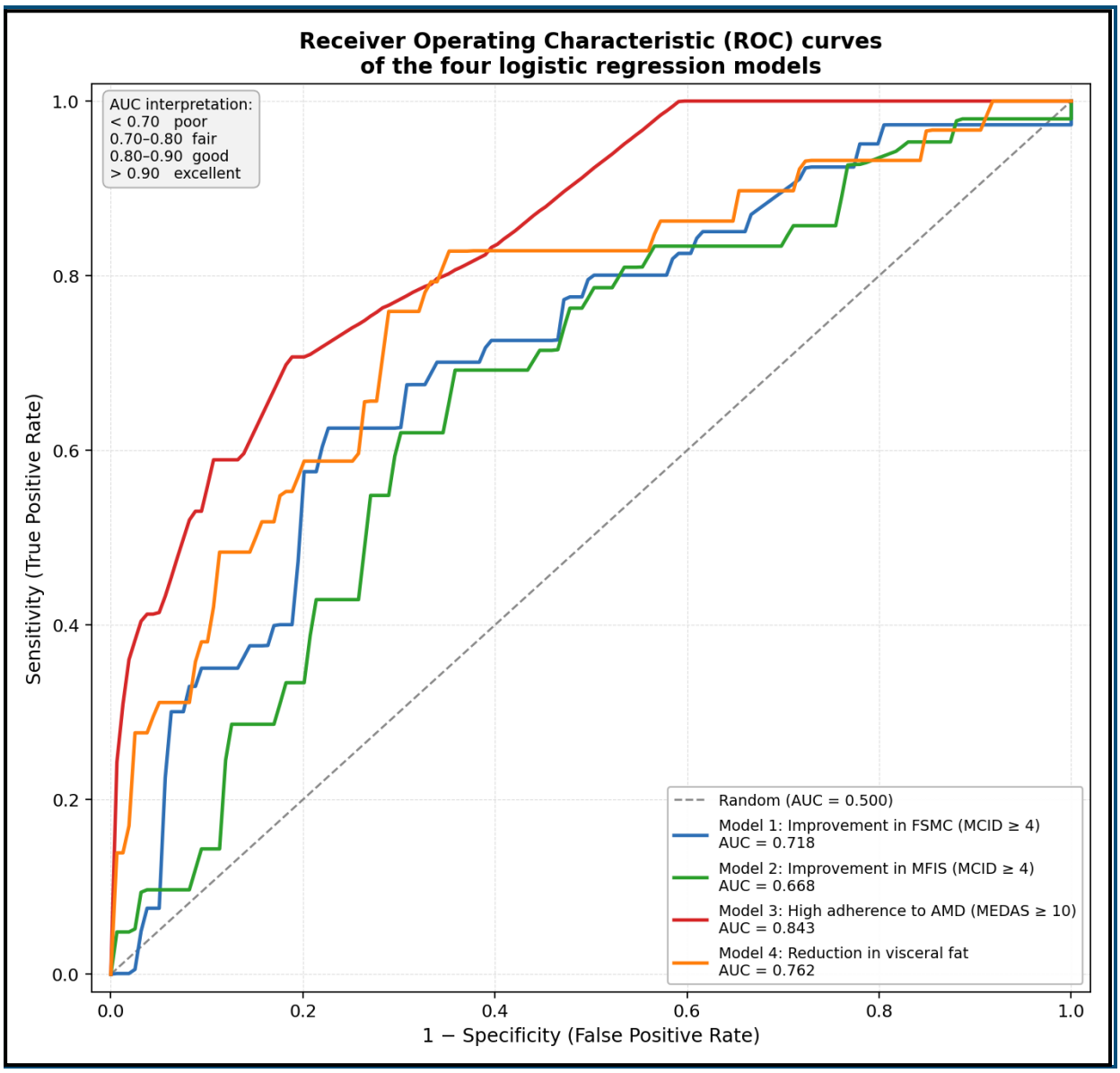

Figure 16. Receiver Operating Characteristic (ROC) curves of the four logistic regression models with AUC values.

The curves depict sensitivity (True Positive Rate) versus 1 – specificity (False Positive Rate) at different threshold values. The area under the curve (AUC – Area Under the Curve) is a numerical indicator of discriminative ability: AUC < 0.70 – weak, 0.70–0.80 – moderate, 0.80–0.90 – good, > 0.90 – excellent. The dashed diagonal line represents random classification (AUC = 0.500). The highest discriminative ability was demonstrated by Model 3 (High adherence to AMD, AUC = 0.843), followed by Model 4 (Visceral fat, AUC = 0.762), Model 1 (FSMC, AUC = 0.718), and Model 2 (MFIS, AUC = 0.668).

11. Summary of the results

The results of the present prospective, longitudinal, observational study in 76 RRMS patients reveal a complex picture of the interrelationships among the adapted Mediterranean dietary regimen, clinical parameters, and disease dynamics. The significant results can be systematised into four main areas:

- **Significant improvements between the two visits.** The results are illustrated in Figure 17. Statistically significant positive changes were found in nine parameters: fatigue by FSMC (−4.1 points; p<0.001; 80.3% improved), fatigue by MFIS (−4.2 points; p<0.001; 84.2% improved), BMI (p<0.001), body fat % (p<0.001), visceral fat % (p=0.001), body water % (p<0.001), muscle mass (p=0.002), IL-17A (−85% reduction; p<0.001; n=15), and DHA (+30.7%; p=0.003). No significant changes were found in the lipid profile, CRP, EPA, pulse, or blood pressure.

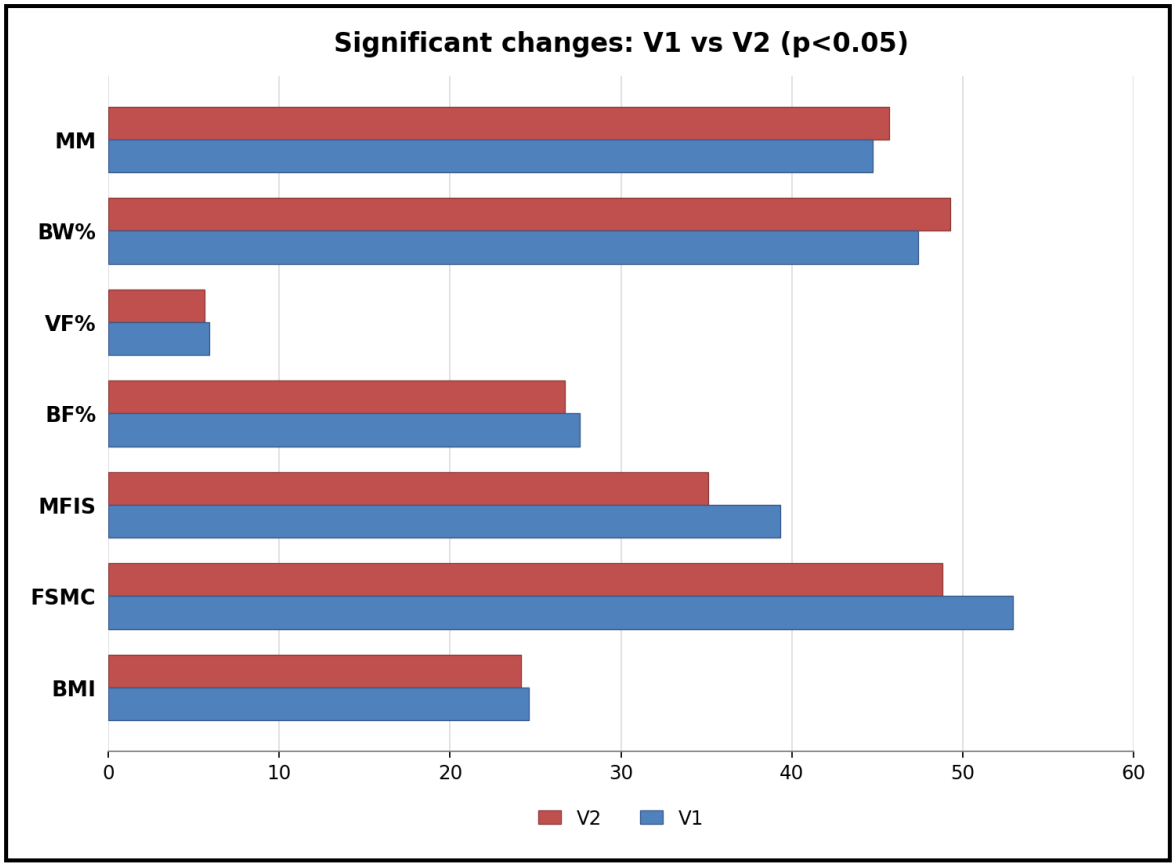

Figure 17. Significant changes between Visit 1 and Visit 2

- **Associations of the AMD adherence results (MEDAS) with the clinical parameters.** The results are illustrated in Figure 18. The MEDAS score demonstrated significant negative correlations with EDSS (r=−0.350; p=0.002), MFIS V1 (r=−0.384; p<0.001), and MFIS V2 (r=−0.473; p<0.001), and showed the strongest correlation among the study variables. Significant correlations were also found between MEDAS and the changes in FSMC (r=−0.326; p=0.004), BMI (r=−0.274; p=0.017), and % visceral fat (r=−0.343; p=0.002), suggesting a dose-dependent effect of dietary adherence. A significant inverse correlation was also observed between MEDAS and total cholesterol at V1 (r = -0.330; p = 0.006).
- **Significant clinical results by MEDAS groups.** The Kruskal-Wallis analysis across the three MEDAS groups confirms significant differences in EDSS (p=0.004), FSMC

V2 (p=0.003), MFIS V1 (p=0.002), and MFIS V2 (p<0.001). The post-hoc analysis with Bonferroni correction reveals that, for MFIS V2, there are significant differences among the three studied groups, providing the most convincing evidence for the effect of the MD on fatigue. Regarding EDSS, the significant differences are limited to the group with high adherence to AMD; only patients with MEDAS 10–14 (low EDSS, 2.59) differ significantly from the other two groups (Figure 18).

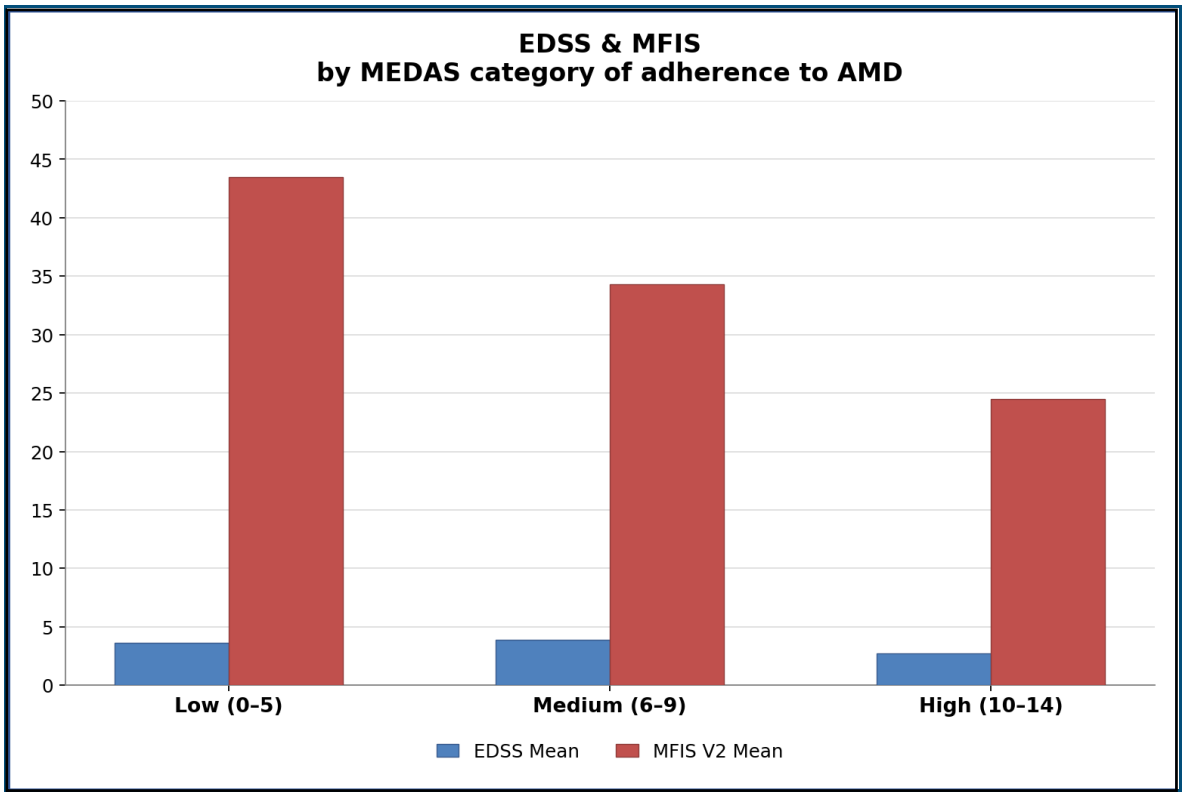

Figure 18. Significant changes in EDSS & MFIS by MEDAS adherence category

- **Significant demographic and clinical determinants.** The analysis of determinants of adherence to AMD reveals that education is the strongest predictor of the MEDAS score – patients with higher education had a significantly higher MEDAS score (9.19 ± 3.31) than those with primary education (5.24 ± 3.13). Marital status is also significantly associated with MEDAS, suggesting better adherence to the dietary regimen among married patients (7.35 versus 5.90; p=0.041). The sex differences are limited to the body composition parameters, with no significant differences in fatigue, EDSS, or MEDAS between men and women.
- **Independent predictors from the multivariate regression analysis.** Binary logistic regression identified independent predictors after adjustment for confounders. The MEDAS score remained an independent predictor of clinically significant improvement in fatigue by MFIS (aOR = 1.22 per point; 95% CI 1.02–1.45; p=0.027) and of the reduction in visceral fat (aOR = 1.24 per point; 95% CI 1.04–1.49; p=0.018), confirming

the independent dose-dependent effect of adherence to AMD. Higher education emerged as the strongest independent predictor of high adherence to AMD (aOR = 8.75; 95% CI 2.25–34.00; p=0.002), and EDSS – as a significant inverse predictor (aOR = 0.47 per point; 95% CI 0.24–0.90; p=0.022), with excellent discriminative ability of the model (AUC = 0.843).

12. Conclusions from the results

Based on the statistical analysis of the data from 76 RRMS patients, the following conclusions can be drawn:

- A significant improvement in fatigue symptoms between the two visits was recorded on the applied assessment scales FSMC ($p<0.001$, $d=-0.94$) and MFIS ($p<0.001$, $d=-1.15$), with the improvement being of large effect and 100% statistical power.
- * A significant improvement was also observed in body composition, as evidenced by reductions in BMI, body fat, and visceral fat, and increases in body water and muscle mass ($p<0.001$–0.002).
- A substantial decrease in IL-17A (−85%; $p<0.001$) was found in the patients studied for this parameter (n=15), and the DHA values increased significantly (+30.7%; p=0.003), suggesting a significant anti-inflammatory and nutritional effect of the applied AMD.
- The role of the MEDAS score as a significant predictor of fatigue (MFIS V2: $r=-0.473$, $p<0.001$), disability (EDSS: $r=-0.350$, p=0.002), and changes in body composition was confirmed.
- The multivariate logistic regression analysis confirms the independent nature of this association: MEDAS remained a significant predictor of clinically significant improvement in fatigue by MFIS (aOR = 1.22; 95% CI 1.02–1.45; p=0.027) and of the reduction in visceral fat (aOR = 1.24; 95% CI 1.04–1.49; p=0.018) after adjustment for EDSS, age, and sex.
- A dose-dependent effect of AMD was observed, as evidenced by significant differences in MFIS at V2 among all three MEDAS groups ($p<0.001$), with a full three-step gradation.
- The role of education and marital status as significant determinants of adherence to AMD was demonstrated, with higher education emerging as the strongest independent predictor – increasing the chance of high adherence to AMD approximately 8.75-fold (aOR = 8.75; 95% CI 2.25–34.00; p=0.002), independently of the degree of disability.
- The degree of disability was identified as an independent inverse predictor of high adherence to AMD – each additional EDSS point reduced the chance by approximately

53% (aOR = 0.47; 95% CI 0.24–0.90; p=0.022), underscoring the need for specialised dietary interventions in patients with higher disability.

- No statistically significant influence of sex on MEDAS, EDSS, and fatigue, assessed with MFIS and FSMC, was demonstrated. Sex, however, emerged as an independent predictor of changes in body composition, with men significantly more likely to have a reduction in visceral fat than women (aOR for women = 0.27; 95% CI, 0.09–0.78; p=0.015).
- The lipid profile and CRP did not change significantly, suggesting that the effects of AMD in RRMS are predominantly neurological in the short term, particularly chronic fatigue symptoms.

13. Concluding remarks on the results

The results of the present prospective, longitudinal, observational study in 76 RRMS patients convincingly demonstrate that the three-month follow-up is associated with clinically and statistically significant improvements in fatigue, body composition, and the studied laboratory biomarkers. The degree of adherence to AMD, assessed using the MEDAS, is a significant and independent predictor of fatigue and disability, as further confirmed by multivariate logistic regression analysis after adjustment for confounders. The established dose-dependent effect, in which each step of adherence to the diet is associated with lower fatigue by MFIS at V2, quantified through the regression analysis (aOR = 1.22 per MEDAS point; 95% CI 1.02–1.45; p=0.027), represents an original contribution of the study and opens up prospects for further non-pharmacological interventional research in this area. The identified determinants of adherence to AMD – higher education (aOR = 8.75; p=0.002) and a lower degree of disability (aOR = 0.47 per EDSS point; p=0.022) – have direct implications for the development of individualised dietary strategies in Bulgarian RRMS patients. The absence of significant changes in the lipid profile and CRP suggests that additional metabolic effects of the Mediterranean dietary pattern may emerge with longer follow-up, a task for future studies.

14. Study strength, limitations, and NNT

Study strength

The post-hoc statistical power analysis revealed that the study is adequately powered for the main parameters of interest. For fatigue by FSMC (Cohen's d=0.94, n=76) and MFIS (Cohen's d=1.15, n=76), the statistical power was 100%, and for BMI (d=0.45, n=76) it was 97.6%, at a significance level of α=0.05. These results confirm that the sample size (n=76) is sufficient for the reliable detection of the observed effects. The effect size by Cohen's d is presented in Table 26.

Table 26. Effect size (Cohen's d) and statistical power for the significant parameters

Parameter	Cohen's d	Magnitude	Power (%)
MFIS	−1.150	large	100.0%
FSMC	−0.939	large	100.0%
BF%	−0.502	medium	–
BW%	+0.693	medium	–
BMI	−0.451	small–medium	97.6%

Interpretation: small d<0.50; medium 0.50–0.79; large ≥0.80

An additional indicator of the reliability of the results is the quality of the multivariate regression models. The discriminative ability, assessed via AUC ROC, ranged from moderate to good (AUC = 0.72–0.84), with excellent calibration by the Hosmer-Lemeshow test ($p > 0.05$ for all models) and no problematic multicollinearity (all VIF < 1.4). Particularly impressive is the model's discriminative ability for high adherence to AMD (AUC = 0.843; pseudo-R^2 = 0.283), which strengthens the clinical significance of the identified independent predictors.

Number Needed to Treat (NNT)

The epidemiological measure NNT was calculated for clinically significant improvement in fatigue, defined as a reduction of at least 4 points, which is considered the minimal clinically important difference (MCID) on the FSMC and MFIS. A conservative estimate of 15% was used for the control rate of spontaneous improvement, based on the natural course of RRMS (Table 27).

- NNT for FSMC (MCID≥4): clinically significant improvement was achieved in 52.6% of patients (40/76), with an absolute risk reduction of ARR=0.376. NNT=2.7, i.e. a dietary intervention needs to be applied in at least 3 patients to achieve a clinically significant improvement in fatigue by FSMC in 1 patient.
- NNT for MFIS (MCID≥4): clinically significant improvement was recorded in 55.3% of patients (42/76). ARR=0.403. NNT=2.5.

At a more conservative MCID threshold of 6 points, 26.3% (FSMC) and 35.5% (MFIS) of patients achieved improvement, with NNT of 9.0 and 5.4, respectively.

Table 27. NNT at different MCID thresholds

MCID threshold	FSMC improved	FSMC NNT	MFIS improved	MFIS NNT
≥3 points	50 (65.8%)	2.0	52 (68.4%)	1.9
≥4 points	40 (52.6%)	2.7	42 (55.3%)	2.5
≥5 points	28 (36.8%)	4.6	33 (43.4%)	3.5
≥6 points	20 (26.3%)	9.0	27 (35.5%)	5.4

NNT calculated at CER=15%, based on a conservative estimate of spontaneous improvement in RRMS, owing to the absence of a parallel control group without a dietary intervention.

Limitations of the study

The results should be interpreted in the context of the following methodological limitations:

- Lack of a control group: the study is single-arm and observational, without a parallel control group, limiting causal inference. The observed improvements may be due in part to regression to the mean, the "Hawthorne effect", or the natural course of the disease.
- Relatively short follow-up period: the three-month period may be insufficient to detect effects on the lipid profile and CRP, for which slower changes are expected. Longer-term follow-up could reveal additional metabolic effects.
- Subjectivity of the dietary assessment: MEDAS is a self-report instrument susceptible to social desirability and recall biases. To reduce the risk of this type of error, a food diary was provided during the study to objectify the assessment, but only a small proportion of participants recorded their food intake daily, so these data were not included in the present assessment. The analysis of the biomarkers included in the study is a step towards increasing the reliability of the dietary results.
- Small subgroup for the IL-17A testing (n=15): the significant decrease in IL-17A found in 15 patients understandably limits the extrapolation of these results and requires confirmation in a larger sample.
- Single-centre study: the inclusion of only patients from University Hospital "Dr Georgi Stranski" – Pleven limits the broad representativeness and applicability of the results to the entire Bulgarian RRMS patient population.
- Limitations of the regression analysis: the sample size (n=76) and the number of binary events limit the number of predictors in the multivariate models, in accordance with the rule of a minimum of 8–10 events per predictor (Peduzzi et al. 1996). Future studies with a larger sample would allow the inclusion of more predictors.

Strengths of the study

Notwithstanding the noted limitations, the study has a number of methodological and scientific strengths:

- The prospective longitudinal design, unlike cross-sectional studies, allows the assessment of dynamics and the establishment of temporal associations.
- The consecutive enrolment of patients *(consecutive sampling)* minimises the systematic selection errors *(selection bias)* and provides a representative sample from real-world clinical practice.
- The parallel assessment of fatigue, body composition, lipid profile, biomarkers, and dietary adherence allows a comprehensive analysis of the interrelationships between the studied parameters.
- The use of the validated instruments MEDAS, FSMC, MFIS, and EDSS ensures methodological reliability.
- The 100% statistical power for the main parameters (FSMC, MFIS) confirms the reliability of the results at n=76.
- The application of multivariate logistic regression analysis allows the independent contribution of each predictor to be isolated after adjustment for confounders. The models' adequate discriminative ability (AUC = 0.72–0.84) confirms the validity of the analyses.
- The originality of this first prospective study in our country on the interrelationship between MD and clinical and laboratory parameters in RRMS lends it high scientific and national significance.

V. INTERPRETATION OF THE OBSERVED EFFECTS

Brief discussion of the significant results

The more than 85% decrease in IL-17A in the studied patients supports the hypothesis that AMD actively suppresses Th17-mediated neuroinflammation. This effect, which we have clearly demonstrated, is confirmed by a number of authors who report data on the positive influence of the individual components of the MD: omega-3 PUFAs (EPA and DHA) as modulators of the Th17/Treg balance (Calder 2010; Calder 2013; Zahoor et al. 2021); the polyphenols in olive oil, inhibiting the NF-κB signalling pathway (Beauchamp et al. 2005; Grubić Kezele and Ćurko-Cofek 2022); and dietary fibre as direct immunomodulators through its fermentation in the large intestine to short-chain fatty acids (Duscha et al. 2020; Ross et al. 2024).

The rich content of antioxidant compounds in AMD (polyphenols, resveratrol, flavonoids, lycopene, vitamin E) counteracts oxidative stress, a central mechanism of neuronal damage and fatigue in MS (Piñar-Morales et al. 2025).

Dietary modulation of the gut microbiome is an important mechanism for the anti-inflammatory effects of AMD. The fibre-rich plant components of the diet support the protective microbial populations (*Lactobacillus, Bifidobacterium, Faecalibacterium*), increase the production of SCFAs (butyrate, propionate), and reduce pro-inflammatory dysbiosis (Ghosh et al. 2020; Buga et al. 2023; Yadav et al. 2023; Bronzini et al. 2023). A reduction in propionic acid in MS is associated with increased fatigue, and supplementation with propionate shows promising effects (Duscha et al. 2020). These mechanisms may explain part of the observed favourable influence on fatigue in our study.

The improvement in body composition, reflected in reduced visceral fat and increased muscle mass and body water, reflects the systemic metabolic effects of AMD (Tosti et al. 2018; Wingo et al. 2018; Mallardo et al. 2024). The favourable change in these metabolic parameters in our study is associated with direct pathogenetic consequences for MS, as accumulated visceral fat increases the production of pro-inflammatory cytokines and adversely affects the disease course.

The significance of the psychological and behavioural aspects of the dietary intervention cannot be excluded either. The active participation of patients in the study, regular contact with the medical team, a sense of control over the disease, and improved self-perception may contribute to the observed effects, especially on subjective parameters such as fatigue (the so-called “Hawthorne effect”). These aspects do not diminish the clinical significance of the intervention but rather support the holistic approach to the care of RRMS patients.

Summary of the discussion

The observed clinical and biochemical effects of AMD in RRMS patients are explained by the synergistic favourable action of the various components of the diet on multiple pathogenetic pathways, including immunomodulation, anti-inflammatory effects, antioxidant protection, microbiome modulation, metabolic regulation, neuroprotection, epigenetic modification, and psychological support. These effects of AMD can hardly be achieved with single nutritional supplements, which supports the concept of the fundamental health benefits of comprehensive adherence to the dietary regimen.

In summary, AMD is one of the best-characterised and scientifically substantiated dietary approaches to the management of MS, is convenient for practical application, is well accepted by patients, and confers a number of favourable health effects.

The seasonal adaptation of the diet to the conditions in Bulgaria in our study is an important step towards region-specific dietary recommendations that consider local dietary traditions, product availability, and socioeconomic characteristics. The main components of AMD, including fresh fruits and vegetables, legumes, fish, olive oil, nuts, and whole grains, are available in our country. Seasonal adaptation enables the use of local seasonal products, improving accessibility and economic viability. Bulgarian traditional cuisine has significant common elements with the MD, including a rich presence of fresh vegetables, legumes (lentils, beans, chickpeas), traditional fermented dairy products (yoghurt, white brined cheese), and whole grains.

This is the first prospective study in our country to investigate the interrelationship between AMD and the clinical and laboratory parameters in RRMS. This originality gives it high scientific and national significance and addresses an important gap in the international literature for the Central and Eastern European region. Our results unequivocally support the benefits of integrating AMD as a preferred dietary pattern in Bulgarian RRMS patients and the need for a multidisciplinary approach with the integration of a dietitian/nutritionist into the MS team, periodic consultations with licensed dietitians, group educational sessions, and online platforms for dietary support, as recommended by leading researchers in the field (Allogmanny and Probst 2024; Stoiloudis et al. 2022). The present study, with its strengths and limitations, offers numerous avenues for future research to strengthen and broaden the evidence base regarding the role of diet in the management of MS in Bulgaria (Table 28).

Table 28. Significant randomised controlled trials (RCTs) and meta-analyses of dietary interventions in multiple sclerosis

Author (year)	Design	N	Intervention / Duration	Assessment scales	Main results
A. Randomised controlled trials (RCTs) with a Mediterranean or modified Mediterranean diet in MS					
Razeghi-Jahromi et al. (2020)	RCT	80	Modified MD vs. control; 12 weeks	FSS, MMSE	Significant reduction in FSS and improvement in cognitive parameters in the intervention group.
Bohlouli et al. (2022)	RCT	50	Modified MD vs. a traditional Iranian diet; 12 weeks	FSS, MSQOL-54, CRP, IL-6, TNF-α	Significant reduction in FSS, improvement in the physical and mental components of MSQOL-54, and significant lowering of CRP, IL-6, and TNF-α in the MD group.
Sharifi et al. (2025)	RCT	90	Modified MD vs. control; 12 weeks	FSS, BDI	Significant reduction in FSS and in depressive symptoms in the MD group.
Moravejolahkami et al. (2020)	RCT	147	Modified MD vs. control	FSS, QoL	Improved QoL and fatigue severity with adherence to a modified MD.

Author (year)	Design	N	Intervention / Duration	Assessment scales	Main results
Navarrete-Pérez et al. (2024)	RCT	approx. 50	MIND diet (MD+DASH hybrid); 12 weeks	Antioxidant status, oxLDL, pro-inflammatory cytokines	Improvement in antioxidant status, reduction in oxidatively modified LDL and pro-inflammatory cytokines.
B. Randomised controlled trials with other dietary patterns in MS					
Wahls et al. (2021) – WAVES	RCT	87 RRMS	Wahls (modified palaeolithic) vs. Swank (low-fat); 24 weeks	Fatigue, QoL, functional parameters	Reduction in fatigue and improvement in QoL with both diets, with no significant difference between them.
Crippes et al. (2023); Titcomb et al. (2021)	Secondary analysis of WAVES	87 RRMS	Wahls vs. Swank; 24 weeks	Fatigue, functional tests	The reduction in fatigue is significantly associated with functional improvement in MS.
Wahls et al. (2018)	RCT	20	Modified palaeolithic diet (Wahls); 12 months	FSS, physical tests	Significant reduction in fatigue and improvement in functional status.
Yadav et al. (2016)	RCT	61	Low-fat plant-based diet; 12 months	FSS, QoL, oxidative stress markers	Favourable effects on fatigue, QoL, and oxidative stress. The diet is practically feasible in MS.
Brenton et al. (2019)	Pilot RCT	36	Ketogenic diet; short duration	Fatigue, QoL, metabolic parameters	Good tolerability and potential effects on fatigue, QoL, and metabolic parameters. Limited sample size.
Fitzgerald et al. (2022)	RCT	42	Intermittent calorie restriction vs. control	T-cell subpopulations, metabolic markers	Changes in T-cell subpopulations and metabolic markers, confirming the biological activity of the intervention.
C. Randomised controlled trials with omega-3 PUFA supplementation in MS					
Golabi et al. (2025)	RCT	60	Omega-3 PUFA supplementation vs. placebo	Fatigue, pro-inflammatory cytokines, physical activity	Significant reduction in fatigue and in pro-inflammatory cytokines and improvement in physical activity.
Shahinfar et al. (2025)	Analysis of an RCT	Secondary analysis	Omega-3 PUFA supplementation	Fatigue, cognitive functions	Confirmation of the effects on fatigue and established favourable effects on cognitive functions.
D. Systematic reviews and meta-analyses of dietary interventions in MS					
Snetselaar et al. (2023)	Network meta-analysis	12 RCTs; 8 diets	Various dietary interventions in MS	Fatigue (FSS, etc.), QoL	Significant favourable effect of dietary interventions on fatigue. Wahls and Swank with the strongest effect; MD – a trend towards improvement.
Moravejolahkami et al. (2024) **Also cites our 2022 study**	Systematic review and meta-analysis	Multiple studies	MD in MS	BMI, FSS	Positive effect of the MD with a reduction in FSS and BMI, especially with a duration over 3

Author (year)	Design	N	Intervention / Duration	Assessment scales	Main results
					months and in patients with higher baseline fatigue.
Kong et al. (2025)	Systematic review and meta-analysis	RRMS	MD in RRMS	FSS, MSQOL-54, CRP, IL-6	Significant reduction in FSS and improvement in the physical component of QoL; a trend towards a reduction in CRP and IL-6.
Shakouri et al. (2026)	Systematic review and meta-analysis	14 studies; 1247 patients	Adherence to the MD in MS	Fatigue, physical component of QoL, CRP	Significant associations between better adherence to the MD and lower fatigue, a better physical component of QoL, and lower CRP levels. A recommendation for integrating the MD into the management of MS.
Kabakibo et al. (2026)	Systematic review	Multiple studies	Anti-inflammatory dietary interventions in MS	Pro-inflammatory cytokines, fatigue	Highlights the role of PUFAs, polyphenols, and dietary fibre in reducing the pro-inflammatory cytokines associated with fatigue.
Guerrero Aznar et al. (2022)	Systematic review and meta-analysis	Multiple studies	Various dietary approaches in MS	Fatigue, QoL, disability	Significant favourable effect of anti-inflammatory diets on fatigue and QoL in MS.
AlAmmar et al. (2021)	Systematic review	RCTs with omega-3 PUFAs	Fish oil / omega-3 PUFA supplementation	Disease activity, nerve function	Reduced disease activity and improved nerve function with omega-3 PUFA supplementation.
Abbasi et al. (2023)	Synthesis of observational and interventional studies	Multiple studies	MD-like diets in MS	Fatigue, QoL	A trend towards improvement in fatigue and QoL with MD-like diets; identified methodological limitations and heterogeneity.
Yousefi Rad et al. (2024)	Meta-analysis	Multiple studies	Dietary Inflammatory Index (DII) and risk of MS	DII	A higher DII is associated with an approximately 50% increased risk of MS / demyelinating autoimmune disease.
Zielińska & Michońska (2023)	Systematic review	Multiple dietary patterns	Various diets in MS	Various	The studied diets show promising but insufficiently proven effects owing to heterogeneity of designs and small samples.

VI. CONCLUSIONS

Based on the results of the present observational study in 76 RRMS patients followed for 12 weeks after the application of a Mediterranean diet seasonally adapted to the conditions in Bulgaria (AMD), the following main conclusions can be drawn:

1. The Mediterranean diet adapted to Bulgarian conditions is associated with clinically and statistically significant improvements in chronic fatigue symptoms among RRMS patients. The improvement was established on both assessment scales used (FSMC and MFIS).
2. The diet has a favourable influence on the body composition of RRMS patients, as evidenced by a significant reduction in body mass index and the percentages of body and visceral fat, and an increase in muscle mass and body-water percentage.
3. The application of AMD is associated with pronounced anti-inflammatory and nutritional effects, as evidenced by a significant reduction in serum interleukin-17A levels and a significant increase in serum DHA levels.
4. The degree of adherence to AMD, assessed via the validated MEDAS-14 questionnaire, emerges as a significant independent predictor of clinically significant improvement in fatigue (MFIS) and of a reduction in visceral fat after adjustment for confounders in the multivariate logistic regression analysis.
5. A dose-dependent effect of AMD on fatigue in RRMS was established, as evidenced by significant differences in MFIS at V2 among the three MEDAS groups (low, medium, high) with a full three-step gradation, providing the most convincing evidence for the biological plausibility of the effect.
6. Higher education is the strongest independent predictor of high adherence to AMD in Bulgarian RRMS patients, increasing the chance of good adherence approximately 8.75-fold, independently of the degree of disability.
7. The degree of disability (EDSS) is a significant independent inverse predictor of adherence to AMD, with each additional EDSS point reducing the chance of high adherence by approximately 53%, which underscores the need for specialised dietary interventions in patients with a higher degree of disability.
8. No statistically significant influence of sex on adherence to AMD, the degree of disability, or fatigue severity was established in Bulgarian RRMS patients. Sex emerges as an independent predictor of changes in body composition, with men significantly more likely than women to experience a reduction in visceral fat, reflecting physiological differences in adipose tissue distribution between the sexes.

9. The lipid profile and C-reactive protein did not change significantly over the three-month dietary intervention, suggesting that the metabolic effects of AMD in RRMS require longer follow-up to be established.

VII. CONCLUDING REMARKS

This dissertation presents the results of the first prospective observational study in Bulgaria to investigate the influence of a seasonally adapted Mediterranean diet on clinical and laboratory parameters in patients with RRMS. The results obtained convincingly demonstrate that the three-month application of AMD is associated with clinically and statistically significant improvements in fatigue, body composition, serum biomarkers, and omega-3 PUFA levels.

Particularly significant are the established dose-dependent effect of adherence to AMD on fatigue – an original contribution of the study – and the identified independent determinants of dietary adherence. Higher education and a lower degree of disability emerge as the leading predictors of good adherence to AMD in Bulgarian RRMS patients.

The results obtained are an expected addition to the growing international evidence for the anti-inflammatory and neuroprotective potential of MD in MS. Our research confirms and enriches the contemporary database with original, region-specific, and clinically applicable findings on the topic that remain insufficiently represented in the literature to date. The adaptation of the classical MD to the seasonal conditions in Bulgaria is a useful and convenient approach, aligned with local dietary culture, the availability of seasonal products, and socioeconomic characteristics, and is well accepted by patients, thereby improving the practical applicability of the intervention.

The excellent safety profile and broad applicability of AMD place it among the most promising non-pharmacological approaches for managing chronic fatigue and optimising body composition in patients with RRMS. These characteristics, combined with the proven cardiometabolic benefits of the Mediterranean dietary pattern, justify the need to integrate AMD into the multidisciplinary management plan for multiple sclerosis in Bulgaria.

The results of the dissertation, despite the methodological limitations of the observational single-arm design, provide a basis for future expanded regional and national studies of non-pharmacological dietary interventions to support contemporary therapeutic strategies in multiple sclerosis.

VIII. CONTRIBUTIONS

The contributions made in the dissertation reflect the complex nature of the study conducted, encompassing molecular, laboratory, statistical, and applied aspects of the relationship between AMD and the clinical characteristics of RRMS patients. The author's original contributions have been publicly confirmed through presentations, working meetings, and interim reports on the project activities carried out.

Contributions of an original nature

1. The first prospective longitudinal study in Bulgaria examined the influence of a Mediterranean diet, seasonally adapted to the country's conditions, on clinical and laboratory parameters in patients with relapsing-remitting multiple sclerosis, thereby enriching the international evidence base with new data for the Central and Eastern European region.
2. An MD adapted to the conditions in Bulgaria was applied, aligned with the seasonal availability of fresh products, the local dietary culture, and the country's socioeconomic conditions.
3. A dose-dependent effect of AMD on fatigue in RRMS, as measured by the MFIS scale, was demonstrated and confirmed by multiple statistical methods.
4. Educational materials, originally developed for Bulgaria, were produced for RRMS patients – an AMD brochure with dietary recommendations adapted to the country's seasonal conditions and a food diary, which can be integrated into the standard care of MS patients in Bulgarian clinical practice.
5. Independent predictors of high adherence to AMD in Bulgarian RRMS patients were identified – higher education and a lower degree of disability – which can be considered in the development of individual dietary approaches and nutritional interventions.
6. For the first time in the country, a pronounced reduction in serum interleukin-17A in RRMS patients after AMD was documented and statistically demonstrated, representing an original contribution supporting the diet's anti-inflammatory potential.
7. A significant increase in serum DHA levels was demonstrated as an objective biomarker of adherence to AMD in the studied patients, in addition to the MEDAS-14 self-assessment questionnaire.

Contributions of a confirmatory and applied nature

1. The effectiveness of AMD on fatigue in RRMS in the conditions of Bulgarian clinical practice was confirmed, consistent with data from international and national studies.
2. The role of education and sociodemographic factors as significant determinants of adherence to healthy dietary regimens in MS patients was confirmed.

3. The favourable influence of AMD on body composition and metabolic status in patients with RRMS was confirmed.
4. The good acceptability and practical applicability of AMD in Bulgarian RRMS patients were established, which are comparable to results from Mediterranean countries.
5. The need for structured dietary support for patients with a higher degree of disability by EDSS, who emerge as the most vulnerable group with respect to adherence to AMD, was demonstrated, and specific approaches were proposed involving family inclusion, simplified dietary recommendations, and social support.
6. A comprehensive methodological approach was employed, including validated metric scales (MEDAS-14, FSMC, MFIS, EDSS), bioimpedance analysis, and contemporary statistical methods, which can serve as a model for future dietary studies in multiple sclerosis and other neurological diseases in Bulgaria.

IX. LIST OF SCIENTIFIC WORKS RELATED TO THE DISSERTATION

1. PUBLICATIONS

In specialised peer-reviewed and indexed scientific journals in Bulgaria

1. Ovcharova EM, Danovska MP, Marinova DL, Pendicheva DI, Tonchev PT, Shepherd NM (2022) Role of diet and supplementation with omega-3 polyunsaturated fatty acids for managing chronic fatigue in patients with relapsing-remitting multiple sclerosis. Journal of Biomedical and Clinical Research 15(2): 99–104. https://doi.org/10.2478/jbcr-2022-0013
2. Ovcharova E, Danovska M, Marinova D, Pendicheva D, Tonchev P, Atanasova M, Russeva AL, Shepherd N, Tzveova R (2022) Adapted Mediterranean diet impact on the symptoms of chronic fatigue, serum levels of omega-3 polyunsaturated fatty acids (PUFAs) and interleukin 17 (IL-17) in patients with relapsing-remitting multiple sclerosis undergoing disease-modifying therapy: a pilot study. Journal of IMAB 28(1): 4297–4304. https://doi.org/10.5272/jimab.2022281.4297
3. Yanakieva M, Danovska M, Ovcharova E, Marinova D, Shepherd N (2023) Tumefactive multiple sclerosis: a diagnostic enigma. A case report. Journal of IMAB 29(2): 4943–4946. https://doi.org/10.5272/jimab.2023292.4943
4. Ovcharova E, Yanakieva M, Danovska M, Marinova-Trifonova D (2024) Mediterranean diet improves the quality of life in patients with relapsing-remitting multiple sclerosis: our experience. Multiple Sclerosis Journal 30(3 Suppl): 1132. [meeting abstract P1799/1098, ECTRIMS 2024] https://journals.sagepub.com/toc/msja/30/3_suppl

2. PARTICIPATION IN SCIENTIFIC FORUMS

International scientific forums

1. Ovcharova E, Yanakieva M, Danovska M, Marinova-Trifonova D (2024) Mediterranean diet improves the quality of life in patients with relapsing-remitting multiple sclerosis: our experience. European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS) Congress, 18–20 September 2024, Copenhagen, Denmark.

National scientific forums

1. Danovska M, Yanakieva M, Ovcharova E, Marinova D, Bhatti N (2023) Schilder's disease: an exceedingly rare and diagnostically challenging variant of multiple sclerosis. In: 20th International Medical Scientific Conference for Students and Young Doctors,

Abstract Book, Neurology and Psychiatry Section. Publishing Center Medical University – Pleven, Pleven.

2. Patanova GE, Yanakieva M, Vasileva V, Marinova-Trifonova D, Ovcharova-Duhlenska EM, Danovska-Mladenova MP (2024) Laughing gas-induced neurotoxicity: a case report. In: XXI International Medical Scientific Conference for Students and Young Doctors 2024, Abstract Book, Neurology, Neurosurgery & Psychiatry Section. Publishing Center Medical University – Pleven, Pleven, 75. ISBN 978-954-756-349-0 (Book), ISBN 978-954-756-350-6 (PDF E-book)

3. RESEARCH PROJECTS RELATED TO THE DISSERTATION

1. Project No. 19/2020, funded by MU – Pleven: Influence of an adapted Mediterranean diet on the symptoms of chronic fatigue, serum levels of omega-3 polyunsaturated fatty acids (PUFAs), and interleukin-17 (IL-17) in patients with the relapsing-remitting form of multiple sclerosis undergoing disease-modifying therapy. https://www.mu-pleven.bg/forms/Proekti%20MU-Pleven%202020-new.pdf

DECLARATION

OF ORIGINALITY AND AUTHENTICITY OF THE DISSERTATION

The undersigned, Dr Emiliya Mladenova Ovcharova-Duhlenska,
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Dissertation: "Assessment of the impact of dietary habits on clinical and laboratory parameters in patients with multiple sclerosis"
for the acquisition of the educational and scientific degree "Doctor", Professional field 7.1. Medicine, Doctoral programme "Neurology"

I DECLARE:

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/Emiliya Ovcharova-Duhlenska, MD/

ASSESSMENT OF THE IMPACT OF DIETARY HABITS ON CLINICAL AND LABORATORY PARAMETERS IN PATIENTS WITH MULTIPLE SCLEROSIS

SUMMARY

BACKGROUND

Multiple sclerosis (MS) is a chronic autoimmune demyelinating disease of the central nervous system, with fatigue affecting up to 90% of patients and substantially impairing quality of life. Despite advances in disease-modifying therapy (DMT), pharmacological options for managing fatigue remain limited and of modest efficacy. Growing international evidence supports the role of the Mediterranean diet (MD) as a non-pharmacological intervention with anti-inflammatory and neuroprotective potential in MS. However, no prospective study has previously evaluated the effects of an MD-based intervention tailored to Bulgarians' dietary culture and seasonal product availability.

AIM

The aim of this dissertation was to evaluate the influence of a seasonally adapted Mediterranean diet (AMD) on fatigue, body composition, and clinico-laboratory parameters in patients with relapsing-remitting multiple sclerosis (RRMS) followed in real-world clinical practice.

MATERIALS AND METHODS

A prospective, longitudinal, observational, single-arm, single-centre study was conducted at the Department of Neurology, University Hospital "Dr Georgi Stranski" – Pleven, between 2020 and 2025. Seventy-six consecutive patients with RRMS, diagnosed according to the revised McDonald criteria (2024), were enrolled and assessed at two time points: baseline (V1) and after 12 weeks of adherence to AMD (V2). Adherence to AMD was evaluated using the validated 14-item Mediterranean Diet Adherence Screener (MEDAS-14). We assessed fatigue with the Fatigue Scale for Motor and Cognitive Functions (FSMC) and the Modified Fatigue Impact Scale (MFIS); neurological disability with the Expanded Disability Status Scale (EDSS); and body composition by bioelectrical impedance analysis (Tanita InnerScan). Serum biomarkers included lipid profile, high-sensitivity C-reactive protein (CRP), interleukin-17A (IL-17A), and omega-3 polyunsaturated fatty acids (EPA, DHA). Statistical analysis included the Wilcoxon signed-rank test, Mann-Whitney U test, Kruskal-Wallis test with Bonferroni post hoc correction, Spearman correlation, and binary logistic regression with univariate and multivariate Odds Ratios (OR/aOR).

RESULTS

The study population included 42 women (55.3%) and 34 men (44.7%) with a mean age of 45.5 ± 7.7 years, a mean disease duration of 9.5 ± 7.6 years, a mean EDSS of 3.52 ± 1.38, and 81.6% on stable DMT. The mean MEDAS score was 6.78 ± 3.08, with 22.4% of patients showing high adherence (MEDAS ≥ 10). We documented statistically significant improvements in 9 parameters: FSMC (−4.1 points, $p < 0.001$, 80.3% improved; Cohen's $d = -0.94$), MFIS (−4.2 points, $p < 0.001$, 84.2% improved; Cohen's $d = -1.15$), BMI ($p < 0.001$), body fat percentage ($p < 0.001$), visceral fat ($p < 0.001$), body

water ($p < 0.001$), muscle mass ($p = 0.002$), serum IL-17A (−85% reduction, $p < 0.001$, $n = 15$), and serum DHA (+30.7% mean, +60.5% median, $p = 0.003$). Lipid profile, CRP, EPA, pulse, and blood pressure remained unchanged. Statistical power for FSMC and MFIS was 100%.

MEDAS score showed significant inverse correlations with EDSS ($r = −0.350$, $p = 0.002$), MFIS at V2 ($r = −0.473$, $p < 0.001$), and changes in BMI and visceral fat. A complete three-step dose-dependent gradient was observed for MFIS at V2 across MEDAS tertiles (low: 43.56; medium: 34.21; high: 24.53 points; all pairwise comparisons $p < 0.05$ after Bonferroni correction), representing the most robust evidence for the effect of AMD on fatigue. MEDAS remained an independent predictor of clinically significant MFIS improvement (aOR = 1.22 per point; 95% CI 1.02–1.45; $p = 0.027$) and visceral fat reduction (aOR = 1.24 per point; 95% CI 1.04–1.49; $p = 0.018$) after adjustment for confounders. Higher education emerged as the strongest independent predictor of high adherence to AMD (aOR = 8.75; 95% CI 2.25–34.00; $p = 0.002$), while EDSS was a significant inverse predictor (aOR = 0.47 per point; 95% CI 0.24–0.90; $p = 0.022$). The model demonstrated good discriminative ability (AUC = 0.843; McFadden pseudo-$R^2 = 0.283$; LR test $p < 0.001$), adequate calibration (Hosmer-Lemeshow $p > 0.05$), and no problematic multicollinearity (VIF < 1.4). For clinically significant fatigue improvement (MCID ≥ 4 points), NNT was 2.7 for FSMC and 2.5 for MFIS, representing favourable values compared with available pharmacological interventions for MS-related fatigue.

Conclusions

This first Bulgarian prospective study on dietary interventions in MS demonstrates that a seasonally adapted Mediterranean diet is associated with clinically and statistically significant improvements in fatigue, body composition, and inflammatory and nutritional biomarkers in patients with RRMS. The dose-dependent effect of MEDAS adherence on fatigue, confirmed across multiple statistical approaches including multivariate logistic regression, represents an original contribution to the international literature. Higher education and lower disability emerged as key independent determinants of dietary adherence, with direct implications for the development of individualised dietary support strategies in the Bulgarian RRMS population. The favourable NNT values (2.5–2.7) and excellent safety profile position AMD as one of the most promising non-pharmacological approaches for managing chronic fatigue in MS. The findings support the integration of AMD into the multidisciplinary management plan for MS in Bulgaria and contribute original data from the Central and Eastern European region to the growing international evidence base on the role of nutrition in multiple sclerosis.

Keywords: RRMS, Mediterranean diet; MEDAS-14, FSMC, MFIS, IL-17A; DHA; EPA, EDSS.